CHARACTERISATION OF ACTIVE PRINCIPLES FOUND IN SOME ETHNOMEDICINAL PLANTS

Thesis submitted for the degree of
Doctor of Philosophy (Science) in Botany
of the University of North Bengal

Submitted by
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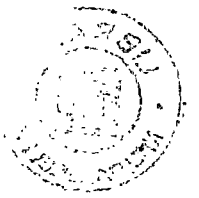

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TO WHOM IT MAY CONCERN

This is to certify that Sri Hariswami Das has carried out his research work under my supervision. His thesis entitled *Characterisation of active principles found in some ethnomedicinal plants* , is based on his original research work and is being submitted to the University of North Bengal for the award of Doctor of Philosophy (Science) degree in Botany, in accordance with rules and regulations of the University of North Bengal.

Usha Chakraborty

(Usha Chakraborty)

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Harishwami Das
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Signature

Place: N.B.U
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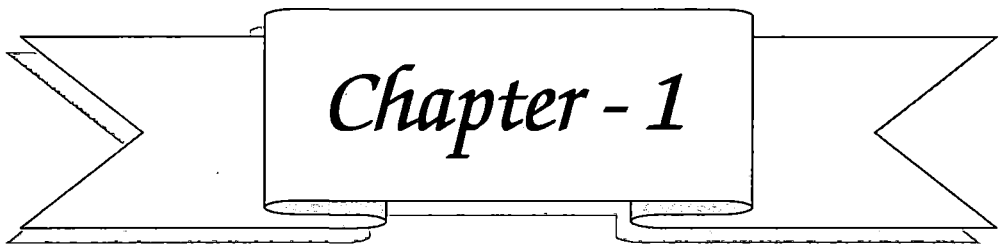


INTRODUCTION

India has one of the oldest, richest and diverse cultural traditions associated with the use of medicinal plants and herbs for human, livestock and plant health. Throughout the world, plants have been in continuous use in one way or the other for the treatment of various ailments. In India, the sacred Vedas, which date back between 3500 B.C. and 800 B.C., give many references of medicinal plants. One of the remotest works in traditional herbal medicine is "Virikshayurveda," compiled even before the beginning of Christian era and formed the basis of medicinal studies in ancient India. The Rig Veda, dating between 3500 B.C. to 1800 B.C.,seems to be the earliest record available on medicinal plants. Ayurveda, the Indian indigenous system of medicine, dating back to the Vedic ages (1500-800 B.C.), has been an integral part of Indian culture (Weiss, 1987). The earliest works of Ayurveda that we know today are the *samhitas* of Caraka and Susruta (before 600 B.C.) modified and supplemented by later authors. Herbs seem to be very important component of medicine in other cultures too; Greek, African and Chinese medicines, etc. Nearly 80% of the world population depends upon traditional system of health care. Allopathic drugs have brought a revolution throughout the world but the plant base medicines have its own unique status (Behera *et al.,* 2006).

Recently Ethno botany has emerged as an interesting and distinctive branch of botany. It is the study of the traditional knowledge acquired through trial and error methods by the primitive people especially aboriginals and tribal. The term today has come to denote the entire realm of direct relationship between plant and man (Manilal, 1989). The scope, concepts and implication of ethnobotany have been expanding at a very fast rate (Jain, 1963, 1965; Harsberger, 1985; Faulks, 1995). In India, quite a few important works on ethnobotany have been published from different corners of the country (e.g. Bodding, 1927; Rajendran *et al.*, 1997; Upadhyay *et al.*, 1997; Sen and Batra, 1997; Rai *et al.*, 1998). Humans, over the ages, have been using plants of their surrounding to cure the diseases they suffer from. It is very difficult to know when humans started using the plants against their disease. In the process of selecting the medicinal plants, they gained a lot of knowledge about medicines and medicinal plants. The traditional knowledge system handed over from generation to generation by traditional communities by oral tradition is still a living tradition in many developing countries particularly biodiversity rich third world nations.

Vast ethnobotanical knowledge exists in India from ancient time. Since the 1950s the study of ethnobotany has intensified. Jain (2007) work over four decades, both in the field and literary studies, has compiled a dictionary of Indian folk-medicine and ethnobotany that includes 2532 plants. India has about 45000 plant species; medicinal properties have been assigned to several thousand. About 2000 figure frequently in the literature; indigenous systems commonly employ 500. Despite early (4500-1500 BC) origins and a long history of usage, in the last two centuries Ayurveda has received little official support and hence less attention from good medical practitioners and researchers. Much work is now being done on the botany, pharmacognosy, chemistry, pharmacology and biotechnology of herbal drugs. The value of ethnomedicine has been realized; work is being done on psychoactive plants, household remedies and plants sold by street drug vendors. Statistical methods are being used to assess the credibility of claims.

A number of organizations within India are concerned with maintaining India's Traditional Medicine Systems. In addition, there is a wide spread development network, and established pharmaceutical industry and a wealth of botanical experts in the country. Until now, however, there has been little effort to document the volume and impact of national or international trade in India's medicinal plants. (Ganesan and Kesavan,2003). According to the latest figures, it costs around 800 million dollars to put a new drug on the market. When companies manufacture a product based on traditional knowledge and convert it into a medicine, they "acquire" a product which is worth a few hundred million dollars (Jain, 1986, 1995). USA based top pharmaceutical companies like Merck, Ranbaxy and Shaman are the classical examples. Such is the enormous potential hidden in these plants gifted by Nature (Ahmad *et al.*, 2003).

The non-disclosure/secrecy surrounding many of the herbal and medicinal formulations with curative properties practiced traditionally prevented the growth of the sector. Modernization followed by industrialization and urbanization has changed the tribal situation in such a way that they are unaware of the traditional knowledge, which had been transferred through generations. At present, this vanishing traditional knowledge survives through few elder peoples. Therefore documentation of traditional medicinal knowledge as well as medicinal plants is needed for the future. The need for conservation of medicinal herbs and traditional knowledge particularly for the developing countries like India, taking into account the socio-cultural and economic conditions, have been discussed at length by Misra (1999). Traditional medicine, which includes ethno-medicine (WHO, 1978) is

important as it provides health services to 75-80% of the world's population (Marine-Bettolo, 1980).

Very few plants used by tribal for medicine are subjected to scientific investigation. Ethnopharmacology is a newly emerging area, which has been defined as the observation, identification, description and experimental investigation of the ingredients on the efficacy of indigenous drugs. It is considered as a scientific backbone in the development of active therapeutics based on traditional medicine.

Plants are the valuable source of new natural products as these have non narcotic, having no side effect and easily available at affordable cost. The drugs of traditional system have been the starting points of the discovery of many important modern drugs. This leads to the investigation of plants and to undertake general biological screening programs of the plants not only in India but all over the world. So, random screening of plant materials in search of biodynamic compounds is necessary. The immense value of the traditional medicinal wisdom can be gauged from the fact that verification of the medicinal property of medicinal plant would involve collection, identification of the plant material, preparation of appropriate extracts and fractions of the plant for testing biological activity using animals or *in vitro* cellular models or by the latest activity guided extraction and through put analysis etc., involving taxonomists, pharmacologists, phytochemists and biochemists. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Hill, 1952).

Plant derived natural products such as flavonoids, terpenoids, and steroids etc have received considerable attention in recent years due to their diverse pharmacological properties including antioxidants. Free radicals have been implicated as the cause of several diseases such as liver cirrhosis, cancer, diabetes and compounds that can scavenge free radicals have great potential in ameliorating these disease processes (Wilson, 1988). Antioxidants play an important role in protecting the human body against damage by reactive oxygen species (Lolliger, 1981). Antioxidants also play an important role in inhibiting and scavenging radicals, thus providing protecting to humans against infection and degenerative diseases.

A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms *in vitro* (Cowan, 1999); and

some plant extracts have shown activity on both gram negative and gram positive organisms (Nascimento *et al.*, 2000). Gabriela *et al.*, (2001) did an antimicrobial evaluation of certain plants used in Mexican traditional medicine for the treatment of respiratory diseases. Characterization of antimicrobial compounds from a common fern, *Pteris biaurita* was done by Chakraborty *et al.* (2007).

Diabetes mellitus is a non communicable disease considered to be one of the five leading causes of death world wide. About 100 million people around the world have been diagnosed with diabetes and by the year 2010 it is projected that 215 million people will have the disease (Zimmet, 1999). Diabetes mellitus is a metabolic disorder and a major cause of disability and hospitalization (Foster, 1994). Diabetes mellitus is generally divided into two different types: insulin-dependent diabetes mellitus (IDDM), and non-insulin-dependent diabetes mellitus (NIDDM). Diabetes mellitus is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both (Baquer *et al.*, 1998). Insulin therapy affords effective glycemic control in IDDM patients, yet its short comings include ineffectiveness on oral administration, short shelf life, need for preservation in refrigeration, fatal hypoglycemia in the event of excess dosage, reluctance to take injection and above all, the resistance due to prolonged administration, limits its usage. Similarly treatment of NIDDM patients with sulfonylureas and biguanides is always associated with side effects (Rang and Dale, 1991). Hence search for a drug with low cost, more active and without side effect is being pursued in several laboratories around the World.

Streptozotocin (STZ) - induced hyperglycaemia has been described as a useful experimental model to study the activity of hypoglycaemic agents (Junod *et al.*, 1969). Streptozotocin (STZ) destroys β-cells of the pancreas and induces hyperglycemia (Palmer *et al*, 1998). Oxidative stress resulting from enhanced free radical formation and/or defects antioxidants defense caused severe tissue damage and may lead to number of diseases like coronary artery disease, atherosclerosis, cancer and diabetes. Increased oxidative stress in streptozotocin diabetic rats has been reported (Garg *et al.*, 2000). In recent years many researchers have examined the effects of plants used traditionally by indigenous healers and herbalists to support liver function and treat diseases of the liver. In most cases, research has confirmed traditional experience and wisdom by discovering the mechanisms and mode of action of these plants as well as reaffirming the therapeutic effectiveness of certain plants or

plant extracts in clinical studies. Several hundred plants have been examined for use in a wide variety of liver disorders. Just a handful has been fairly well researched (Scott, 1999).

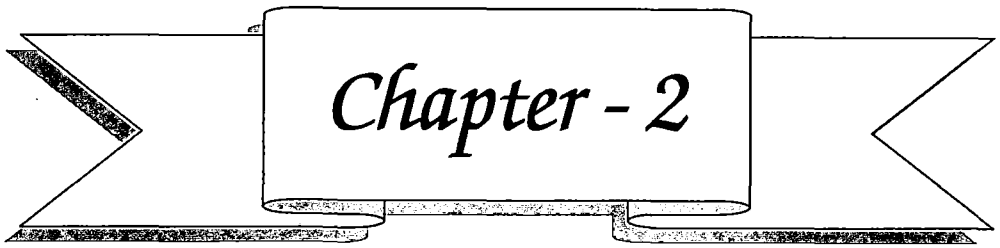
To determine the mechanism of action of hypoglycemic drugs, levels of lipid peroxides, glutathione content, the related enzymatic antioxidants superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), glycogen content in liver and kidney tissues are important. Administration of various antioxidants such as vitamin C, vitamin E, reduced glutathione and selenium in diabetes along with hypoglycemic drugs is useful as supportive therapy (Ihara *et al.*, 2000; Head, 2000). Many indigenous Indian medicinal plants have been used to manage diabetes (Nagararajan *et al.*, 1987; Anjali and Manoj, 1995) and some of them have been tested and the active principles isolated. *Aegel marmelos* L. (Rutaceae) has been shown to be anti-hyperglycemic and to induce release of insulin (Sharma *et al.*, 1996). An isolated compound momordin from *Momordica charantia* L. (Cucurbitaceae) has been shown to posses' anti-fungal activity (Chandravadana *et al.*, 1997) and the plant extract also reduced blood sugar (Chandrasekar *et al.*, 1989). *Trigonella foenum-graecum* wild (Leguminosae) has been shown to have an anti-hyperglycemic effect in normal and alloxan-induced diabetic rats (Abdel-Barry *et al.*, 1997).

The varied climatic zone of India has large bio diversity and rich natural resources. Dakshin Dinajpur is a district of West Bengal which falls within the latitude $25^0 10' 5''$N and the longitude $89^0 0' 30''$E, surrounded by the Malda and Uttar Dinajpur districts of West Bengal from the West and by the neighboring country Bangladesh from the north, south, and east. The district has the oldest and richest cultural traditions of using medicinal plants. In this regard no research work has been done from the selected area of this district, so the present investigation was undertaken to know their traditional knowledge about the uses of medicinal plants, generally predominant in those selected areas. The proposed work was divided into two phases:

In the first phase the traditional knowledge about medicinal plants of the villagers was collected from the selected areas of the district and in the second phase detailed investigation of a few selected plants was carried out. The objectives of the present work were: (i) to collect information from the locals for the uses of medicinal plants of Dakshin Dinajpur district; (ii) to gather information on the nature of plants/ Plant parts used and complete details of such plants; (iii) to select some plants used by villagers, showing good medicinal

properties, (iv) to make detailed studies of the plants especially on morphology, biochemistry and pharmacology, (v) to isolate the active principles from such plants and partially characterize the isolated compound(s), (vi) to test the antimicrobial activities of the active principles, (vii) to test the presence of antioxidants, (viii) to determine the efficacy for suppressing hyperglycemia.

At the onset, a literature review pertaining to the line of work has been presented. Experiments have been performed based on standard methodologies. Statistics have been done wherever necessary.



LITERATURE REVIEW

From the very beginning of human evolution, man has turned to plants for all his uses- whether it be food, clothing or as medicines to combat illnesses. Hence, the knowledge about the use of medicinal plants dates back to centuries, but unfortunately, most of it is not recorded. With the beginnings of the modern world as we know today, when miracle medicines came to the front, their uses suppressed the use of traditional medicines, which became confined to small ethnic groups, who in turn kept their knowledge secret. However, when understanding dawned on humans that the so called miracle medicines have several side-effects, people turned to the use of plants once again. Hence this is an important area which offers much scope to researchers and doctors. Knowledge stored in the heads of ethnic people are now being tried to be brought into print and to be corroborated scientifically. Hence, a lot of literature is now available in this line.

In the following pages, an attempt has been made to compile the available knowledge in this field.

2.1. Ethnobotanical study

According to Jain (1994) India has about 45,000 plant species of which medicinal properties have been assigned to several thousand. About 2000 figure frequently in the literature; indigenous systems commonly employ 500, despite early (4500-1500 BC) origins and a long history of usage, in the last two centuries. Ayurveda has received little official support and hence less attention from good medical practitioners and researchers. Much work is now being done on the botany, pharmacognosy, chemistry, pharmacology and biotechnology of herbal drugs. The value of ethnomedicine has been realized; work is being done on psychoactive plants, household remedies and plants sold by street drug vendors. Statistical methods are being used to assess the credibility of claims. A scrutiny of folk claims found 203 plants for evaluation. Less well known ethnomedicines have been identified that are used to treat intestinal, joint, liver and skin diseases. Gupta *et al.* (1993) did a medicinal plant inventory of Kuna Indians. Results of an ethnopharmacognostic survey of 90 plants used by the Kuna Indians of San Blas Islands, who lived in Ailigandi, were listed. Results of a literature search were also reported, including medical uses, known constituents and pharmacological effects.

Barrett and David (1996) worked on the ethnomedical, Biological, and Clinical Support for Medicinal Plant Use on Nicaragua's Atlantic Coast. The use of medicinal plants

on the Atlantic coast of Nicaragua was compared with the use of these plants worldwide. Similar medical usage was documented for 66 species in dozens of countries on several continents. Additionally, medicinal uses of 34 plants in eastern Nicaragua were noted to have applications consistent with biological or clinical activity. The similarities in ethnomedicinal applications and the evidence from biomedical investigations support the concept that the use of medicinal plants by indigenous peoples of Nicaragua was not haphazard, but rather a consistent and perhaps legitimate system of medical treatment.

An ethnopharmacobotanical survey of the medicinal plants and food medicines of the northern part of Lucca Province, north-west Tuscany, central Italy, was carried out (Pieroni, 2000). The geographical isolation of that area has permitted the survival of a rich folk phytotherapy involving medicinal herbs and also vegetable resources used by locals as food medicine. Among these were the uncommon use of *Ballota nigra* leaves as a trophic protective; the use of *Lilium candidum* bulbs as an antiviral to treat shingles (*Herpes zoster*); *Parmelia* sp. as a cholagogue; *Crocus napolitanus* flowers as antiseptic; *Prunus laurocerasus* drupes as a hypotensive; and the consumption of chestnut flour polenta cooked with new wine as bechic. Many wild gathered greens were eaten raw in salads, or in boiled mixtures, as 'blood cleansing' and 'intestine cleansing' agents. Of particular interest was the persistence of the archaic use of *Bryonia dioica* root against sciatica, and the use of ritual plant therapeuticals as good omens, or against the 'evil eye.' Over 120 species represented the heritage of the local folk pharmacopoeia in upper Garfagnana. Anthropological and ethnopharmacological considerations of the collected data were also discussed. In another survey Pieroni *et al.* (2007) have investigated the medicinal perceptions of vegetables traditionally consumed by South-Asian migrants living in Bradford, Northern England. Dietary habits change rapidly amongst migrant communities in Western countries, and those changes can cause major concerns for public-health policymakers because they frequently lead to increases in diet-related diseases like diabetes. Such was the case in most South-Asian communities in the UK. In that study, they carried out an ethnobiological survey of the vegetables traditionally consumed among the Indian and Pakistani communities of Bradford, in Western Yorkshire, UK. Their purpose was to analyse in depth details of the traditional culinary use of vegetables within those households, and to assess the health perceptions of them. Semi-structured interviews with a total of 150 South-Asian women were carried out. Twenty-five vegetables were recorded, as well as their traditional culinary use and their frequency of use. They found that a few of these vegetables, particularly those presenting

bitter or aromatic tastes, were perceived to have remarkable medicinal value particularly against diabetes. Their study also found important generational differences in the women's knowledge of the culinary processes related to these foods, confirming that the consumption of traditional vegetables was inextricably embedded in cultural heritage and the representation of identity among migrants. According to the authors their findings might offer evidence of a link between the choice of food and the foods' perceived medicinal value among South-Asian migrants that might also provide important information for health care professionals when designing strategies for improving health care counteracting type 2 diabetes. They strongly believe such strategies should take into account socio-cultural components and *emic* health beliefs, as well as patients' views of traditional dietary ingredients.

Rajan *et al.* (2002), in a review, opined that a high degree of biodiversity, marked by varied flora and fauna of good therapeutic potential as well as the varied number of indigenous groups of people in that area, makes it very popular among herbalists. The district had six anthropologically well defined ethnic groups namely *Todas, Kotas, Kurumbas, Irulas, Paniyas* and *Kattunayakas* living here possibly since 1200 B.C. That review highlighted the ethnobiological profile of six indigenous populations and their dependence on ambient flora and fauna for traditional health care needs. It had been observed that about 2700 therapeutically potent plant species are available in that hill station of which almost all have come from local medicine. Some have been explored scientifically. However, about 150 plant species were still to be explored for their therapeutic potential. The ethnography, phytochemical and therapeutic uses as well as the anthropological perspectives of the local medicines have been discussed in that review. A study by Harsha *et al.*, (2003) deals with the herbal remedies for skin diseases in Uttara Kannada district of Karnataka--a Southern State in India, which was located in the hearts of Western Ghats. In an ethnobotanical exploration of that area 52 herbal preparations from 31 plants belonging to 21 families have been recorded. That also included 17 new claims to the ethnomedical knowledge. The parts used and methods of preparation were discussed along with the family and local name for all the plants.

Sixty-six medicinal plant species traditionally collected and used by the Red-headed Yao people in Jinping County, Yunnan Province, SW China, were investigated and studied (Chun-lin and Li, 2004) through the approaches of ethnobotany, anthropology and participatory rural appraisal (PRA). Among these plants, 27 species were recorded to have

medicinal values for the first time recorded in literature, 23 species were found to have different medicinal functions from those recorded in the literature. Many medicinal herbs are simultaneously wild food plants. The local Yao people take medicinal baths on some special days very common to treat and prevent diseases. The Red-headed Yao medicinal herb doctors have conserved medicinal plants and their habitats over the years. Most of the folk healers were old women, who were concerned about passing on their secrets to the younger generation. They fear that the younger generations have not learned enough about the herbal traditions to keep the practice going. The authors suggested that plants used by the Red-headed Yao people need to be further studied phytochemically and pharmacologically.

Menezes *et al.* (2005) worked on the chemical and pharmacological survey on Brazilian medicinal plants using ethnopharmacological information as a tool. The project encompasses plants from the following families: Palmae, Lamiaceae, Acanthaceae, Leguminosae and Gesneriaceae. Regarding the pharmacology, several models have been used like antinociceptive, anti-inflammatory, antioxidant, molluscicidal, anti-diabetes, anti-microbial and nitric oxide production inhibition. Results showed that utilizing ethnopharmacological information was a very important way to search for new bioactive molecules. It was noteworthy to mention the activity of Açaí fruit extracts in the inhibition of nitric oxide production. It was also possible to identify flavonoids responsible for the antidiabetic activity in plants belonging to the family Leguminosae. Acanthaceae extracts showed important antinociceptive and anti-inflammatory activities, as they were very rich in steroids and triterpenes. The same could be said about plants belonging to Lamiaceae that gave several examples of this kind of pharmacological property due to its steroid and triterpenoid compounds. One species of Lamiaceae also produced a great amount of dihydroxylated triterpenoids with great molluscicidal potencial. Palmae species, rich in fatty acids and steroids led to enriched extracts responsible for the anti-BPH activities. Plants belonging to Gesneriaceae were antioxidant due to their flavonoid content. Polar extracts and isolated molecules, isolated from many species were able to donate hydrogen radical to DPPH.

An investigation on the medicinal plants of Khasi hills of Meghalaya, India was undertaken by Kayang *et al.* (2005). Indigenous people were generally very knowledgeable about the wild medicinal plants around them, many of which have local names and were important to the people medically or were featured in folklore. According to the authors' traditional knowledge was the best starting point for effective *in situ* conservation, which

requires accurate and up to date information on the status of medicinal plant populations, the extent and nature of plant use by local communities and the capacity of the resource base to support different economic activities. Their knowledge could be used in the evaluation and in creation of awareness of the importance of medicinal plant as it was generally easier for the public to relate to the cultural significance than the results of scientific trials. An understanding of the many aspects of human influences on biodiversity and the underlying driving forces of the influences was of crucial importance for setting priorities and directing efforts towards conservation and sustainable use.

A survey was conducted (Prayoonrat, 2005) to categorize medicinal weeds found in Chonburi Province, Thailand. Places sampled were residential areas, agricultural lands, plantations, along the seashore and some aquatic habitats were also included. In total 227 species were cataloged belonging to 174 genera in 63 families. Of these weeds, 10 families, 36 genera and 40 species were monocotyledon in contrast to 53 families, 138 genera and 187 species for dicotyledon plants. From the total of weeds catalogued 8 species were found only in the coastal area. The medicinal characteristics of each weed species were studied in collaboration with traditional herb doctors as well as from historical manuscripts. Frequency of occurrence and medicinal characteristics of the weeds were discussed. Chamratpan and Homchuen (2005) have worked on the ethnobotany in upper northeastern Thailand. Some rural people residing in the villages of upper northeastern Thailand still used herbs for preventing and curing many diseases. On the basis of interviews from traditional healers and elders living in seven villages in three provinces of Thailand, authors reported that the medicinal plants were used in five ways – as a rubbing or poultice, a decoction, an alcoholic tincture, a massage or eaten fresh. Rubbing was the common application used for plants such as; (1) slender amaranth (*Amaranthus viridis*) - leaf was applied for removing pain, reducing swelling and pain of insect bites, (2) crushed hophead Philippine violet (*Barleria lupulina*) and phaya yo (*Clinacanthus nutans*) which were applied for herpes (ngu-swat), (3) immature dry black fruit of sugar apple (*Annona squamosa*) called mummy was scrubbed and externally applied on suppurated skin as an effective suppurant. Examples of the fresh ingestion or similar methods were – (1) crushed fresh Siam weed (*Chromolaena odorata*) - leaf with alum was chewed and applied on the wound as an antidote for snake bite, (2) veld grape (*Cissus quadrangularis*) vine with ripe tamarind pulp was eaten for curing hemorrhoids, (3) root and vine of khruea sai tan (*Aganosma marginata*) which was eaten to get rid of schizophrenia. An example of a decoction was khi non (*Uraria crinita*) which was

an effective remedy for severe colon cancer. An example of massage was the mixture of Indian sarsaparilla or thao en on (*Cryptolepis buchanani*), derris (*Derris scandens*), Thai ginger (*Zingiber montanum*) and turmeric (*Curcuma longa*) were applied to paralysis. Alcoholic tincture of krachai dam (*Boesenbergia rotunda*) roots was used as sex-stimulant.

Chandra Prakash (2006) has published a review on developing the medicinal plants sector in northern India. The medicinal properties of plant species have made an outstanding contribution in the origin and evolution of many traditional herbal therapies. Their traditional knowledge systems have started to disappear with the passage of time due to scarcity of written documents and relatively low income in those traditions. Over the past few years, however, the medicinal plants have regained a wide recognition due to an escalating faith in herbal medicine in view of its lesser side effects compared to allopathic medicine in addition the necessity of meeting the requirements of medicine for an increasing human population. Through the realization of the continuous erosion of traditional knowledge of plants used for medicine in the past and the renewed interest at the present time, a need existed to review that valuable knowledge of medicinal plants with the purpose of developing medicinal plants sectors across the different states in India. The major objectives of the review therefore were to explore the potential in medicinal plants resources, to understand the challenges and opportunities with the medicinal plants sector, and also to suggest recommendations based upon the present state of knowledge for the establishment and smooth functioning of the medicinal plants sector along with improving the living standards of the underprivileged communities. The review revealed that northern India harbors a rich diversity of valuable medicinal plants, and attempts were made at different levels for sustainable utilization of that resource in order to develop the medicinal plants sector.

The traditional use of medicinal plants by the Jaintia tribes in North Cachar Hills district of Assam, northeast India was investigated by Sajem and Gosai (2006).The study of ethnobotany relating to any tribe is in itself a very intricate or convoluted process. The paper documents the traditional knowledge of medicinal plants that are in use by the indigenous Jaintia tribes residing in few isolated pockets of northeast India. The study was done through structured questionnaires in consultations with the tribal practitioners and has resulted in the documentation of 39 medicinal plant species belonging to 27 families and 35 genera. For curing diverse form of ailments, the use of aboveground plant parts was higher (76.59%) than the underground plant parts (23.41%). Of the aboveground plant parts, leaf was used in the majority of cases (23 species), followed by fruit (4). Different underground plant forms such

as root, tuber, rhizome, bulb and pseudo-bulb were also found to be in use by the Jaintia tribe as a medicine. Altogether, 30 types of ailments have been reported to be cured by using these 39 medicinal plant species. The study thus underlines the potentials of the ethnobotanical research and the need for the documentation of traditional ecological knowledge pertaining to the medicinal plant utilization for the greater benefit of mankind.

An ethnobotanical investigation has been carried out to collect information on the use of medicinal plants among tribes in Southern Western Ghats of India (Madurai district, Tamil Nadu) by Ignacimuthu *et al.* (2006). An ethnobotanical survey was carried out Information presented in their paper was gathered from the paliyar tribes using an integrated approach of botanical collections, group discussions and interviews with questionnaires in the years 1998 - 1999. The informants interviewed were 12 among whom 4 were tribal practitioners. A total of 60 ethnomedicinal plant species distributed in 32 families were documented in that study. The medicinal plants used by paliyars were listed with Latin name, family, local name, parts used, mode of preparation and medicinal uses. Generally, fresh part of the plant was used for the preparation of medicine. They observed that the documented ethnomedicinal plants were mostly used to cure skin diseases, poison bites, stomachache and nervous disorders. The results of that study showed that these tribal people still depend on medicinal plants in Madurai district forest areas. An ethnobotanical survey was undertaken by Muthu *et al* (2006) to collect information from traditional healers on the use of medicinal plants in Kancheepuram district of Tamil Nadu during October 2003 to April 2004. The indigenous knowledge of local traditional healers and the native plants used for medicinal purposes were collected through questionnaire and personal interviews during field trips.The investigation revealed that, the traditional healers used 85 species of plants distributed in 76 genera belonging to 41 families to treat various diseases. The documented medicinal plants were mostly used to cure skin diseases, poison bites, stomachache and nervous disorders. In their study the most dominant family was Euphorbiaceae and leaves were most frequently used for the treatment of diseases. Their study showed that many people in the studied parts of Kancheepuram district still continue to depend on medicinal plants at least for the treatment of primary healthcare. The traditional healers were dwindling in number and there was a grave danger of traditional knowledge disappearing soon since the younger generation was not interested to carry on that tradition.

Tahraoui *et al.* (2007) have made an ethnopharmacological survey of plants used in the traditional treatment of hypertension and diabetes in south-eastern Morocco (Errachidia

province). The survey was undertaken in the Errachidia province in south-eastern Morocco in order to inventory the main medicinal plants used in folk medicine to treat arterial hypertension and diabetes mellitus. Four hundred individuals, who knew about and/or had used the medicinal plants for the indicated diseases, including some herbal healers, were interviewed throughout different regions of the province. The inventory of medicinal plants was summarized in a synoptic table, which contains the scientific, vernacular and common name of the plant, its ecological distribution, the part of the plant and the preparation used and the therapeutic indication. Extensive investigations have brought to light 64 medicinal plants belonging to 33 families; of these, 45 were used for diabetes, 36 for hypertension, and 18 for both diseases. Of those plants, 34% grew in the wild, 44% were cultivated, and 22% were not indigenous to the area and were brought from other parts of Morocco or from outside the country. The survey showed that 78% of the patients regularly use these medicinal plants. In that region, the most frequently used plants to treat diabetes include *Ajuga iva*, *Allium cepa*, *Artemisia herba-alba*, *Carum carvi*, *Lepidium sativum*, *Nigella sativa*, *Olea europaea*, *Peganum harmala*, *Phoenix dactylifera*, *Rosmarinus officinalis*, and *Zygophyllum gaetulum*, and those to treat hypertension include *Ajuga iva*, *Allium cepa*, *Allium sativum*, *Artemisia herba-alba* Asso, *Carum carvi*, *Nigella sativa*, *Olea europea*, *Rosmarinus officinalis*, *Origanum majorana*, *Peganum harmala*, and *Phoenix dactylifera*. The local people recognize the toxic plants and were very careful in using such plants, which were *Citrullus colocynthis*, *Datura stramonium*, *Nerium oleander*, *Nigella sativa*, *Peganum harmala* and *Zygophyllum gaetulum*. Their survey showed that traditional medicine in the south-eastern Moroccan population had not only survived but had thrived in the transcultural environment and intermixture of many ethnic traditions and beliefs.

Ballab and Chaurasia (2007) have investigated on the traditional medicinal plants of cold desert Ladakh. They reported on traditional remedies of cold, cough and fever among Boto (the Buddhists) tribal community of Leh-Ladakh region of India. Ladakh is one of the least populated regions of our country where major population lives in far-flung villages and higher elevations. Health care of tribal population is mainly dependent on traditional system of medicine which is popularly known as Amchi system of medicine. The Amchi system is principally based on Tibetan system of medicine. Fifty-six valuable species belonging to 21 families were identified with relevant information and documented by the authors with regard to their botanical name, family, collection number, local name, parts used and utilization by 'Amchis' (herbal practitioners) in treatment of cold, cough and fever.

An ethnobotanical survey of plants used to treat asthma in Andhra Pradesh, India has done by Savithramma (2007). Tribal and non-tribal inhabitants of Andhra Pradesh used nearly 80 medicinal plants for treating asthma. The tribal people have a strong faith and belief in the traditional health care system, through herbal treatment. Plant species were generally used along with other materials and plant products in different combinations to effective cure. Herbalists reported that plant ingredients were used in the form of dry powder, decoction and juice in the treatment of asthma. The knowledge of most asthma drug plants used in herbal treatment and their method of using them were confined to some of the local healers. Some of the plants mentioned by local healers however, were extensively used nationally in the preparation of Ayurvedic medicines including those to treat asthma. Clinical and pharmacological data were available for these plants. Most of the plants used for treating asthma by local herbalists appear not to have been recorded hitherto.

2.2. Phytochemical Study

Preliminary phytochemical investigation of the leaves of *Synclisia scabrida* indicated the presence of two alkaloids in the water extracts and five alkaloids in the ethanol extracts (Sokomba *et al.*, 1986). The alkaloidal fraction obtained from the cold ethanol extract furnished on column-chromatography, a homogeneous amorphous solid which has been designated as alkaloid C. Alkaloid C showed positive test for alkaloids. The UV and IR spectra and colour reactions of alkaloid C indicated that the compound may be a phenolic bisbenzylisoquinoline alkaloid. All the extracts delayed the onset and shortened the duration of apomorphine-induced stereotyped behaviour in chicks. In addition, 40 mg kg^{-1} i.p. of the ethanolic extract induced catalepsy in rats. The cold water extract (CWE) synchronized the EEG of the hyperstriatum, optic tectum and the reticular formation while the EMG activity was slightly enhanced. The hot ethanol alkaloidal extract (HEE) inhibited the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The minimum inhibitory concentration of HEE on *Pseudomonas aeruginosa* strains I and II were 5 and 2.5 µg/ml while for *Staphylococcus aureus* strains I and II were 5 and 10 µg/ml, respectively. Up to 1 g kg^{-1} i.p. of the extract failed to induce any lethal effect in chicks and rats. Those effects of the leaf extracts of *Synclisia scabrida* Miers support some of the local uses of the plant by traditional medical practitioners. Hirschmann and co-workers (1987) made preliminary pharmacological studies on *Eugenia uniflora* leaves. *Eugenia uniflora* was widely used in Paraguayan folk

medicine. A hydroalcoholic extract of the leaves showed some central nervous system activity in hippocratic screening when given intraperitoneally, but little to no acute or subacute toxicity in doses up to 4200 mg/kg orally in BALB c mice. The LD_{50} of the extract was 220 mg/kg i.p. in mice. A decoction or infusion of the leaves was recommended for treating gout by native herbalists. The known flavonoids quercitrin, quercetin, myricitrin and myricetin were found to be responsible for the xanthine oxidase inhibitory action of the plant extract.

From ancient times, *Swarnabhasma* (gold ash) has been used in several clinical manifestations including loss of memory, defective eyesight, infertility, overall body weakness and incidence of early aging. *Swarnabhasma* has been used by Ayurvedic physicians to treat different diseases like bronchial asthma, rheumatoid arthritis, diabetes mellitus, nervous disorders, etc. In that investigation, *Swarnabhasma* was prepared after proper purification and calcination as per Ayurvedic pharmacy which consisted of Realger (As_2S_2), Lead oxide (Pb_3O_4), pure gold (Au) and latex of *Calotropis gigantea.* Qualitative analyses indicated that *Swarnabhasma* contained not only gold but also several microelements (Fe, Al, Cu, Zn, Co, Mg, Ca, As, Pb, etc.). Infrared spectroscopy showed that the material was free from any organic compound. The metal content in the *bhasma* was determined by atomic absorption spectrometry. Acute oral administration of *Swarnabhasma* showed no mortality in mice (up to 1 ml /20 g b.w. of *Swarnabhasma* suspension containing 1mg of drug). Chronic administration of *Swarnabhasma* also showed no toxicity as judged by SGPT, SGOT, serum creatinine and serum urea level and histological studies. In an experimental animal model, chronic *Swarnabhasma*-treated animals showed significantly increased superoxide dismutase and catalase activity, two enzymes that reduce free radical concentrations in the body (Mitra *et al.*, 2002).

The pharmacognostical and phytochemical studies on some medicinal plants of Tirunel veli hills in Tamilnadu in India was carried out by Britto *et al.* (2005). Aqueous extracts of *Securinega virosa, Phyllanthus reticulatus* and *Breynia retusa* were screened for their hepatoprotective properties against carbontetra-chloride induced liver damage in Wistar albino rats. All the extracts gave promising results, and hence the plants were taken up for phytochemical investigation.Fractionation of the extracts and crystallization of the compounds yielded five compounds that were identified as amyrin, thea alcohol, octacosanol, β-sitosterol and β-sitosterol 3- β-D glucopyranoside. Among the three plants screened, *Seurinega virosa* had the best repair mechanism on blood biochemistry, and also correlated

with the pathological changes seen in the liver. Histopathological examinations of the liver from the group of rats that received the extract of *Securinega virosa* revealed almost normal architecture of the liver.

The phenolic contents and antioxidant activity of some food and medicinal plants was worked by Bajpai *et al.* (2005). To identify promising sources of antioxidants, some food and medicinal plants were studied for total phenolic contents and antioxidant activity. The leaves, bark and fruits of *Terminalia arjuna*, *Terminalia bellerica*, *Terminalia chebula* and *Terminalia muelleri*, the leaves and fruits of *Phyllanthus emblica*, and the seeds of *Syzygium cumini* were found to have high total phenolic contents (72.0-167.2 mg/g) and high antioxidant activity (69.6-90.6%). Leaves of *Eucalyptus globulus* were a rich source of rutin, *Moringa oleifera* for kaempferol, aerial parts of *Centella asiatica* for quercetin, fruits of *T. bellerica* and *T. chebula* for gallic acid, and bark of *T. arjuna*, leaves and fruits of *T. bellerica* and bark, leaves and fruits of *T. muelleri* for ellagic acid.

The phytochemical screening and pharmacological evaluations for the antifertility effect of the methanolic root extract of *Rumex steudelii* have studied by Gebrie *et al.* (2005). The practice of traditional medicine for the control of fertility in most parts of Ethiopia was based on the uses of plant medicines for many years. The fact that herbal medicines have been employed for such a long time does not guarantee their efficacy and safety. The aim of their study was, therefore, to carry out phytochemical screening, efficacy and safety studies on one of the traditionally used antifertility plants: *Rumex steudelii.* The secondary metabolites of the root of that plant were determined. The methanolic extract of the roots of that plant were investigated for their antifertility activity in female rats and oral LD_{50} was determined in mice. The identification of the secondary metabolites showed that the roots of the plant contained phytosterols and polyphenols. It also produced antifertility effect in a dose dependent manner and the contraceptive effect was manifested for a definite period of time. Furthermore, the extract prolonged significantly the estrus cycle ($p < 0.05$) and the diestrous phase ($p < 0.01$) of the rats. The wet weights of the ovaries and uterus were shown to be reduced significantly ($p < 0.01$) and ($p < 0.05$), respectively. The oral LD_{50} of the extract was found to be 5 g/kg in mice. All those observations suggest that the extract has antifertility effect and were safe at the effective antifertility doses employed in that study.

Onocha *et al.* (2005) examined the phytochemical antimicrobial properties of extracts of *Combretum racemosum. Combretum racemosum* (Combretaceae), a straggling shrub

widespread across Africa was traditionally reputed to be anthelmintic and antimicrobial for genito-urinary and gastrointestinal infections. The methanol and ethyl acetate crude extracts obtained from the whole plant were evaluated invital to determine inhibition of human pathogenic micro organisms made up of five bacteria and three fungi. The extracts inhibited the eight test organisms to different degrees. All the bacteria strains were sensitive to both extracts at concentration ranging from 25 to 125 mg/ml using the agar broth cup diffusion procedure. The sensitivity of *Salmonella typhii, Escherica coli* and *Pseudomonas aeruginosa* (gram negative) to both extracts were not concentration dependent, whereas sensitivity of *Bacillus subtilis* and *Staphylococcus aureus* (gram positive) were concentration dependent with activity being higher at higher concentrations of ethyl acetate extract. Only the methanol extract exhibited intrinsic antifungal properties on *Candida albicans, Asperigillus niger* and *Dermatophyte* sp. with activity comparable to that of the reference drug tioconazole trosyd. Preliminary phytochemical screening of both extracts indicated the presence of alkaloids, steroids, cardiac glycosides, saponins and tannins.

Takeda *et al.* (2007) have worked on the chemical constituents of an Uzbek medicinal plant, *Perovskia scrophularifolia.* From the aerial parts of *Perovskia scrophularifolia*, a traditional medicinal plant in Uzbekistan, 11 known compounds, including 4 abietane-type diterpenoids, 1 flavone and 6 glycosides, were isolated and identified.

The anticoronaryspastic and antibronchospastic activities of ethanolic and aqueous extracts of *Valeriana officinalis* L. roots were investigated (Circosta *et al.,* 2007) in anaesthetized guinea-pigs and the results were correlated with the qualitative/quantitative chemical composition of the extracts in order to account for some of the common uses of that plant. The protective effects of orally administered ethanolic and aqueous extracts (50, 100 and 200 mg/kg) were evaluated against pitressin-induced coronary spasm and pressor response in guinea-pigs and were compared with those of nifedipine. Furthermore, the protective effects against histamine-induced and Oleaceae antigen challenge-induced bronchospasm were evaluated. Finally, the two valerian extracts were analytically characterized by qualitative and quantitative chromatographic analysis.The results showed that the two valeriana extracts possessed significant anticoronaryspastic, antihypertensive and antibronchospastic properties. These were similar to those exhibited by nifedipine and were due to the structural features of the active principles they contain.That study justified the traditional use of that plant in the treatment of some respiratory and cardiovascular disorders.

2.2.1. Antioxidants in plants

Oxidative stress may result from the over production of reactive oxygen radicals caused by metabolic stress, cellular dysfunction and tissue damage in several disease state. Free radicals have been implicated as the cause of several diseases such as liver cirrhosis, atherosclerosis, cancer and diabetes, and compounds that can scavenge free-radicals have great potential in ameliorating these disease processes (Wilson, 1988).

α-Tocopherol was identified as the main antioxidant in hexane extracts of leaves of sixteen Mediterranean plant species (Chevolleau *et al.*, 1993). The α-tocopherol content was determined by a two-step procedure involving column and gas chromatography with α-tocopherol acetate as internal standard. The tocopherol content of the extracts was in the range of 0.0–4.7%, and that of the dry leaves was 0–846 ppm. The highest α-tocopherol content was found in the leaves of a Mediterranean oak, *Quercus ilex*. The antioxidative activity, which was previously investigated, was correlated with the α-tocopherol content. Correlation coefficients were 0.947 and 0.904 for extracts and leaves, respectively. Chen *et al.*, (2006) worked on the progress of vitamin E metabolic engineering in plants. Vitamin E was important for human and animal health. Many human diseases, such as certain cancers and neurodegenerative and cardiovascular disease, were associated with the insufficient intake of vitamin E. The daily requirements for vitamin E in men and women have been increased to 15-30 mg. Because the primary source of dietary vitamin E comes from plants, there was a need to increase vitamin E production through plant engineering in order to meet the demand in human consumption. Numerous studies have been carried out in that field, leading to many successful examples. In that review, they summarized the recent progress in vitamin E metabolic engineering in plants aimed at improving the vitamin E content and regulating composition of vitamin E.

Smirnoff and Wheeler (2000) reviewed the role of ascorbic acid (vitamin C) which was an abundant component of plants. It reached a concentration of over 20 mM in chloroplasts and occurred in all cell compartments, including the cell wall. It had proposed functions in photosynthesis as an enzyme cofactor (including synthesis of ethylene, gibberellins and anthocyanins) and in control of cell growth. A biosynthetic pathway via GDP-mannose, GDP-L-galactose, L-galactose, and L-galactono-1,4-lactone has been proposed only recently and was supported by molecular genetic evidence from the ascorbate-deficient vtc 1 mutant of Arabidopsis thaliana. Other pathways via uronic acids could provide

minor sources of ascorbate. Ascorbate, at least in some species, was a precursor of tartrate and oxalate. It had a major role in photosynthesis, acting in the Mehler peroxidase reaction with ascorbate peroxidase to regulate the redox state of photosynthetic electron carriers and as a cofactor for violaxanthin de-epoxidase, an enzyme involved in xanthophyll cycle-mediated photoprotection. The hypersensitivity of some of the vtc mutants to ozone and UV-B radiation, the rapid response of ascorbate peroxidase expression to (photo)-oxidative stress, and the properties of transgenic plants with altered ascorbate peroxidase activity all support an important antioxidative role for ascorbate. In relation to cell growth, ascorbate was a cofactor for prolyl hydroxylase that posttranslationally hydroxylates proline residues in cell wall hydroxyproline-rich glycoproteins required for cell division and expansion. Additionally, high ascorbate oxidase activity in the cell wall was correlated with areas of rapid cell expansion. It remains to be determined if this was a causal relationship and, if so, what was the mechanism. Identification of the biosynthetic pathway now opens the way to manipulating ascorbate biosynthesis in plants, and, along with the vtc mutants, that should contribute to a deeper understanding of the proposed functions of that multifaceted molecule.

Increasing vitamin C (ascorbic acid) content of plants through enhanced ascorbate recycling was done by Chen *et al.* (2003). Vitamin C (ascorbic acid) is essential to prevent disease associated with connective tissue (e.g., scurvy), improves cardiovascular and immune cell functions, and is used to regenerate α-tocopherol (vitamin E). In contrast to most animals, humans lack the ability to synthesize ascorbic acid as a result of a mutation in the last enzyme required for ascorbate biosynthesis. Vitamin C, therefore, must be obtained from dietary sources and, because it cannot be stored in the body, it must be obtained regularly. Once used, ascorbic acid can be regenerated from its oxidized form in a reaction catalyzed by dehydroascorbate reductase (DHAR). To examine whether overexpression of DHAR in plants would increase the level of ascorbic acid through improved ascorbate recycling, a DHAR cDNA from wheat was isolated and expressed in tobacco and maize, where DHAR expression was increased up to 32- and 100-fold, respectively. The increase in DHAR expression increased foliar and kernel ascorbic acid levels 2- to 4-fold and significantly increased the ascorbate redox state in both tobacco and maize. In addition, the level of glutathione, the reductant used by DHAR, also increased, as did its redox state. Those results demonstrate that the vitamin C content of plants could be elevated by increasing expression of the enzyme responsible for recycling ascorbate.

Shamsi *et al.* (2006) investigated the effect of different doses of vitamin C on the biochemical parameters of normal and streptozotocin (STZ)-induced diabetic rats. Biochemical analysis was used to study the effect of this vitamin on the biochemical parameters of normal and diabetic rats. Liver and kidney enzymes were elevated after the onset of diabetes. Moderate doses of vitamin C significantly ($P < 0.0008$) reduced plasma gamma-glutamyl level in diabetic rats. Moreover, vitamin C significantly ($P < 0.01$) reduced the blood urea nitrogen level of diabetic rats. The plasma level of electrolytes, such as calcium and sodium, also changed significantly ($P < 0.00001$) after oral administration of vitamin C. Antioxidants, such as vitamin C, may ameliorate the biochemical parameters of diabetic rats.

Fresh fruit and vegetables are a major source of ascorbic acid (vitamin C), an important antioxidant for the human diet and also for plants. Ascorbic acid content in fruit exhibits a quantitative inheritance. Quantitative trait loci (QTL) for ascorbic acid content have been mapped in three tomato populations derived from crosses between cultivated tomato varieties (*Solanum lycopersicum* accessions) and three related wild species or subspecies by Stevens *et al.* (2007). The first population consists of a set of introgression lines derived from *Solanum pennellii*, each containing a unique fragment of the wild species genome. The second population was an advanced backcross population derived from a cross between a cultivated tomato and a *Solanum habrochaites* (formerly *Lycopersicum hirsutum*) accession. The third population was a recombinant inbred line population derived from the cross between a cherry tomato line and a large fruited line. Common regions controlling ascorbic acid content have been identified on chromosomes 2, 8, 9, 10, and 12. In general, the wild alleles increased ascorbic acid content, but some improvement could also be provided by *S. lycopersicum*. Most QTLs appeared relatively stable over years and in different environments. Mapping of candidate genes involved in the metabolism of ascorbic acid has revealed a few colocations between genes and QTLs, notably in the case of a monodehydroascorbate reductase gene and a QTL present in two of the populations on chromosome 9 (bin 9-D), and a previously mapped GDP-mannose epimerase and a QTL on chromosome 9 (bin 9-J).

The antiinflammatory and antioxidant activity of plants used in traditional medicine in Ecuador were investigated by Heras *et al.* (1998). Ethanolic extracts from 15 plant species, representing eight different families, used in traditional medicine in Ecuador were evaluated for antiinflammatory and antioxidant activities. *Conyza floribunda*, *Eupatorium articulatum*, *Bonafousia longituba*, *Bonafousia sananho*, *Tagetes pusilla* and *Piper lenticellosum* extracts

showed a significant antiinflammatory activity *in vivo* in the carrageenan-induced paw oedema model in mice. The extracts were also tested *in vitro* for their ability to inhibit lipid peroxidation and to scavenge superoxide and hydroxyl radicals. *E. articulatum* extract possesses both activities. *Baccharis trinervis, E. articulatum* and *Phytolacca rivinoides* extracts were active as antioxidants.

Peterson and Dwyer (1998) showed that flavonoids are plant phytochemicals that cannot be synthesized by humans. The six classes of flavonoids (flavanones, flavones, flavonols, isoflavonoids, anthocyanins, and flavans) vary in their structural characteristics around the heterocyclic oxygen ring. Flavanones occur predominantly in citrus fruits, flavones in herbs, isoflavonoids in legumes, anthocyanins and catechins in fruits and flavonols in all fruits and vegetables. Food preparation and processing of fresh fruits and vegetables may decrease flavonoid content by 50% owing to leaching into water or removal of portions of the plant that are rich in them. Grains and oils seeds have flavonoids, but processing removes or reduces them. Other plant food groups contain differing amounts of flavonoids. Honey, chocolate, and sweets that contain some plant constituents have flavonoids. Flavonoids were not present in animal foods. Dietary intake estimates vary from 23 mg/day to 1000 mg/day but the number of compounds and classes assessed vary, and all estimates currently are incomplete. In vitro and animal studies have demonstrated that flavonoids have antioxidant and antimutagenic activities. Their studies suggested that flavonoids might reduce the risk of cardiovascular disease and stroke. Flavonoid classes vary in their absorption and their metabolism was still obscured. They were conjugated in the liver or kidney and excreted into bile or urine. Colonic bacteria split the heterocyclic ring and degrade the flavonoids to phenyl acids which might be absorbed, conjugated, and excreted or metabolized further by colonic bacteria.

The antioxidant activity of *Tinospora cordifolia* roots in experimental diabetes investigated by Prince and Venugopal (1999). They made an attempt to study the antioxidant properties of *Tinospora cordifolia* roots, an indigenous plant used in Ayurvedic medicine in India in alloxan diabetic rats. Oral administration of an aqueous *T. cordifolia* root extract (TCREt) (2.5 and 5.0 g/kg) for 6 weeks resulted in a decreased in the levels of plasma thiobarbituric acid reactive substances, ceruloplasmin and α-tocopherol in alloxan diabetic rats. The root extract also caused an increase in the levels of glutathione and vitamin C in alloxan diabetes. The root extract at a dose of 5.0 g/kg showed the highest effect. The effect

of TCREt was more effective than glibenclamide. Insulin restored all the parameters to near normal levels.

Sarada and others (2002) have investigated the antioxidant effect of β-carotene on hypoxia induced oxidative stress in male albino rats. Hypoxia was known to induce oxidative stress in organisms leading to tissue injury. In their study β-carotene (BC) given at 10 mg/kg body weight (BW) in reducing the oxidative stress induced by hypoxia was evaluated on male albino rats. Hypoxia exposure caused an increased in malondialdehyde (MDA) levels in plasma and tissues, a concurrent decreased in blood glutathione (GSH), glutathione peroxidase (GPx), plasma protein and plasma BC content. Hemoglobin concentration, Red blood corpuscles (RBC) and White blood corpuscles (WBC) count were also increased under hypoxia. BC supplementation reversed the trend, inducing a significant decrease ($P<0.05$) in MDA and subsequent increase in plasma and tissue GSH levels in animals exposed to hypoxia. Blood GPx and plasma protein also increased significantly in BC supplemented animals. BC supplementation did not alter the changes in Hb concentration, RBC and WBC count. BC has potent antioxidant activities in reducing the oxidative stress induced by hypobaric hypoxia.

The significance of Carotenoid-Mediated Modulation of Membrane Physical has done by Strzaka (2003). Carotenoids, apart of their antenna function in photosynthesis, play an important role in the mechanisms protecting the photosynthetic apparatus against various harmful environmental factors. They protect plants against overexcitation in strong light and dissipate the excess of absorbed energy; they scavenge reactive oxygen species formed during photooxidative stress and moderate the effect of extreme temperatures. One of the important factors involved in the protective action of carotenoids is their influence on the molecular dynamics of membranes. To obtain complex information about interactions between carotenoids and lipids in a membrane, different techniques were used. In this review, the data resulting from EPR–spin label spectrometry, ^{31}P- and ^{13}C-NMR, differential scanning calorimetry, and computer simulation of the membrane molecular dynamics are presented. The effects of selected, structurally different carotenoid species on various physical parameters of model and natural membranes were described and their relevance to protection against some environmental stresses was discussed.

The antioxidant activity related to plant part, life form and growing condition in some diabetes remedies were investigated by McCune and Johns (2007). Selection, collection and

preparation of 35 plant species used by traditional healers in the boreal regions of Canada for treatment of the symptoms of diabetes were supported empirically by antioxidant activity of the plants. Because antioxidants fluctuate with growth parameters and environmental factors, these remedies were evaluated in relation to the affect of plant part, life form and growing condition on the level of activity. The parts used here more frequently as medicines were roots and bark. Activity (IC_{50}) of the bark extracts used medicinally averaged to 21.38 ± 3.84 ppm while root extracts used medicinally had an IC_{50} of 185.11 ± 32.18 ppm in a free radical DPPH assay. In contrast the analysis of extracts of overall parts (medicinal or not) in these species found leaves and bark to have the least activity (112.22 ± 30.63 ppm and 123.02 ± 21.13 ppm, respectively). The highest activity was found in tree extracts (24.88 ± 3.32 ppm) as compared to herbs and shrubs, and increased activity was found in plant extracts from growing conditions of decreased water/fertility. The antioxidant activity of those traditional plant remedies have the potential to be partially deduced through environment signals interpreted by the traditional herbalist.

The phenolic compounds of *Sideritis ozturkii* and their *in vivo* anti-inflammatory and antinociceptive activities were studied by Kupeli *et al.* (2007). Acetone extract from aerial parts of *Sideritis ozturkii* Aytaç & Aksoy and its fractions were investigated for its *in vivo* anti-inflammatory and antinociceptive activities. For the anti-inflammatory activity assessment, carrageenan-induced hind paw edema and for the antinociceptive activity, *p*-benzoquinone-induced abdominal constriction tests were used. Acetone extract of the plant and its phenolic fraction were found to possess significant inhibitory activity on those *in vivo* models in mice. Ozturkoside A (chrysoeriol 7-*O*-[2‴-*O*-caffeoyl-6‴-*O*-acetyl-β-d-glucopyranosyl-(1 → 2)-β-d-glucopyranoside]); ozturkoside B (chrysoeriol 7-*O*-[2‴-*O*-caffeoyl-β-d-glucopyranosyl-(1 → 2)-β-d-glucopyranoside]); and ozturkoside C (chrysoeriol 7-*O*-[2‴-*O*-*p*-coumaroyl-6‴-*O*-acetyl-β-d-glucopyranosyl-(1 → 2)-β-d-glucopyranoside]) were isolated from the active phenolic fraction. The structures of isolated compounds were elucidated by spectroscopic techniques (UV, IR, 1D- and 2D-NMR, MS). Ozturkoside C showed notable antinociceptive and anti-inflammatory activities without inducing any apparent acute toxicity or gastric damage. Although the activity of ozturkosides A and B were found insignificant in statistical analysis, some inhibitory effect was observed. Accordingly, it was suggested that these components in phenolic fraction might possibly share the antinociceptive and anti-inflammatory activities together.

2.2.2. Antimicrobials

The antimicrobial activity of plants popularly used in Guatemala for the treatment of dermatomucosal diseases were determined by Armando *et al.* (1987). Ethnobotanical surveys were conducted among traditional healers and local market vendors; about 200 plants used in Guatemala for the treatment of dermatomucosal diseases were detected. Eighty nine plants were selected for *in vitro* screening for antimicrobial activity against the microorganisms usually causing skin and mucosal infections. Ethanolic macerations were prepared and impregnated in absorbent paper; once dried, these were applied over standardized inocula of *Candida albicans, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus.* After incubation, inhibition zones were measured, demonstrating that 28 of the plants exhibited some *in vitro* inhibition of the tested microorganisms. Rios and co-workers (1987) investigated on the antimicrobial activity of selected plants employed in the Spanish mediterranean area. Eighty-one plants from the Spanish Mediterranean area employed as antimicrobial agents in folk medicine have been identified. The *in vitro* antimicrobial activity of chloroform and methanol extracts of the plants was studied using the agar dilution method against six selected microorganisms. Thirty extracts had activity against some of the microorganisms tested. Bioautography showed that the antimicrobial activity was probably due to flavonoids, terpenoids and phenolic acids.

Extracts of *Mitracarpus villosus* leaves and inflorescences were investigated (Irobi and Daramola, 1993) individually for *in vitro* antifungal activities by agar-diffusion and tube-dilution techniques. Ethanolic extracts produced definite antifungal activities against *Trichophyton rubrum, Microsporum gypseum, Candida albicans, Aspergillus niger* and *Fusarium solani.* The aqueous extracts and the glycerol vehicle control did not inhibit any of the fungi tested. The zones of inhibition produced by the ethanol extracts ranged from 10 to 20.5 mm while ketoconazole control ranged from 9 to 19 mm. The minimum inhibitory concentration of the extracts ranged from 0.50 to 4.0 mg/ml while their minimum fungicidal concentration values ranged from 1 to 8 mg/ml. Those results indicate that the extracts were fungistatic at lower concentrations and fungicidal at higher concentrations.

Gundidza and Gaza (1993) investigated the antimicrobial activity of *Dalbergia melanoxylon* extracts. The antibacterial and antifungal properties of the niethanol citric acid, aqueous, dichloromethane and petroleum ether extracts from the bark of *Dalbergia melanoxylon* were determined by using seeded agar plates with wells into which were placed

the extract, and flasks of yeast extract and sucrose broth for mycelial growth of the fungi. After incubation for 24 h, the diameter of the inhibition zone was measured for the antibacterial tests and after 7 days, the dry weight of the mycelia was measured and a percentage of inhibition calculated using controls where no extracts were added. The results obtained showed that the citric acid extract exhibited strongest antimicrobial activity. The ethanol fraction showed significant antibacterial activity but was not significantly active against fungi. The dichloromethane extract exhibited no activity against bacteria but showed notable activity against fungi. The petroleum ether fraction showed no antimicrobial activity.

The screening of some Cuban medicinal plants for antimicrobial activity was done by Martinez *et al.* (1996). The antimicrobial activities of 23 extracts of 12 Cuban plant species reported in traditional medicine were tested. The agar diffusion method was used to assess the activity against four bacteria and one yeast: *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*. The results, evaluated as the diameter of the inhibition zone of microbial growth, showed that nine extracts were active against Gram-positive bacteria but only two of these proved to be also active against Gram-negative bacteria. None of the extracts inhibited the growth of the yeast. The most susceptible bacterium was *Staphylococcus aureus* and the best antibacterial activity was shown by *Schinus terebenthifolius*. Essawi and Srour (2000) did a screening of some Palestinian medicinal plants for antibacterial activity. Antibacterial activity of organic and aqueous extracts of 15 Palestinian medicinal plants was carried against eight different species of bacteria: *Bacillus subtilis*, two *Escherichia coli* species, *Staphylococcus aureus* (methicillin resistant), two *S. aureus* (methicillin sensitive) species, *Pseudomonas aeruginosa*, and *Enterococcus fecalis*. Of the 15 plants tested, eight showed antibacterial activity. Each plant species showed unique activity against different bacteria. The most active antibacterial plants against both gram-positive and gram-negative bacteria were *Thymus vulgaris* and *Thymus origanium*. The organic and aqueous extract from the same plants showed different activities; the organic extract showed the same or greater activity than the aqueous extract. Finally, the hole–plate diffusion method showed larger activity than the disc diffusion method.

Ngane *et al.* (2000) investigated on the evaluation of antifungal activity of extracts of two Cameroonian Rutaceae. Aqueous-ethanol 90% extracts of leaves, roots and stem barks of *Zanthoxylum leprieurii* and *Zanthoxylum xanthoxyloides* were examined for their antifungal properties against nine fungi by dilution methods on a solid medium and in a liquid medium. Their results indicated that those extracts, to varying extents, inhibit the in vitro growth of

Candida albicans, *Cryptococcus neoformans* and seven filamentous fungi tested. Only the extracts obtained from the roots and stem barks of *Z. xanthoxyloides* showed antifungal activity on the germs studied, with minimal inhibitory concentration varying, respectively, from 0.5 to 1 mg/ml for the roots and from 0.125 to 1 mg/ml for the stem barks. Quiroga *et al.* (2001) made a screening of antifungal activities of some selected medicinal plants. Plants synthesise a vast array of secondary metabolites that were gaining importance for their biotechnological applications. The antifungal activity of the ethanolic extracts of ten Argentinean plants used in native medicine was reported. Antifungal assays included radial growth inhibition, disk and well diffusion assays and growth inhibition by broth dilution tests. The chosen test fungi were yeasts, microfungi and wood-rot causing Basidiomycetes. Extracts of *Larrea divaricata*, *Zuccagnia punctata* and *Larrea cuneifolia* displayed remarkable activity in the assays against the majority of the test fungi. In addition to the former plants, *Prosopanche americana* also inhibited yeast growth. Kariba *et al.*, (2001) did an in vitro antifungal activity of *Schizozygia coffaeoides* Bail. (Apocynaceae) extracts. Leaf extracts of *Schizozygia coffaeoides* were investigated for antifungal activity using the disc diffusion assay technique. Petroleum ether 40–60°C, dichloromethane–ethyl acetate (1:1) and methanol extracts were fungitoxic to *Trichophyton mentagrophytes*, *Microsporum gypseum*, *Cladosporium cucumerinum* and *Candida albicans*. The extracts were fungistatic in action.

Gabriela *et al.* (2001) did an antimicrobial evaluation of certain plants used in Mexican traditional medicine for the treatment of respiratory diseases. Eighteen crude extracts, including six hexanic, six chloroformic and six methanolic from six different plant species used in Mexican traditional medicine for the treatment of respiratory infections, were evaluated for potential antimicrobial activity against *Staphylococcus aureus, Enterococcus faecalis, Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Escherichia coli,* and *Candida albicans*. The minimal inhibitory concentration was determined for each extract using a two-fold dilution assay. The results showed that 16 crude extracts (89%) exhibited antimicrobial activity against at least one of the microorganisms tested at concentrations of 5 mg/ml or below. The extracts from *Gnaphalium oxyphyllum*, *Gnaphalium americanum*, and *Crescentia alata* possessed strong antimicrobial activity against the pathogens tested.

The antimicrobial activity of crude ethanolic extracts of 16 Siberian medicinal plants was tested against five species of microorganisms: *Bacillus cereus, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa*, and *Candida albicans* (Kokoska *et al.*, 2002). Of the 16 plants tested, 12 showed antimicrobial activity against one or more species

of microorganisms. The most active antimicrobial plants were *Bergenia crassifolia, Chelidonium majus, Rhaponticum carthamoides, Sanguisorba officinalis*, and *Tussilago farfara.*

The antimicrobial activity of some medicinal plants of the island Soqotra have wxamined by Mothana and Lindequist (2005). Twenty-five selected plants belonging to 19 families were collected from different localities of the island Soqotra, dried and extracted with the solvents chloroform, methanol and hot water to yield 80 extracts. The extracts were tested for their antimicrobial activity against several Gram-positive and Gram-negative bacteria and against one yeast species using agar diffusion method. Antibacterial activity was demonstrated especially against Gram-positive bacteria including multiresistant *Staphylococcus* strains. The greatest activity was exhibited by the methanolic extracts of *Boswellia elongata, Boswellia ameero, Buxus hildebrandtii, Commiphora parvifolia, Jatropha unicostata, Kalanchoe farinacea, Pulicaria stephanocarpa, Punica protopunica, Withania adunensis* and *Withania riebeckii.* Only the methanolic extract of *Buxus hildebrandtii* displayed significant antifungal activity.

Billo *et al.* (2005) have done a screening of some New Caledonian and Vanuatu medicinal plants for antimycobacterial activity. Twenty plants, belonging to sixteen families, used in traditional New Caledonian and Vanuatu medicine for treatment of symptoms potentially related to tuberculosis (cough, fever or inflammation) were screened for antimycobacterial activity. They also screened an original endemic plant, *Amborella trichopoda*, only member of the monogeneric family Amborellaceae and considered the most primitive living angiosperm. In total, 55 extracts were evaluated for inhibitory activity against *Mycobacterium bovis* BCG strain at a concentration of 100 µg/ml. Methanolic and dichloromethane extracts of *Amborella trichopoda, Codiaeum peltatum, Myristica fatua*, and essential oils *Myoporum crassifolium* showed an activity at that concentration. Methanolic extract of *Amborella trichopoda* fruits presented a significant activity with a minimal inhibitory concentration included between 1 and 2.5 µg/ml. In the same conditions, thiat activity was comparable with those of the reference drugs pyrazynamide and ethambutol, at 20 and 2.5 µg/ml respectively.

A series of 61 Indian medicinal plants belonging to 33 different families used in various infectious disorders, were screened for their antimicrobial properties. Screening was carried out at 1000 and 500 ug/ml concentrations by agar dilution method against *Bacillus*

cereus var *mycoides, Bacillus pumilus, Bacillus subtilis, Bordetella bronchiseptica, Micrococcus luteus, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Streptococcus faecalis, Candida albicans, Aspergillus niger* and *Saccharomyces cerevisiae*. Twenty-eight plant extracts showed activity against at least one of the test organisms used in the screening. On the basis of the results obtained, they concluded that the crude extracts of *Dorema ammoniacum, Sphaeranthus indicus, Dracaena cinnabari, Mallotus philippinensis, Jatropha gossypifolia, Aristolochia indica, Lantana camara, Nardostachys jatamansi, Randia dumetorum* and *Cassia fistula* exhibited significant antimicrobial activity and properties that support folkloric use in the treatment of some diseases as broad-spectrum antimicrobial agents. That probably explains the use of those plants by the indigenous people against a number of infections (Kumar *et al.* (2006).

Buwa and Staden (2006) have done an investigation on the antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South Africa. Aqueous, ethanolic and ethyl acetate extracts of 13 plants used in South Africa for the treatment of venereal diseases were screened for antibacterial and antifungal activity. Among the plants tested, *Gunnera perpensa, Harpephyllum caffrum, Hypoxis latifolia* and *Ledebouria ovatifolia* showed the best antibacterial activity. The aqueous extracts of *Gunnera perpensa* and *Harpephyllum caffrum* were most active against all the tested bacteria. In antifungal screening, good activity was shown by the ethanolic extracts of *Bersama lucens* and *Harpephyllum caffrum.* Only in the case of *Harpephyllum caffrum* did aqueous extracts have activity against *Candida albicans.*

The antibacterial effects of some Cameroonian medicinal plants against common pathogenic bacteria was tested by Moses *et al.*, 2006). They screened forty crude extracts of twenty Cameroonian medicinal plants commonly used to treat bacterial infections for broad spectrum antibacterial activity, as a more affordable alternative against resistant organisms. The extracts were screened on common pathogenic gram negative and gram positive bacteria initially by the disc diffusion method followed by the tube dilution method. Using discs containing 30µg of extract, *Escherichia coli* showed sensitivity to 23 extracts with diameter of zone of inhibition ranging from 7 – 19mm, fifteen of which were up to or > 10mm. *Pseudomonas aeruginosa* was sensitive to 11 extracts, whereas *Salmonella typhimurium* and *Staphylococcus aureus* were not sensitive to any of the extracts. Based on the zones of inhibition the activity of the extracts were equivalent to 30 to 138 %

efficacy of the standard antibiotic discs. The lowest Minimum Inhibitory Concentration (MIC) recorded was 2 mg/ml for *Euphorbia hirta* against S. *aureus* and *P. aeruginosa* and the lowest Minimum Bactericidal Concentration (MBC) was 6 mg/ml for six extracts from *Ageratum conyzoides*, *Aframomum citratum*, *Euphorbia hirta*, *Momordica charantia*, *Mangifera indica* and *Khaya senegalensis* against three bacterial species. Three extracts had broad spectrum bacteriostatic activity (MICs ≤ 4 mg/ml) while in terms of MBCs none of the extracts showed broad spectrum bactericidalactivity. They conclude that most of the tested plants used as traditional antibacterials have a bacteriostatic effect on gram-negative pathogenic bacteria.

Cordia gilletii De Wild (Boraginaceae) root bark was traditionally used in Democratic Republic of Congo (DRC) for the treatment of various disorders, including malaria, diarrhea, wounds and skin diseases; part of those activities might rely on antimicrobial and antioxidant properties (Okusa *et al.*, (2007). Successive extracts of root barks powder with *n*-hexane, dichloromethane, ethyl acetate, methanol and water were tested for antimicrobial activity, both direct and indirect (antibiotic resistance reversal), against 10 strains of bacteria and 1 strain of fungi by broth microdilution and agar diffusion methods. The eventual synergy between plant extracts and antibiotics was investigated by the determination of the fractional inhibitory concentration index (FIC index). The methanol extract showed direct antimicrobial activity against all tested microorganisms with minimum inhibitory concentrations (MIC) ranging between 125 and 1000 µg/ml, whereas the ethyl acetate and the dichloromethane extracts showed activity on four and three strains, respectively. 200 µg/ml of *n*-hexane and dichloromethane extracts decreased the MICs of penicillin and streptomycin 4–64-fold for methicillin-resistant *Staphylococcus aureus*. A synergistic effect was found between the methanol extract and tetracycline, whereas additive effects were observed for the other combinations tested. The methanol and dichloromethane extracts showed the greater antioxidant activity by scavenging the free radical DPPH with IC_{50} values of 3.2 and 8.1 µg/ml, respectively. Those results support the use of the plant in the treatment of infectious diseases and wounds; they warrant further studies as to the nature of active compounds.

2.2.3. Anti-hyperglycemic activities in plant extracts

Khaleeva *et al.* (1987) worked on the comparative evaluation of the hypoglycemic activity of the vegetal complex of *Phaseolus vulgaris* and chlorpropamide in experimental diabetes. Experiments on rabbits with alloxan diabetes showed that the plant complex (PC) reduced the level of glycemia after single administration for 6-8 h by 27-32%. A similar effect was demonstrated with chlorpropamide. However the PC produced a longer hypoglycemic effect. In course treatment the PC returned the blood level of glucose (5.14 +/- 0.62 mmol/l) to normal on the 11th day whereas with chlorpropamide this indicator was almost normal (6.6 +/- 1.1 mmol/l) on the 15^{th} day only. A rapid decrease in the blood glucose concentration caused by the PC was observed in AIS induced hyperglycemia. The PC demonstrated its sugar reducing action by extrapancreatic means.

The hypoglycemic effects of a decoction and an ethanol extract of *Trigenolla foenum graceum* seeds on the serum glucose levels of normal and alloxan diabetic mice were studied. Ajabnoor and Tilmisany (1988) investigated the effect of *Trigonella foenum graceum* on blood glucose levels in normal and alloxan-diabetic mice. A single 0.5 ml oral dose of 40-80% decoctions to normal as well as alloxanized mice was followed by hypoglycemia developed over a 6-h period. Reduction in blood glucose concentration was highly significant, was maximum at 6 h and was dose-dependent. The hypoglycemia caused by the ethanol extract (200-400 mg/kg) in alloxanized mice was also dose-dependent and 200 mg/kg was comparable in effect to 200 mg/kg tolbutamide. Noor and Ashcroft (1989) worked on the antidiabetic effects of *Tinospora crispa* in rats. In Malaysia, an aqueous extract of *Tinospora crispa* stems was taken orally to treat diabetes mellitus. In their study, normal and alloxan-diabetic rats were used to evaluate the hypoglycaemic properties of the extract. A hypoglycaemic effect was observed in moderately diabetic rats with concomitant improvement in insulinaemia. After a 2-week treatment with the extract (4 g/l in the drinking water), these rats also showed improvement in glucose tolerance. Moreover, acute intravenous treatment with the extract (50 mg/kg) caused an increase in plasma insulin levels. The data support the traditional belief that *T. crispa* extract could improve diabetic conditions by virtue of its action on the endocrine pancreas.

The blood sugar lowering potentiality of selected Cucurbitaceae plants of Indian origin were investigated (Chandrasekar *et al.*, 1989). Using five experimental models, the blood sugar lowering efficacy of eight plants of Cucurbitaceae family has been assessed. The

ethanolic extract of *Cucumis sativus* Linn, *Cucumis melo utilissimum* Roxb, *Cucumis melo* Linn, *Benincasa hispida Thunb Cogn* and *Tricosanthes anguina* Nees, when administered in 250 mg/kg dose, orally to rats failed to lower blood sugar or to depress the peak value, after glucose load. However, ethanolic extract of *Momordica charantia* Linn plant and *Coccinia indica* Whit and Arn root significantly lowered blood sugar in fasted model and depressed the peak value in glucose loaded model. Ethanolic extract of *Tricosanthes dioica* Roxb plant caused a significant lowering of blood sugar in fasted rats and depressed the peak value in glucose loaded single and longterm fed groups of rats. The ethanolic extract of the aerial part of *T. dioica* also induced significant depression in the peak values in the glucose loaded models.

Shabana *et al.* (1990) examined the hypoglycaemic activity of some selected plants in normal fasting and alloxanised rats. 31 desert plants belonging to 17 families were collected from different Egyptian localities. 21 plants extracts were orally given to normal rats, and 15 were tested on fasted and to alloxanised rats. The results were compared with a standard oral hypoglycaemic drug (Daonil, Hoechst) used as a positive control. The following findings were obtained: 8 plants exhibited persistent hypoglycaemic effects, *Lycium shawii, Salvia (S.) aegyptiaca, Pergularia tomentosa, Convolvulus (C.) althaeoides, Haloxylon salicornicum, Ephedra alata, Scrophularia deserti,* and *Crotalaria aegyptiaca.* Transient hypoglycaemic effects appeared only 1 hour after administration in response to 4 plants, *Silena succulenta, Lygos raetam, C. lanatus,* and *Pulicaria incisa.* In the cases of *Chradenus baccatus* and *Zygophyllum album,* slow hypoglycaemic activity was produced and appeared 3 hours after administration. 5 plants showed hypoglycaemic effects viz, *Thymus capitatus, Launaea nudicaulis, Conyza dioscorides, Nitraria retusa,* and *Limonium tubiflorum.* Among the 15 plant extracts tested on alloxanised diabetic rats only 4 showed hypoglycaemic effects more potent than those of the administered dose of Daonil. Those were *Matthiola livida, S. aegyptiaca, Astragalus species,* and *Arthrocnemum glaucum.* The hypoglycaemic effect of *S. aegyptiaca* in fasting rats has been confirmed also in alloxanised diabetic animals. That emphasises the importance of conducting both experiments in order to obtain a reliable conclusion.

The hypoglycemic activity of olive leaf was studied (Gonzalez *et al.*, 1992). Maximum hypoglycemic activity was obtained from samples collected in the winter months, especially in February. One of the compounds responsible for that activity was oleuropeoside, which showed activity at a dose of 16 mg/kg. That compound also demonstrated antidiabetic

activity in animals with alloxan-induced diabetes. The hypoglycemic activity of that compound may result from two mechanisms: (a) potentiation of glucose-induced insulin release, and (b) increased peripheral uptake of glucose.

Ponnachan (1993) worked on the effect of leaf extract of *Aegle marmelose* in diabetic rats. Alloxan induced animal model was used to evaluate the potential antidiabetic effect of *A. marmelose* leaf extract. The diabetic animals were given insulin injection and another group *A. marmelose* leaf extract orally. It maintained the weight of the animals near to the control rats but a significant decrease in weight was noted in diabetic animals without any treatment. The blood glucose level in treated animals were near to that of control ones. Also a significantly increased glucose tolerance was observed in animals orally given the leaf extract prior to the experiment. A significant decrease in liver glycogen (1.24 +/- .07 g/100 g of wet tissue) was observed in diabetic rats which were brought to almost the normal level (1.84 +/- .14 g/100 g) with leaf extract treatment. Blood urea and serum cholesterol increased (62.66 +/- 3.50 and 192.67 +/- 13.64 mg/dl) significantly in alloxan diabetic rats. The leaf extract treatment decreased the blood urea and serum cholesterol (37.83 +/- 3.97 and 99.20 +/-8.43 mg/dl) to that of controls ones. A similar effect was seen with insulin treatment. Their results indicated that the active principle in *A. marmelose* leaf extract had similar hypoglycaemic activity to insulin treatment.

The glycaemic balance in streptozotocin-diabetic rats treated with an aqueous extract of *Ficus carica* (fig tree) leaves were investigated (Perez *et al.*, 1998). The hypoglycaemic effect of an aqueous extract of *Ficus carica* leaves was studied in streptozotocin-diabetic rats. The extract induced a significant hypoglycaemic effect after either oral- or intraperitoneal (i.p.) administration. Body weight loss was prevented in treated diabetic rats and the survival index was significantly affected by plasma insulin levels. Results show that *Ficus carica* aqueous extract has a clear hypoglycaemic activity in treated versus non-treated diabetic rats. The mechanism involved in such an effect was not elucidated. Saha *et al.*, (1998) have invaestigated the hypoglycaemic activity of *Leucas lavandulaefolia* Rees. in streptozotocin-induced diabetic rats. The antidiabetic activity of *Leucas lavandulaefolia* Rees extract on streptozotocin (STZ) induced diabetic rats has been investigated. A methanol extract of the herb at doses of 200 and 400 mg/kg, and glybenclamide (1 mg/kg) were administered concurrently to STZ-diabetic rats. The extract caused a significant reduction of blood glucose levels in streptozotocin-induced diabetic rats by 29.8% ($p<0.001$) compared with control groups. Makonnen *et al.* (1998) have tested the hypoglycaemic effect of *Moringa stenopetala*

aqueous Extract in rabbits. The hypoglycaemic effect of *Moringa stenopetala* extract was assessed in nondiabetic rabbits by blood glucose analysis. *In vivo* experiments were carried out in rabbits that received the test material and the standard, glibenclamide. The plant extract, although less potent than glibenclamide, was found to lower blood glucose concentration. The hypoglycaemic effect was observed to increase with time and with an increase in the dose of the extract.

Hypoglycemic effect of water extract of the root of *Pandanus odorus* RIDL. (Thai name: Toei-hom, Pandanaceae) was examined in normal and streptozotocin-diabetic rats (Peungvicha *et al.*, 1996). In the hypoglycemic test without glucose load, an administration of the extract at doses of 0.125-0.5g/kg p.o. did not affect significantly the plasma glucose level in normal rats, whereas the extract significantly lowered the plasma glucose level at a dose of 0.5g/kg p.o. in diabetic rats. In oral glucose tolerance test, an administration of the extract at a dose of 0.5g/kg p.o. significantly lowered the plasma glucose level in normal rats. The extract at doses of 0.5 and 1.0g/kg p.o. also significantly lowered the plasma glucose level in diabetic rats. A reference drug, glibenclamide at a dose of 5 mg/kg p.o. showed a significant hypoglycemic effect in both normal and diabetic rats. Repeated administration of the extract at doses of 0.25 and 0.5 g/kg p.o. for 7d produced a significant hypoglycemic effect in diabetic rats. Glibenclamide (5 mg/kg p.o.) also caused a significant hypoglycemia in the diabetic rats. LD50 (95% confidence limit) after intraperitoneal injection was 1.87 (1.26-2.76)g/kg in male and female rats and 1.62 (1.18-2.24)g/kg in male and female mice, respectively. The LD50 after oral administration was over 8 g/kg in both sexes of rat and mice. Peungvicha *et al.* (1998) also worked on the hypoglycemic effect of the water extract of *Piper sarmentosum* in rats. The hypoglycemic effect of the water extract of the whole plant of *Piper sarmentosum* Roxb. (Piperaceae, Thai name: Chaplu) was examined in normal and streptozotocin-diabetic rats. In an oral glucose tolerance test, a single oral administration of the water extract at doses of 0.125 and 0.25 g: kg significantly lowered the plasma glucose level in the normal rats. A reference drug, glibenclamide, at a dose of 5 mg: kg (per os, p.o.) also showed a significant hypoglycemic effect in the normal rats. In contrast, a single oral administration of the water extract at these doses and glibenclamide did not significantly lower the plasma glucose level in the diabetic rats. However, the repeated oral administration of the water extract at a dose of 0.125 g: kg for 7 days produced a significant hypoglycemic effect in the diabetic rats. Glibenclamide (5 mg: kg, p.o.) also caused significant

hypoglycemia in the diabetic rats. That result demonstrated that the water extract of whole plant of *Piper sarmentosum* had a hypoglycemic effect in rats.

The anti-hyperglycemic effect of 12 edible plants was studied on 27 healthy rabbits, submitted weekly to subcutaneous glucose tolerance tests after gastric administration of water, tolbutamide or a traditional preparation of the plant. Tolbutamide, *Cucurbita ficifolia, Phaseolus vulgaris, Opuntia streptacantha, Spinacea oleracea, Cucumis sativus* and *Cuminum cyminum* decrease significantly the area under the glucose tolerance curve and the hyperglycemic peak. *Brassica oleracea* var. *botrytis, Allium cepa* and *Allium sativum* only decrease the hyperglycemic peak. The glycemic decreases caused by *Psidium guajava, Brassica oleracea* and *Lactuca sativa* var. *romana* were not significant ($p > 0.05$). The integration of a menu that includes the edible plants with hypoglycemic activity for the control and prevention of diabetes mellitus may be possible and recommendable (Roman-Ramos *et al.*, 1995).

The hypoglycemic effect of *Ocimum sanctum* leaf extract in normal and streptozotocin diabetic rats have done by Chattopadhyay (1993). Oral administration of alcoholic extract of leaves of *O. sanctum* led to marked lowering of blood sugar level in normal, glucose fed hyperglycemic and streptozotocin induced diabetic rats. Further the extract potentiated the action of exogenous insulin in normal rats. The activity of the extract was 91.55 and 70.43% of that of tolbutamide in normal and diabetic rats respectively. Chattopadhyay (1999) have worked on the comparative evaluation of some blood sugar lowering agents of plant origin. A comparison of blood sugar lowering activity of four important medicinal plants (*Azadirachta indica, Gymnema sylvestre, Catharanthus roseus* and *Ocimum sanctum*) were carried out against normal and streptozotocin-induced diabetic rat models. The plant extracts decreased the blood sugar level in varying degrees. Blood sugar lowering unit (BLU) of activity of each leaf extract and tolbutamide was calculated by ED50 values. Statistical analysis revealed significant ($P < 0.05$) variation among the treatments as well as doses with regard to their blood sugar lowering capacity. *A. indica* leaf extract was found to have the most potent blood sugar-lowering activity followed by *C. roseus, G. sylvestre* and *O. sanctum.*

Swanston-Flatt *et al.* (1989a) worked on the glycaemic effects of traditional European plant treatments for diabetes. Twelve plants used for the traditional treatment of diabetes mellitus in northern Europe were studied using normal and streptozotocin diabetic

mice to evaluate effects on glucose homeostasis. The plants were administered in the diet (6.25% by weight) and/or as decoctions or infusions in place of drinking water, to coincide with the traditional method of preparation. Treatment for 28 days with preparations of burdock (*Arctium lappa*), cashew (*Anacardium occidentale*), dandelion (*Taraxacum officinale*), elder (*Sambucus nigra),* fenugreek (*Trigonella foenum-graecum*), guayusa (*Ilex guayusa*), hop (*Humulus lupulus*), nettle (*Urtica dioica*), cultivated mushroom (*Agaricus bisporus*), periwinkle (*Catharanthus roseus*), sage (*Salvia officinale*), and wild carrot (*Daucus carrota*) did not affect the parameters of glucose homeostasis examined in normal mice (basal plasma glucose and insulin, glucose tolerance, insulin-induced hypoglycaemia and glycated haemoglobin). After administration of streptozotocin (200 mg/kg) burdock and nettle aggravated the diabetic condition, while cashew, dandelion, elder, fenugreek, hop, periwinkle, sage and wild carrot did not significantly affect the parameters of glucose homeostasis studied (basal glucose and insulin, insulin-induced hypoglycaemia, glycated haemoglobin and pancreatic insulin concentration). Guayusa and mushroom retarded the development of hyperglycaemia in streptozotocin diabetes and reduced the hyperphagia, polydipsia, body weight loss, and glycated haemoglobin. Mushroom also countered the initial reduction in plasma insulin and the reduction in pancreatic insulin concentration, and improved the hypoglycaemic effect of exogenous insulin. Those studies suggested the presence of potentially useful antidiabetic agents in guayusa and mushroom. Swanston-Flatt *et al.* (1989b) made the evaluation of traditional plant treatments for diabetes in streptozotocin diabetic mice. Seven plants and a herbal mixture used for traditional treatment of diabetes were studied in streptozotocin diabetic mice. The treatments were supplied as 6.25% by weight of the diet for 9 days. Consumption of diets containing bearberry (*Arctostaphylos uva-ursi*), golden seal (*Hydrastis canadensis*), mistletoe (*Viscum album*) and tarragon (*Artemisia dracunculus*) significantly reduced the hyperphagia and polydipsia associated with streptozotocin diabetes, but bayberry (*Cinnamomum tamala*), meadowsweet (*Filipendula ulmaria*), senna (*Cassia occidentalis*) and the herbal mixture did not alter these parameters. Bearberry, mistletoe and tarragon retarded the body weight loss but none of the eight treatments significantly altered plasma glucose or insulin concentrations. Those studies suggest that bearberry; golden seal, mistletoe and tarragon may counter some of the symptoms of streptozotocin diabetes without, however, affecting glycemic control. Swanston-Flatt *et al.* (1990) further worked on the traditional plant treatments for diabetes studies in normal and streptozotocin diabetic mice. The effects on glucose homeostasis of eleven plants used as traditional treatments for diabetes mellitus were evaluated in normal

and streptozotocin diabetic mice. Dried leaves of agrimony (*Agrimonia eupatoria*), alfalfa (*Medicago sativa*), blackberry (*Rubus fructicosus*), celandine (*Chelidonium majus*), eucalyptus (*Eucalyptus globulus*), lady's mantle (*Alchemilla vulgaris*), and lily of the valley (*Convallaria majalis*); seeds of coriander (*Coriandrum sativum*); dried berries of juniper (*Juniperus communis*); bulbs of garlic (*Allium sativum*) and roots of liquorice (*Glycyrhizza glabra*) were studied. Each plant material was supplied in the diet (6.25% by weight) and some plants were additionally supplied as decoctions or infusions (1 g/400 ml) in place of drinking water to coincide with the traditional method of preparation. Food and fluid intake, body weight gain, plasma glucose and insulin concentrations in normal mice were not altered by 12 days of treatment with any of the plants. After administration of streptozotocin (200 mg/kg i.p.) on day 12 the development of hyperphagia, polydipsia, body weight loss, hyperglycaemia and hypoinsulinaemia were not affected by blackberry, celandine, lady's mantle or lily of the valley. Garlic and liquorice reduced the hyperphagia and polydipsia but did not significantly alter the hyperglycaemia or hypoinsulinaemia. Treatment with agrimony, alfalfa, coriander, eucalyptus and juniper reduced the level of hyperglycaemia during the development of streptozotocin diabetes. That was associated with reduced polydipsia (except coriander) and a reduced rate of body weight loss (except agrimony). Alfalfa initially countered the hypoinsulinaemic effect of streptozotocin, but the other treatments did not affect the fall in plasma insulin. The results suggested that certain traditional plant treatments for diabetes, namely agrimony, alfalfa, coriander, eucalyptus and juniper, can retard the development of streptozotocin diabetes in mice.

The effects of cinnamon bark and olive leaf have been investigated on streptozotocin-induced tissue injury, and some biochemical and haematological changes in rats (Onderoglu *et al.*, 1999). The effects on glycaemia were also evaluated. Long-term administration of olive leaf caused significant improvement in tissue injury induced by streptozotocin treatment; the effect of cinnamon bark was less extent. No effects on blood glucose levels were detected. However, significant decreases in some increased biochemical and haematological parameters of streptozotocin-treated rats were observed. Aspartate aminotransferase, urea and cholesterol levels were significantly decreased by treatment with both plant materials, and alanine aminotransferase by treatment with olive leaf. Cinnamon bark also caused a significant decrease in platelet counts. In addition, any visible toxicity, except decrease in body weight gain, attributable to the long-term use of plant materials was not established in normal rats. The data indicate that long-term use of olive leaf and

cinnamon bark may provide benefit against diabetic conditions. Determination of underlying mechanism(s) of beneficial effects, toxicity to other systems and clinical assessments of related plant materials were major topics requiring further studies.

Avella *et al.* (1991) worked on the effects of *Cajanus cajan* L. and of *Cassia fistula* L. on carbohydrate metabolism in mice. They reported the results of pharmacologic evaluation of two medicinal plants, *Cajanus cajan* (L.) Millsp and *Cassia fistula*, which are used in Panamanian folk medicine for the treatment of diabetes. It was found that the aqueous fraction of the leaves and stems of *C. cajan* did not produce any hypo blood sugar effect in normoglycemic mice; instead, it produced a hyperglycemia with doses of 500 mg/kg and 1000 mg/kg (p less than 0.001). Only with a dose of 300 mg/kg a short lived decrease in the glycemia was seen at one hour. On the contrary, the folk use of the leaves of *C. fistula* for diabetes seems to have some correlation with the popular frek use. The aqueous fraction produced a significant decrease in the glycemia (p less than 0.001) at 4 and 24 hours with doses of 300 and 500 mg/kg, and at one and four hours after the dose of 1000 mg/kg (p less than 0.001). In the glucose tolerance test, the aqueous fraction of *C. cajan* produced a significant and short lasting decrease (p less than 0.05) with the dose of 300 mg/kg, while the dose of 500 mg/kg did at 0.25, 0.5 and 1 hour (p less than 0.01). The 1000 mg/kg dose produced a significant increase in glucose tolerance at 1 and 2 hours (p less than 0.05). The aqueous fraction of *C. fistula* produced a significant decrease (p less than 0.05) with the dose of 500 mg/kg at 0.25 and 0.5 hours. The 1000 mg/kg dose produced a significant increase (p less than 0.001) at 0.25 and 2 hours. David *et al.*, (1994) studied the anti-diabetic properties of the African mistletoe in streptozotocin-induced diabetic rats. The African mistletoe, *Loranthus bengwensis* L. (Loranthaceae), has been widely used in Nigerian folk medicine to treat diabetes mellitus. The aqueous extract or infusion (1.32 g/kg per day) of the leaves of this plant parasitic on lemon, *Citrus limon* (L.) Brum f. (Rutaceae), guava, *Psidium guajava* L. (Myrtaceae) and jatropha, *Jatropha curcas* L. (Euphorbiaceae), respectively, were supplied *ad libitum* to separate groups of both non-diabetic and streptozotocin-induced diabetic rats, as their only source of fluid for a period of 28 days. The infusions of mistletoe parasitic on both lemon and guava trees significantly decreased serum glucose levels in non-diabetic ($P < 0.05$) and diabetic ($P < 0.001$) rats, whereas that prepared from mistletoe parasitic on jatropha did not. The data indicated that African mistletoe possesses significant anti-diabetic activity in streptozotocin-induced diabetic rats; its anti-diabetic activity appears to be highly dependent on the host plant species.

El-Fiky *et al.* (1996) worked on the effect of *Luffa aegyptiaca* (seeds) and *Carissa edulis* (leaves) extracts on blood glucose level of normal and streptozotocin diabetic rats. Their study showed the effect of oral administration of the ethanolic extracts of *Luffa aegyptiaca* (seeds) and *Carissa edulis* (leaves) on blood glucose levels both in normal and streptozotocin (STZ) diabetic rats. Treatment with both extracts significantly reduced the blood glucose level inSTZ diabetic rats during the first three hours of treatment. *L. aegyptiaca* extract decreased blood glucose level with a potency similar to that of the biguanide, metformin. The total glycaemic areas were 589.61 +/- 45.62 mg/dl/3 h and 660.38 +/- 64.44 mg/dl/3 h for L. aegyptiaca and metformin, respectively, vs. 816.73 +/- 43.21 mg/dl/3 h for the control ($P < 0.05$). On the other hand, in normal rats, both treatments produced insignificant changes in blood glucose levels compared to glibenclamide treatment.

The mechanism of dose-dependent hypoglycemic effect, the margin of safety and ED50 of three structurally unrelated compounds, tolbutamide (TB), centpiperalone (CP) and a swerchirin-containing fraction (SWI) from the plant *Swertia chirayita*, were investigated in experimental models (Saxena *et al.*, 1996). After a single oral administration of TB, CP and SWI to groups of normal and streptozotocin (STZ)-induced mild and severe diabetic rats, the blood sugar lowering effect and ED50 of the agents were determined. Plasma Immuno Reactive Insulin (IRI) levels and the degree of islet beta cell degranulation were assayed using RIA and histochemical staining, respectively, in normal rats treated with the agents.The percent blood sugar lowering, increase in IRI levels and beta cell degranulation were highest in CP treated normal rats (69, 124 and 75%, respectively). In addition, CP was the only agent found active in STZ-induced severely diabetic rats ($P < 0.01$). In STZ-mild diabetic rats, however, TB was more effective than CP and SWI. By analysis of data using Anova method, it was concluded that CP is more effective than SWI ($P < 0.01$) and TB. However, SWI an impure natural product showed better blood sugar lowering than tolbutamide which was a drug in use. Mukherjee *et al.* (1997) worked on the effect of *Nelumbo nucifera* rhizome extract on blood sugar level in rats. Oral administration of the ethanolic extract of rhizomes of *Nelumbo nucifera* markedly reduced the blood sugar level of normal, glucose-fed hyperglycemic and streptozotocin-induced diabetic rats, when compared with control animals. The extract improved glucose tolerance and potentiated the action of exogenously injected insulin in normal rats. When compared with tolbutamide, the extract exhibited activity of 73 and 67% of that of tolbutamide in normal and diabetic rats, respectively.

Alarcon-Aguilara *et al.* (1998) studied the anti-hyperglycemic effect of 28 medicinal plants used in the treatment of diabetes mellitus. Each plant was processed in the traditional way and intragastrically administered to temporarily hyperglycemic rabbits. The results showed that eight out of the 28 studied plants significantly decrease the hyperglycemic peak and/or the area under the glucose tolerance curve. Those plants were: *Guazuma ulmifolia, Tournefortia hirsutissima, Lepechinia caulescens, Rhizophora mangle, Musa sapientum, Trigonella foenum graceum, Turnera diffusa*, and *Euphorbia prostrata.* The results suggested the validity of their clinical use in diabetes mellitus control, after their toxicological investigation. Alarcon-Aguilar *et al.* (2002) made another investigation on the hypoglycaemic effects of extracts of four Mexican medicinal plants in normal and alloxan-diabetic mice. The hypoglycaemic activities of four water ethanol extracts (WEE) prepared from *Bidens pilosa* L., *Salvia officinalis* L., *Psacalium peltatum* H.B.K. (Cass) and *Turnera diffusa* Willd. were investigated in healthy and alloxan-diabetic mice The WEE of *S. officinalis* significantly reduced the blood glucose of fasting normal mice 120 (15.7%) and 240 min (30.2%) after intraperitoneal administration ($p < 0.05$). The WEE of *P. peltatum* and *B. pilosa* also significantly diminished glycaemia in healthy mice at 240 min (19.6% and 13.8%, respectively). In mildly diabetic mice, the WEE of *P. peltatum* lowered the basal blood glucose level 120 (16%) and 240 min (54%) after intraperitoneal administration ($p < 0.05$ and $p < 0.01$, respectively). The WEE of *B. pilosa* and *S. officinalis* also significantly diminished the hyperglycaemia in mildly diabetic mice at 240 mins (32.6% and 22.7%, respectively). The administration of those three extracts to animals with severe hyperglycaemia did not cause a significant decrease. The WEE of T. diffusa did not show any hypoglycaemic activity. Thus, three of the WEE studied conserved the hypoglycaemic activity originally detected in the traditional preparations of the studied antidiabetic plants. It appeared that those extracts require the presence of insulin to show hypoglycaemic activity. In a further study Alarcon-Aguilar *et al.* (2005) reported on the acute and chronic hypoglycemic effect of *Ibervillea sonorae* root extracts-II. *Ibervillea sonorae*'s root, or "wareque" (Cucurbitaceae), was widely used in Mexican traditional medicine for the control of diabetes mellitus. In their study, the hypoglycemic effects produced by the acute and chronic administration of various extracts of *Ibervillea sonorae* were investigated. Both the traditional preparation (aqueous decoction) and the raw extract (juice) from the root resulted in significant reductions of glycemia in healthy mice after intraperitoneal administration at a dose of 600 mg/kg. Additionally, ground dried root was used to obtain a dichloromethane (DCM) extract and a

methanol (MeOH) extract. The DCM extract induced a clear reduction of glycemia in healthy ($P < 0.05$) and in alloxan-diabetic mice. The intraperitoneally administered DCM extract caused a severe hypoglycemia that produced lethality in all the treated animals when doses of 300 and 600 mg/kg body weight were used. Since the DCM extract showed a marked hypoglycemic activity, it was administered daily per os to alloxan diabetic rats, employing corn oil and tolbutamide as controls. After 41 days of DCM extract administration at a dose of 300 mg/kg/day, diabetic rats showed improvement in glycemia, body weight, triglycerides, and GPT in comparison with the diabetic control group. Total cholesterol, GOT, and uric acid blood levels were not affected.

Galega officinalis (galega, Goat's Rue, French Lilac) was well known for its hypoglycaemic action and has been used as part of a plant mixture in the treatment of diabetes mellitus. Palit *et al.* (1999) have done the novel weight-reducing activity of *Galega officinalis* in mice. During pharmacological investigations of an ethanolic extract of a powdered mixture of equal proportions of *G. officinalis, Cressa cretica, Mangifera indica* and *Syzygium jambolanum*, a weight reducing effect of galega was discovered. Galega herb (10% w/w in the diet) caused a significant reduction in body weight in both normal and genetically obese (ob/ob) animals treated for 28 days when compared with respective controls ($P < 0.01$). In normal mice, the weight loss was reversible and initially associated with a transient reduction in food intake but was then maintained even in the presence of increased eating above the control level. Pair-fed normal mice receiving galega for seven days also showed significant weight loss ($P < 0.01$, compared with the control) in the presence of increasing food intake. In sharp contrast, weight loss in galega-treated ob/ob mice was accompanied by a persistent reduction in food intake over the 28-day treatment period. Post-mortem examinations of all galega-treated mice revealed a striking absence of body fat. Serum glucose was significantly reduced in both strains of mice receiving galega for 28 days ($P < 0.01$), whereas serum insulin was significantly reduced only in obese mice ($P < 0.01$). In summary, together with its established hypoglycaemic effects, galega had a novel weight reducing action that, in normal mice, was largely independent of a reduction in food intake. The mechanism of the weight reducing action of galega was unclear but involves loss of body fat.

The hypoglycaemic activity of a 20% dried leaf infusion of *Bauhinia candicans* Benth. (Leguminosae), *Galega officinalis* L. (Leguminosae), *Morus alba* L. (Moraceae) and *Rubus ulmifolius* Schott. (Rosaceae), used for diabetes in Chilean popular medicine, was

evaluated in alloxan and streptozotocin induced hyperglycaemic rats. In normal rats the different infusions did not modify significantly the glycaemia in the period studied, but in diabetic rats different results were observed, depending on the diabetogenic drug used. *B. candicans* and *R. ulmifolius* infusions elicited remarkable hypoglycaemic effects in both experimental models. *B. candicans* presented a greater decrease of glycaemia in alloxan diabetic rats (39%) and *R. ulmifolius* showed a similar activity in both alloxan and streptozotocin diabetic rats (28% and 29%). Activity-guided fractionation of *R. ulmifolius* showed that petroleum ether extracts elicited a marked hypoglycaemic effect (35%) in the streptozotocin induced model (Lemus *et al.*, 1999).

The insulin-like biological activity of culinary and medicinal plant aqueous extracts *in vitro* has done by Broadhurst *et al.* (2000). To evaluate the possible effects on insulin function, 49 herb, spice, and medicinal plant viz,*Cinnamomun zeylanicum*, *Hamamelis virginiana* (witchhazel), *Camellia sinensis* (green and black teas), *Pimenta dioica* (allspice), *Laurus nobilis L.* (bay leaves), *Myristica fragrens* (nutmeg), *Syzgium aromaticum* (cloves), mushrooms, and brewer's yeastextracts were tested in the insulin-dependent utilization of glucose using a rat epididymal adipocyte assay. Cinnamon was the most bioactive product followed by witch hazel, green and black teas, allspice, bay leaves, nutmeg, cloves, mushrooms, and brewer's yeast. The glucose oxidation enhancing bioactivity was lost from cinnamon, tea, witchhazel, cloves, bay leaf and allspice by poly(vinylpyrrolidone) (PVP) treatment, indicating that the active phytochemicals were likely to be phenolic in nature. The activity of sage, mushrooms, and brewers's yeast was not removed by PVP. The positive effects of specific plant extracts on insulin activity suggested a possible role of these plants in improving glucose and insulin metabolism. Malalavidhane *et al.* (2001) have shown that an aqueous extract of the green leafy vegetable *Ipomoea aquatica* was as effective as the oral hypoglycaemic drug tolbutamide in reducing the blood sugar levels of Wistar rats. Their study was undertaken to compare the oral hypoglycaemic activity of an aqueous extract of the green leafy vegetable *Ipomoea aquatica* (dose equivalent to 3.3 g starting material /kg body weight) with that of the known oral hypoglycaemic drug tolbutamide (15 mg/kg body weight) in glucose challenged Wistar rats (3 g/kg body weight, administered 30 min after the administration of *Ipomoea aquatica* or tolbutamide). One and half hours after administration of glucose (equivalent to 2 h after administration of plant extract or tolbutamide), the mean blood glucose level of the *Ipomoea aquatica* treated group was 47.5% lower than that of the control group treated with distilled water. The tolbutamide treated group showed a mean blood glucose level which was only 33.8% lower than that of the control group. However,

statistical analysis indicated that the blood glucose levels of the *Ipomoea aquatica* treated group were not significantly different from that of the tolbutamide treated group. Their results showed that the aqueous extract of *Ipomoea aquatica* is as effectve as tolbutamide in reducing the blood glucose levels of glucose-challenged Wistar rats.

The hypoglycaemic activity of *Punica granatum* Linn. (Family Punicaceae) seed extract on rats made diabetic by streptozotocin (STZ) was investigated (Das *et al.*, 2001). The methanol extract of the seed at doses of 300 and 600 mg/kg, and chlorpropamide 200 mg/kg was administered to STZ diabetic rats. The seed extract (150, 300 and 600 mg/kg, orally) caused a significant reduction of blood glucose levels in STZ induced diabetic rats by 47% and 52%, respectively, at the end of 12 h. Okyar (2001) made a study on the effect of *Aloe vera* leaves on blood glucose level in type I and type II diabetic rat models. *Aloe vera* (L.) Burm. fil. (= *A. barbadensis* Miller) (Liliaceae) was native to North Africa and also cultivated in Turkey. Aloes have long been used all over the world for their various medicinal properties. In the past 15 years, there have been controversial reports on the hypoglycaemic activity of *Aloe* species, probably due to differences in the parts of the plant used or to the model of diabetes chosen. In their study, separate experiments on three main groups of rats, namely, non-diabetic (ND), type I (IDDM) and type II (NIDDM) diabetic rats were carried out. *A. vera* leaf pulp and gel extracts were ineffective on lowering the blood sugar level of ND rats. A. vera leaf pulp extract showed hypoglycaemic activity on IDDM and NIDDM rats, the effectiveness being enhanced for type II diabetes in comparison with glibenclamide. On the contrary, *A. vera* leaf gel extract showed hyperglycaemic activity on NIDDM rats. Based on their results, authers concluded that the pulps of *Aloe vera* leaves devoid of the gel could be useful in the treatment of non-insulin dependent diabetes mellitus. Rathi *et al.* (2002) made a study on the prevention of experimental diabetic cataract by Indian Ayurvedic plant extracts. The efficacy of *Momordica charantia* (MC), *Eugenia jambolana* (EJ), *Tinospora cordifolia* (TC) and *Mucuna pruriens* (MP) was assessed in the prevention of murine alloxan dibetic cataract. Alloxan (120 mg/kg) was used as the diabetogenic agent. While controls and diabetic controls did not receive any plant extract, treated rats received lyophilized aqueous extract of MC and EJ (200 mg/kg p.o.), alcohol extract of TC (400 mg/kg) and MP (200 mg/kg p.o.) every day until 4 months. Serum glucose concentration was assessed and cataracts examined with both the naked eye and through a slit lamp. Of the eight animals in the diabetic control group, four developed cortical cataract (stage IV) by day 90 while the remaining four developed it by day 100. The incidence rate of cataract in MC, EJ,

TC and MP treated groups at 120 days was only 0, 0, 1 and 2. Oral feeding of MC, EJ, TC and MP extracts for 1 month produced a fall of 64.33%, 55.62%, 38.01% and 40.17%, respectively, in the serum glucose levels in comparison with the 48 h level. After 2 months of treatment, the respective values were 66.96%, 59.85%, 40.41% and 45.63%. MC and EJ prevented the development of cataract while the protective effect was less with TC and MP along with a significant reduction of plasma glucose levels ($p < 0.001$).

Caesalpinia bonducella F. (Leguminosae) is a medicinal plant, widely distributed throughout India and the tropical regions of the world. Its seed kernels were used in the management of diabetes mellitus, in the folklore medicine of Andaman and Nicobar as well as the Caribbean Islands. Parameshwar *et al.* (2002) have worked on the oral antidiabetic activities of different extracts of *Caesalpinia bonducella* seed kernels. The seed kernel powder was reported to have hypoglycaemic activity in experimental animals. Four extracts (petroleum ether, ether, ethyl acetate and aqueous) of the seed kernels were prepared and tested for their hypoglycaemic potentials in normal as well as alloxan induced diabetic rats. In normal rats, only ethyl acetate and aqueous extracts showed a minimum significant hypoglycaemic effect, compared to that of glibenclamide. In diabetic rats, both the polar extracts (ethyl acetate and aqueous) as well as glibenclamide, showed significant hypoglycaemic effect, besides, reversing the diabetes induced changes in lipid and liver glycogen levels. As far as the non-polar extracts were concerned, the ether extract showed a marginal antidiabetic activity, while the petroleum ether extract failed to show any. Since both the polar extracts were, chemically, found to contain triterpenoidal glycosides, they presumed that they might be the active principles contributing to the antidiabetic actions. In *in vitro* antioxidant studies, the aqueous extract was found to be devoid of any free radical scavenging activity, while the ethyl acetate extract showed a maximum of 49% activity at the end of 1 h. Although the antioxidant potential of ethyl acetate extract might contribute to overcome the diabetes linked oxidative stress, it needed not necessarily contribute to its hypoglycaemic activity.

Kar *et al.* (2003) have worked on the comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats. In their experiments 30 hypoglycaemic medicinal plants (known and less known) have been selected for thorough studies from indigenous folk medicines, Ayurvedic, Unani and Siddha systems of medicines. In all the experiments with different herbal samples (vacuum dried 95% ethanolic extracts), definite blood glucose lowering effect within 2 weeks have been confirmed in alloxan

diabetic albino rats. Blood glucose values were brought down close to normal fasting level using herbal samples at a dose of 250 mg/kg once, twice or thrice daily, as needed. While evaluating comparative hypoglycaemic activity of the experimental herbal samples, significant blood glucose lowering activities were observed in decreasing order in the following 24 samples-*Coccinia indica, Tragia involucrata, G. sylvestre, Pterocarpus marsupium, T. foenum-graecum, Moringa oleifera, Eugenia jambolana, Tinospora cordifolia, Swertia chirayita, Momordica charantia, Ficus glomerata, Ficus benghalensis, Vinca rosea, Premna integrifolia, Mucuna prurita, Terminalia bellirica, Sesbenia aegyptiaca, Azadirachta indica, Dendrocalamus hamiltonii, Zingiber officinale, Aegle marmelos, Cinnamomum tamala, Trichosanthes cucumerina* and *Ocimum sanctum.* The studies besides confirming hypoglycaemic activities of the experimental herbal samples have helped to identify more potent indigenous hypoglycaemic herbs (in crude ethanolic extract) from the comparative study of the reported experimental results.

The effect of an antidiabetic extract of *Catharanthus roseus* on enzymic activities in streptozotocin induced diabetic rats has investigated by Singh *et al.* (2001). Hypoglycemic activity was detected in dichloromethane: methanol extract (1:1) of leaves and twigs of *Catharanthus roseus* (family Apocynaceae), a traditionally used medicinal plant, using streptozotocin (STZ) induced diabetic rat model. Extract at dose of 500 mg/kg given orally for 7 and 15 days showed 48.6 and 57.6% hypoglycemic activity, respectively. Prior treatment at the same dose for 30 days provided complete protection against STZ challenge (75 mg/kg/i.p.×1). Enzymic activities of glycogen synthase, glucose 6-phosphate-dehydrogenase, succinate dehydrogenase and malate dehydrogenase were decreased in liver of diabetic animals in comparison to normal and were significantly improved after treatment with extract at dose 500 mg/kg p.o. for 7 days. Results indicate increased metabolization of glucose in treated rats. Increased levels of lipid peroxidation measured as 2-thiobarbituric acid reactive substances (TBARS) indicative of oxidative stress in diabetic rats were also normalized by treatment with the extract. The leaf juice or water decoction of *Catharanthus roseus* L. (Apocyanaceae) was used as a folk medicine for the treatment of diabetes all over the world. The leaf juice of *C. roseus* has also been evaluated by Srinivas *et al.* (2003) for its hypoglycemic activity in normal and alloxan-induced diabetic rabbits. The blood glucose lowering activity of the leaf juice was studied in normal and alloxan-induced (100 mg/kg, i.v.) diabetic rabbits, after oral administration at doses of 0.5, 0.75 and 1.0 ml/kg body weight. Blood samples were collected from the marginal ear vein before and also at 4, 6, 8,

10, 12, 16, 18, 20 & 24 h after drug administration and blood glucose was analyzed by Nelson-Somogyi's method using a visible spectrophotometer. The data was compared statistically by using Student's *t*-test. The leaf juice of *C. roseus* produced dose-dependent reduction in blood glucose of both normal and diabetic rabbits and comparable with that of the standard drug, glibenclamide. The results indicated a prolonged action in reduction of blood glucose by *C. roseus* and the mode of action of the active compound(s) of *C. roseus* was probably mediated through enhance secretion of insulin from the β-cells of Langerhans or through extrapancreatic mechanism. The study clearly indicated a significant antidiabetic activity with the leaf juice of *Catharanthus roseus* and supports the traditional usage of the fresh leaves by Ayurvedic physicians for the control of diabetes.

In another study Satyanarayana *et al.* (2003) have made an evaluation of herbal preparations for hypoglycemic activity in normal and diabetic rabbits.The leaf juice of *Catharanthus roseus* and the seed powder of fenugreek were tested for their hypoglycemic action individually and in combination in normal and alloxan-induced diabetic rabbits. Blood glucose was determined in all the groups before and after treatment with *Catharanthus roseus* and fenugreek at doses of 0.5, 0.75 and 1.0ml/kg and 50,100 and 150mg/kg, respectively, at 0, 0.5, 1, 2, 3 ,4,6,8,10,12,14,16,18,20,22 and 24h after being fasted for 18h. The effect was found to be dose-dependent with both the treatments at the doses administered. The percentage blood glucose reduction produced by the combination of *Catharanthus roseus* (0.5ml/kg) and fenugreek (50mg/kg) was more than the sum of their individual percent blood glucose reduction in both normal and diabetic rabbits, suggesting that the combination produced a synergistic action.

The antidiabetic activity of *Boerhaavia diffusa* L. in experimental diabetes was studied by Pari and Satheesh (2004). The purpose of the study was to investigate the effects of daily oral administration of aqueous solution of *Boerhaavia diffusa* L. leaf extract (BLEt) (200 mg/kg) for 4 weeks on blood glucose concentration and hepatic enzymes in normal and alloxan induced diabetic rats. A significant decrease in blood glucose and significant increase in plasma insulin levels were observed in normal and diabetic rats treated with BLEt. Treatment with BLEt resulted in a significant reduction of glycosylated haemoglobin and an increase in total haemoglobin level. The activities of the hepatic enzymes such as hexokinase was significantly increased and glucose-6-phosphatase, fructose-1, 6-bisphosphatase were significantly decreased by the administration of BLEt in normal and diabetic rats. An oral glucose tolerance test (OGTT) was also performed in the same groups, in which there was a

significant improvement in glucose tolerance in rats treated with BLEt. A comparison was made between the action of BLEt and antidiabetic drug—glibenclamide (600 μg/kg). The effect of BLEt was more prominent when compared to glibenclamide.

Effects of ten different plant extracts in the regulation of serum cortisol and glucose concentrations were evaluated in male mice by Gholap and Kar (2004). While the extracts of *Inula racemosa, Boerhaavia diffusa* and *Ocimum sanctum* decreased the serum concentration of both cortisol and glucose, *Aegle marmelos, Azadirachta indica* and *Gymnema sylvestre* extracts could exhibit hypoglycaemic activity without altering the serum cortisol concentration. It appeared that the hypoglycaemic effects of former three plant extracts were mediated through their cortisol inhibiting potency, whereas the mechanism for other plant extracts could be different. Lipid-peroxidation was not enhanced by any of the plant extracts (some were in fact, antiperoxidative in nature). As *I. racemosa, B. diffusa* and *O. sanctum* exhibited antiperoxidative, hypoglycaemic and cortisol lowering activities, it was suggested that these three plant extracts may potentially regulate corticosteroid induced diabetes mellitus.

The aqueous extract of *Murraya koenigii* leaves was also evaluated for hypoglycemic activity in normal and alloxan induced diabetic rabbits (Kesari *et al.*, 2005). The plant was promising as it was widely and regularly used as a spice for food flavoring and as such it appears to be without any side effects and toxicity. The scientific evaluation of its hypoglycemic activity was, explored and also compared with the effect of a standard hypoglycemic drug, tolbutamide. A single oral administration of variable dose levels (200, 300 and 400 mg/kg) of aqueous extract led to lowering of blood glucose level in normal as well as in diabetic rabbits. The maximum fall of 14.68% in normal and 27.96% in mild diabetic was observed after 4 h of oral administration of 300 mg/kg. The same dose also showed a marked improvement in glucose tolerance of 46.25% in sub-diabetic (AR) and 38.5% in mild diabetic rabbits in glucose tolerance test after 2 h. The findings from this study suggest that the aqueous extract of these leaves might be prescribed as adjunct to dietary therapy and drug treatment for controlling diabetes mellitus. Mukherjee *et al.* (2006) have leads from Indian medicinal plants with hypoglycemic potentials. The plants provide a potential source of hypoglycemic drugs because many plants and plant derived compounds have been used in the treatment of diabetes. Several medicinal plants have found potential use as hypoglycemic in the Indian system of medicines, including ayurveda. Many Indian

plants have been investigated for their beneficial use in different types of diabetes and reports occur in numerous scientific journals. That article aims to provide a comprehensive review on various plant species from Indian biosphere and their constituents, which have been shown to display potent hypoglycemic activity. The use of herbs as hypoglycemic was a major avenue in Indian perspectives particularly for treating diabetes, which require to be explored more effectively as there are so many literatures available on these aspects. That paper describes the chemistry, activity and usage of the constituents isolated from these plants from India for the treatment of diabetes.

Another investigation on the effect of feeding *Murraya koeingii* and *Brassica juncea* diet on kidney functions and glucose levels in streptozotocin diabetic mice has been carried out by Grover *et al.* (2003). Purpose of the study was to investigate the effects of daily oral feeding 15% of powdered leaves of *Murraya koeingii* (MK) (commonly called as Curry patta) and10%powder of seeds of *Brassica juncea* (BJ) (commonly called as Rai) for 60 days on serum glucose concentrations and kidney functions in streptozotocin (STZ; 100 mg/kg) diabetic rats. Serum glucose levels, body weight, urine volume, serum creatinine, and urinary albumin (UAE) levels were monitored on day 0, 10, 25, 40, and 70 of the experiment. After 60 days of STZ administration, urine volume per day andUAE levels were significantly higher (P <0.0005) in diabetic controls (DC) as compared to normal controls (NC). Although feeding of the MK/BJ showed a trend towards improvement in most of the parameters, results were not statistically different from theDCexcept in serum creatinine values in BJ-fed rats on day 70. Thus, those plants could be best utilized by promoting them as preferable food adjuvants for diabetic patients.

Kayoko *et al.* (2005) examined the effects of long-term oral administration of green tea cultivated in different districts in Japan on body weight, blood lipid and glucose levels on db/db mice. Previous research using short-term animal experiments has indicated that oral administration of green tea can suppress elevation of blood sugar. However, few data were available on the antihyperglycemia effect of green tea in long-term experiments. In their study effects of long-term (up to 16 weeks) administration of green tea preparations on body weight, blood sugar and lipid content of db/db and db/+m mice were examined. Preparations were prepared by the same procedure from tea leaves cultivated in different districts in Japan. Significant differences ($P < 0.05$) were observed in the blood parameters and body weight between control and green tea groups of db/db mice. In the case of normal mice, however, no

significant difference was observed. In addition, the response to the administration of green tea depended on the source and composition of tea leaves.

In another work Ravi *et al.* (2005) examined the antihyperlipidemic effect of *Eugenia jambolana* seed kernel on streptozotocin-induced diabetes in rats. Abnormalities in lipid profile are one of the most common complications in diabetes mellitus, which is found in about 40% of diabetics. In their study, anti-hyperlipidemic efficacy of *Eugenia jambolana* seed kernel (EJs-kernel) was evaluated in streptozotocin (STZ)-induced diabetic rats and the efficacy was compared with standard hypoglycemic drug, glibenclamide. The effect of oral administration of ethanolic extract of EJs-kernel (100 mg/kg body weight) was examined on the levels of cholesterol, phospholipids, triglycerides and free fatty acids in the plasma, liver and kidney tissues of STZ (55 mg/kg body weight)-induced diabetic rats. The plasma lipoproteins and tissues fatty acid composition were also monitored. STZ-induced diabetic rats, showed significant increase in the levels of cholesterol, phospholipids, triglycerides and free fatty acids which were considerably restored to near normal in EJs-kernel or glibenclamide treated animals. The plasma lipoproteins (HDL, LDL, VLDL-cholesterol) and fatty acid composition were altered in STZ-induced diabetic rats and these levels were also reverted back to near normalcy by EJs-kernel or glibenclamide treatment. They concluded that, EJs-kernel possesses hypolipidemic effect, which might be due to the presence of flavonoids, saponins, glycosides and triterpenoids in the extract. The hypolipidemic effect mediated by EJs-kernel might also be anticipated to have biological significance and provide a scientific rationale for the use of EJs-kernel as an anti-diabetic plant.

Dimo *et al.* (2007) have investigated the effect of *Sclerocarya birrea* (Anacardiaceae) stem bark methylene chloride/methanol extract on streptozotocin-diabetic rats. *Sclerocarya birrea* (Anacardiaceae) was used as a traditional treatment of diabetes in Cameroon. They investigated the possible antidiabetic effect of the stem bark extract in diabetic rats. Diabetes was induced by intravenous injection of streptozotocin (STZ, 55 mg/kg) to male Wistar rats. Experimental animals (six per group), were treated by oral administration of plant extract (150 and 300 mg/kg body weight) and metformin (500 mg/kg; reference drug) for comparison, during 21 days. The stem bark methanol/methylene chloride extract of *Sclerocarya birrea* exhibited at termination, a significant reduction in blood glucose and increased plasma insulin levels in diabetic rats. The extract also prevented body weight loss in diabetic rats. The effective dose of the plant extract (300 mg/kg) tended to reduce plasma

cholesterol, triglyceride and urea levels toward the normal levels. Four days after diabetes induction, an oral glucose tolerance test (OGTT) was also performed in experimental diabetic rats. The results showed a significant improvement in glucose tolerance in rats treated with *Sclerocarya birrea* extract. Metformin, a known antidiabetic drug (500 mg/kg), significantly decreased the integrated area under the glucose curve. Those data indicated that *Sclerocarya birrea* treatment might improve glucose homeostasis in STZ-induced diabetes which could be associated with stimulation of insulin secretion.

The amount of glycogen and its synthesis from glucose was studied in white muscle (extensor digitorum longus -- EDL) and red muscle (soleus -- SOL) of normal rats and rats with alloxan diabetes by the anthrone method (Sofrankova, 1975). The amount of glycogen was higher in the white muscle of normal rats, both after a 24 hours' fast (0.37+/-0.02 mg/g as against 0.29+/-0.01 mg/g in the SOL) and with feeding ad libitium (0.72+/-0.05 mg/g as against 0.58+/-0.03 mg/g in the SOL). After a 24 hours' fast, the glycogen content of both muscles was non-significantly higher in alloxan-diabetic rats than in normal animals, whereas in diabetic animals fed *ad libitum* it was significantly lower than in normal rats fed in the same manner (0.54+/-0.07 mg/g in the EDL and 0.33+/-0.03 mg/g in the SOL). The difference between the glycogen content of the white and red muscle of diabetic rats was also in favour of the white muscle. Muscle glycogenesis from intragastrically administered glucose was higher in the red muscle in all the experimental groups. In normal fed *ad libitum* the glycogen content of the EDL did not change after glucose administration, but in the SOL it raised from 0.58+/-0.03 to 0.83+/-0.05 mg/g. In fasting (24 hours) normal rats it rose sharply in both muscles, from 0.037+/-0.02 to 0.57+/-0.03 mg/g in the EDL and from 0.29+/-0.01 to 0.87+/-0.06 mg/g in the SOL. In fasting (24 hours) diabetic animals, the glycogen content rose after glucose in the SOL only, from 0.36+/-0.01 to 0.66+/-0.06 mg/g. The differences found in glycogen synthesis in the white and red muscle of normal and diabetic rats were discussed mainly from the aspect of the existence of a relationship between the glycogen concentration and glycogen synthetase activity.

Rapin (1998) has worked on the effects of repeated treatments with an extract of *Ginkgo biloba* (EGb 761) and bilobalide on liver and muscle glycogen contents in the non-insulin-dependent diabetic rat. The effects of repeated (15-day) oral treatments with an extract of *Ginkgo biloba* (EGb 761; 50 mg/kg/day) or with its terpenoid constituent, bilobalide (2 mg/kg/day), were assessed in normal rats and in rats that had been previously injected with streptozotocin (50 mg/kg, i.p. in saline solution), a dose which provided a

model of non-insulin-dependent diabetes mellitus (NIDDM). In that model of diabetes, blood glucose was significantly increased while the circulating insulin level remains unchanged. Glucose penetrates cells because of decreased glycogen turnover, a metabolic abnormality that can be revealed by using an oral glucose tolerance test (OGTT). In control rats, hyperglycemia was accompanied by increased glycogen synthesis, as evidenced by increased concentrations of that storage substance in liver and skeletal muscle. Repeated treatment with EGb 761 or bilobalide increased the glycogen contents of both liver and muscle. That effect of bilobalide was additive to that of hyperglycemia in muscle. In diabetic rats, hyperglycemia did not modify glycogen synthesis, indicating impaired glucose utilization. Repeated treatment with EGb 761 or bilobalide partially prevented this impairment and led to increased in glycogen content in both liver and muscle under control conditions an during OGTT with 2 g/kg glucose. The moleculasr mechanism underlying these actions of EGb 761 could be related to an antioxidant effect (i.e., suppression of free radical formation) or to free radical-scavenging, since EGb 761 was known to have such effects and since free radicals have been implicated in the cytotoxic activity of streptozatocin. However, the increase in glucose uptake induced by bilobalide might have been related to increased glycogen synthesis.

The effects of acute insulin deficiency on the kidney have been investigated in animal models of experimental diabetes; however, the impact of long-term diabetes has not been determined. Nannipieri *et al.* (2001) have examined the influence of long term diabetes on renal glycogen metabolism. They measured renal glycogen contents in streptozotocin (STZ)-diabetic rats 3 weeks (n = 12) or 9 months (n = 12) after the induction of diabetes, and in 2 groups of control rats of similar age (n = 16 and n = 12, respectively), in the fed state and after a 24-hour fast. Diabetic rats had high glucose levels, low insulin but normal glucagon concentrations in portal blood. In the fasting state, kidney glycogen content was very low in both young control and young diabetic rats (54 ± 15 and 189 ± 26 μg/g, respectively, mean ± SD); in contrast, glycogen levels were markedly elevated in rats with long-standing diabetes as compared to old nondiabetic animals (2,628 ± 1,023 ± and 1,968 ± 989 μg/g of diabetic rat, fasting and fed, respectively, $p < 0.001$ vs. 0 ± 0 and 4 ± 6 μg/g of control rats). On electron microscopy, large glycogen clusters were localized to the renal tubules. Kidney phosphorylase activity was higher, and synthase activity lower in diabetic than control rats ($p < 0.05$ for both), whereas kidney glycogen was strongly related to plasma glucose levels, suggesting that the enzyme changes were secondary to glycogen accumulation itself. Renal hexosephosphates and fructose-2,6-bisphosphate contents were both increased in long-term

diabetic rats ($p < 0.05$), implying enhanced fluxes through both glycolysis and gluconeogenesis. In chronic, untreated diabetes glycogen accumulates in the renal tubules; prolonged hyperglycemia was the sole driving force for this phenomenon.

Vats *et al.* (2003) have worked on the effect of *T. foenum graecum*, a plant mentioned in Ayurveda on glycogen content of tissues and the key enzymes of carbohydrate metabolism. In the study, FG (1 g/kg PO) was assessed for its effect on glycogen levels of insulin dependent (skeletal muscle and liver), insulin independent tissues (kidneys and brain) and enzymes such as glucokinase (GK), hexokinase (HK), and phosphofructokinase (PFK). Administration of FG led to decrease in blood glucose levels by 14.4 and 46.64% on 15th and 30th day of the experiment. Liver and 2-kidney weight expressed as percentage of body weight was significantly increased in diabetics ($P < 0.0005$) versus normal controls and this alteration in the renal weight ($P < 0.0005$) but not liver weight was normalized by feeding of FG. Renal glycogen content increased by over 10 folds while hepatic and skeletal muscle glycogen content decreased by 75 and 68% in diabetic controls versus controls and these alteration in glycogen content was partly prevented by FG. Activity of HK, GK and PFK in diabetic controls was 35, 50 and 60% of the controls and FG partially corrected this alteration in PFK, HK and GK. In a further study also Vats *et al.*, (2004) studied the anti-cataract activity of *Pterocarpus marsupium* bark and *Trigonella foenum-graecum* seeds extract in alloxan diabetic rats. Long-term complications were frequently encountered in diabetes mellitus and were difficult to treat. An aqueous extract of *Pterocarpus marsupium* Linn bark (1 g kg-1 day-1), *Ocimum sanctum* Linn leaves (200 mg kg-1 day-1) and alcoholic extract of *Trigonella foenum-graecum* Linn seeds (2 g kg-1 day-1) were given to alloxan (120 mg kg-1) diabetic rats until the development of cataract. Serum glucose and body weight were monitored at regular intervals while cataract was examined through naked eye as well as slit lamp at 75, 100 and 115 days after alloxan administration. Administration of all the three plant extracts exerted a favorable effect on body weight and blood glucose, the effects were best with *Pterocarpus marsupium* followed by *Trigonella foenum-graecum* and *Ocimum sanctum.* On the course of cataract development, *Pterocarpus marsupium* followed by *Trigonella foenum-graecum* exerted anti-cataract effect evident from decreased opacity index while *Ocimum sanctum* failed to produce any anti-cataract effect in spite of significant antihyperglycemic.

The effect of aminoguanidine on lipid peroxidation in streptozotocin-induced diabetic rats has done by Ihm *et al.* (1999). One potential mechanism of the increased lipid

peroxidation in diabetes was lipid-linked advanced glycosylation and oxidation. Aminoguanidine (AMGN), the prototype inhibitor of advanced glycosylation end product (AGE) formation, has been shown to prevent oxidative modification of low-density lipoprotein (LDL) *in vitro* at a moderate concentration. It was unknown whether AMGN may act as an antioxidant against lipid peroxidation under hyperglycemia *in vivo*. To investigate the *in vivo* effect of AMGN on lipid peroxidation in diabetes, they administered AMGN (1 g/L in drinking water) or vitamin E (400 mg/d for 5 d/wk) to streptozotocin (STZ)-induced diabetic rats for 9 weeks and measured plasma lipid hydroperoxides by ferrous oxidation with xylenol orange II (FOX method) and red blood cell (RBC) membrane malondialdehyde (MDA) and related aldehydes as thiobarbituric acid-reactive substances (TBARS). Plasma lipid hydroperoxide was higher in STZ-induced diabetic rats versus control rats (mean +/- SD, 7.53 +/- 2.03 v 5.62 +/- 0.44 micromol/L, $P < .05$; n = 8 to 14). RBC membrane TBARS were also higher in STZ-induced diabetic rats than in control rats (2.67 +/- 0.46 v 1.81 +/- 0.19 nmol/mL, $P < .05$). Plasma lipid hydroperoxide was lower in AMGN-treated (6.23 +/- 0.59 micromol/L, $P < .05$) and vitamin E-treated (5.29 +/- 0.27 micromol/L, $P < .05$) diabetic rats than in untreated diabetic rats. RBC membrane TBARS were also lower in AMGN-treated (1.93 +/- 0.12 nmol/mL, $P < .05$) diabetic rats than in untreated diabetic rats. There was no significant difference in plasma glucose, cholesterol, and triglyceride levels among diabetic groups. Although the mechanism(s) of action of AMGN on lipid peroxidation *in vivo* should be studied further, authors suggested that AMGN may have an additional beneficial effect as an antioxidant against lipid peroxidation in a prevention trial for diabetic vascular complications.

The antidiabetic effect of aqueous extract of seed of *Tamarindus indica* in streptozotocin-induced diabetic rats have examined by Maiti *et al.* (2004). In their study, aqueous extract of seed of *Tamarindus indica* Linn. was found to have potent antidiabetogenic activity that reduced blood sugar level in streptozotocin (STZ)-induced diabetic male rat. Supplementation of that aqueous extract by gavage at the dose of 80 mg/0.5 ml distilled water/100 g body weight per day in STZ-induced diabetic rat resulted in a significant diminution of fasting blood sugar level after 7 days. Continuous supplementation of that extract for 14 days resulted in no significant difference in this parameter from control level. Moreover, this supplementation produced a significant elevation in liver and skeletal muscle glycogen content, activity of liver glucose-6-phosphate dehydrogenase in respect to diabetic group. Activities of liver glucose-6-phosphatase, liver and kidney glutamate

oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities were decreased significantly in the aqueous extract supplemented group in respect to diabetic group. All those parameters were not resettled to the controlled level after 7 days of that extract supplementation but after 14 days of that supplementation, all the above mentioned parameters were restored to the control level.

Catechins, polyphenolic compounds belonging to flavanoid family, have been reported to posses' insulin-like properties and their antidiabetic actions have been documented. Catechins have received much attention as strong anti-oxidative agents. Since oxidative stress has been implicated in the development of diabetic complications and GSH plays an important role in protection against oxidative damages, the researchers have studied the *in vitro* effect of (-) epicatechin and insulin on the reduced glutathione content in normal and type 2 diabetic erythrocytes. The GSH content was significantly lower ($p < 0.001$) in type 2 diabetic patients as compared to normal individuals. *In vitro* insulin treatment (10(-9) M) resulted in increase in the GSH content in both normal and type 2 diabetic erythrocytes. (-)Epicatechin (1mM) also resulted in an increase in erythrocyte GSH content in both normal and type 2 diabetic erythrocytes. Insulin gave a pronounced dose-responsive effect: maximum increase in GSH content at physiological hormone concentration and a lower increase at higher and lower insulin concentrations. (-)Epicatechin did not show a similar dose-responsive effect. Although the exact mechanism by which (-) epicatechin causes elevation of erythrocyte GSH was not clear nevertheless that finding may have important therapeutic implications. A higher content of dietary flavanoids might thus protect diabetic patients against long-term complications (Rizvi and Zaid, 2001).

The mechanism of action of antiatherogenic and related effects of *Ficus bengalensis*Linn. flavonoids in experimental animals syudied by Daniel *et al.* (2003). One month treatment of alloxan diabetic dogs with a glycoside, viz.leucopelargonin derivative (100 mg/kg/day) isolated from the bark of *F.bengalensis* decreased fasting blood sugar and glycosylated haemoglobin by 34% and 28% respectively. Body weight was maintained in both the treated groups while the same was decreased significantly by 10% in the control group. In cholesterol diet fed rats, as the atherogenic index and the hepatic bile acid level and the faecal excretion of bile acids and neutral sterols increased, the HMGCoA reductase and lipogenic enzyme activities in liver and lipoprotien lipase activity in heart and adipose tissue and plasma LCAT activity and the incorporation of labelled acetate into free and ester cholesterol in liver decreased significantly. On treatment with the two ficus flavonoids,

viz.leucopelargonin and leucocyanin derivatives and another flavonoid quercetin (100 mg/kg/day) the above said effects except on bile acids and sterols and lipogenic enzymes were significantly reversed in the cholesterol fed rats. However in the treated rats the hepatic level of bile acids and the faecal excretion of bile acids and neutral sterols still further increased and the action of lipogenic enzyme glucose 6 phosphate dehydrogenase was still further decreased. Those effects of leucopelargonidin and quercetin were better than that of the second. Toxicity studies were required to be carried out to find out if the ficus flavonoids could be used as health promoters as they are hypocholesterolemic and antioxidant in action. Koyama *et al* (2004) have done an evaluation on the effects of green tea on gene expression of hepatic gluconeogenic enzymes in vivo. It has recently been reported that the major green tea polyphenolic constituent, epigallocatechin 3-gallate (EGCG), mimics the cellular effects of insulin including the reductive effect on the gene expression of rate-limiting gluconeogenic enzymes in a cell culture system. They showed that administration of green tea that contains EGCG caused a reduction in the level of mRNAs for gluconeogenic enzymes, phosphoenolpyruvate carboxykinase and glucose-6-phosphatase in the mouse liver. EGCG alone was also found to down-regulate the gene expression of these enzymes but not so curcumin or quercetin. The results of their study supported the idea that green tea intake might be beneficial in the prevention of diabetes mellitus.

The antioxidant activity of *Tinospora cordifolia* roots in experimental diabetes have been worked out by Prince and Menon (2001). They made an attempt to study the antioxidant properties of *Tinospora cordifolia* roots, an indigenous plant used in Ayurvedic medicine in India in alloxan diabetic rats. Oral administration of an aqueous *T. cordifolia* root extract (TCREt) (2.5 and 5.0 g: kg) for 6 weeks resulted in a decreased in the levels of plasma thiobarbituric acid reactive substances, ceruloplasmin and a-tocopherol in alloxan diabetic rats. The root extract also caused an increased in the levels of glutathione and vitamin C in alloxan diabetes. The root extract at a dose of 5.0 g: kg showed the highest effect. The effect of TCREt was more effective than glibenclamide. Insulin restored all the parameters to near normal levels. They also reported that oral administration of 2.5 g and 5.0 g/kg body weight of the aqueous extract of the roots for 6 weeks resulted in a significant reduction in thiobarbituric acid reactive substances (TBARS) and an increased in reduced glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) in alloxan diabetic rats. The effect of *Tinospora cordifolia* root extract (TCREt) was most prominently seen in the case of rats given 5.0 g/kg body weight. The effect of TCREt was more effective than glibenclamide.

Thus their study showed that TCREt exhibits antioxidant action in alloxan diabetes. Annida and Prince (2005) evaluated the antioxidant effect of *Trigonella foenum-graecum* by estimating thiobarbituric acid-reactive substances and reduced glutathione and measuring the activities of catalase and superoxide dismutase in liver, heart, and kidney in diabetic rats. Fenugreek leaf powder supplementation significantly lowered lipid peroxidation and significantly increased the antioxidant system in diabetic rats. The effect at a dose of 1 g/kg of body weight of fenugreek leaf powder was similar to that of glibenclamide. Insulin restored all the parameters to near normal values. Thus, fenugreek leaf powder reduced oxidative stress in experimental diabetes.

The antihyperglycaemic and anti-oxidant properties of *Andrographis paniculata* in normal and diabetic rats were investigated by by Xiang-Fan and Kwong-Huat (2000). Oxidative stress was believed to be a pathogenetic factor in the development of diabetic complications. They investigated the ethanolic extract of the aerial parts of *Andrographis paniculata* for antihyperglycaemic and anti- oxidant effects in normal and streptozotocin-induced type I diabetic rats. Normal and diabetic rats were randomly divided into groups and treated orally by gavage with vehicle (distilled water), metformin (500 mg/kg bodyweight) or the extract (400 mg/kg bodyweight), twice a day for 14 days. At the end of the 14 day period, the extract, like metformin, significantly increased bodyweight ($P< 0.01$) and reduced fasting serum glucose in diabetic rats ($P< 0.001$) when compared with vehicle, but had no effect on bodyweight and serum glucose in normal rats. Levels of liver and kidney thiobarbituric acid-reactive substances (TBARS) were significantly increased ($P< 0.0001$, $P< 0.01$, respectively), while liver glutathione (GSH) concentrations were significantly decreased ($P< 0.005$) in vehicle-treated diabetic rats. Liver and kidney TBARS levels were significantly lower ($P< 0.0001$, $P< 0.005$, respectively), whereas liver GSH concentrations were significantly higher ($P< 0.05$) in extract- and metformin-treated diabetic rats compared with vehicle-treated diabetic rats. *Andrographis paniculata* significantly decreased kidney TBARS level ($P< 0.005$) in normal rats. Hepatic superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities were significantly lower in vehicle-treated diabetic rats compared with vehicle-treated normal rats. The extract, as well as metformin, significantly increased the activity of SOD and CAT, but had no significant effect on GSH-Px activity in diabetic rats. The extract and metformin did not produce significant changes in the activity of these anti-oxidant enzymes in normal rats. Their results showed that oxidative stress was evident in streptozotocin-diabetic rats and indicate that the ethanolic extract of *A.*

paniculata not only possessed an antihyperglycaemic property, but might also reduced oxidative stress in diabetic rats.

The glutathione redox cycle plays a major role in scavenging hydrogen peroxide (H_2O_2) under physiological conditions. It was demonstrated by Tachi *et al.*, 2001 that high glucose concentration in the culture medium reduced the level of H_2O_2 scavenging activity of human vascular smooth muscle cells (hVSMCs). They also showed that a high glucose concentration reduced the intracellular glutathione (GSH) content and the rate of uptake of cystine, which itself was a rate-limiting factor that maintains the GSH level. In their study, they investigated whether the hyperglycemic condition in diabetic rats impairs the glutathione content in the aortic tissue in vivo. Wistar rats were divided into the following three groups: streptozotocin-induced diabetic rats (STZ-D, n=7), insulin-treated STZ-D rats (I-STZ-D, n=8), and non-diabetic controls (C, n=7). Fourteen days after streptozotocin injection, the aortic tissue was extracted and the GSH content in the aortic tissue was measured. Furthermore, the relationship between the GSH content in the aortic tissue and blood glucose level in Otsuka Long-Evans Tokushima Fatty (OLETF) rats aged 30 weeks, which developed diabetes spontaneously, was investigated. The GSH content in the aortic tissue of the STZ-D group (0.99+/-0.14 nmol/mg protein) was significantly lower than that of the control group (1.68+/-0.15 nmol/mg protein). Insulin treatment to the diabetic rats restored the GSH content in the aortic tissue (I-STZ-D group; 1.45+/-0.11 nmol/mg protein). Among the 22 Wistar rats, the GSH content in the aortic tissue was negatively correlated with the blood glucose level (r=-0.69, pless than 0.01, n=22). Among the OLETF rats, a similar negative correlation between the GSH content in the aortic tissue and blood glucose level was seen (r=-0.64, pless than 0.05, n=10). They demonstrated *in vivo* that the hyperglycemic condition in STZ-induced diabetic Wistar rats and OLETF rats reduced the GSH content in aortic tissue. That suggested reduced glutathione redox cycle function of aorta.

Pari and Umamaheswari (2000) studied the antihyperglycaemic activity of *Musa sapientum* flowers, commonly known as banana was widely used in Indian folk medicine for the treatment of diabetes mellitus. Oral administration of 0.15, 0.20 and 0.25 g/kg body weight of the chloroform extract of the flowers for 30 days resulted in a significant reduction in blood glucose and glycosylated haemoglobin and an increase in total haemoglobin. The extract prevented a decrease in body weight, and also resulted in a decrease in free radical formation in the tissues. Thus the study showed that banana flower extract (BFEt) had an antihyperglycaemic action. The decrease in thiobarbituric acid reactive substances (TBARS)

and the increase in reduced glutathione (GSH), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) clearly showed the antioxidant property of BFEt. The effect of BFEt was more prominently seen in the case of animals given 0.25 g/kg body weight. BFEt was more effective than glibenclamide.

The antioxidant effect of an aqueous extract of *Phaseolus vulgaris* pods was studied in rats with streptozotocin-induced diabetes (Venkateswaran and Pari, 2002). Oral administration of *Phaseolus vulgaris* pod extract (PPEt; 200 mg/kg body weight) for 45 days resulted in a significant reduction in thiobarbituric acid reactive substances and hydroperoxides. The extract also caused a significant increase in reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase in the liver and kidneys of rats with streptozotocin-induced diabetes. Those results clearly showed the antioxidant property of PPEt. The effect of PPEt at 200 mg/kg body weight was more effective than glibenclamide. Pari and Venkateswaran (2004) further investigated the protective role of *Phaseolus vulgaris* on changes in the fatty acid composition in experimental diabetes. The investigation was carried out to evaluate the effect of *Phaseolus vulgaris* on blood glucose, plasma insulin, cholesterol, triglycerides, free fatty acids, phospholipids, and fatty acid composition of total lipids in liver, kidney, and brain of normal and streptozotocin (STZ) diabetic rats was determined. The results showed that there was a significant increase in tissue cholesterol, triglycerides, free fatty acids, and phospholipids in STZ diabetic rats. The analysis of fatty acids showed that there was a significant increase in the concentrations of palmitic acid (16:1), stearic acid (18:0), and oleic acid (18:1) in liver, kidney, and brain, whereas the concentrations of linolenic acid (18:3) and arachidonic acid (20:4) were significantly decreased. Oral administration of the aqueous extract of *P. vulgaris* pods (200 mg/kg of body weight) for 45 days to diabetic rats decreased the concentrations of lipids and fatty acids, *viz.*, palmitic, stearic, and oleic acids, whereas linolenic and arachidonic acids were elevated. Similarly, the administration of *P. vulgaris* pod extract (PPEt) to normal animals resulted in a significant hypolipidemic effect. These results suggested that PPEt exhibits hypoglycemic and hypolipidemic effects in STZ diabetic rats. That also prevents the fatty acid changes produced during diabetes. The effect of PPEt at 200 mg/kg of body weight was better than that of glibenclamide.

Sabu and Kuttan (2002) have investigated the anti-diabetic activity of medicinal plants and its relationship with their antioxidant property. Methanolic extract (75%) of *Terminalia chebula, Terminalia belerica, Emblica officinalis* and their combination named

'Triphala' (equal proportion of above three plant extracts) were used extensively in Indian system of medicine. They were found to inhibit lipid peroxide formation and to scavenge hydroxyl and superoxide radicals in vitro. The concentration of plant extracts that inhibited 50% of lipid peroxidation induced with Fe (2+)/ascorbate were found to be 85.5, 27, 74 and 69 micro g/ml, respectively. The concentration needed for the inhibition of hydoxyl radical scavenging were 165, 71, 155.5 and 151 ug/ml, and that for superoxide scavenging activity were found to be 20.5, 40.5, 6.5 and 12.5 ug/ml, respectively. Oral administration of the extracts (100 mg/kg body weight) reduced the blood sugar level in normal and in alloxan (120 mg/kg) diabetic rats significantly within 4 h. Continued, daily administration of the drug produced a sustained effect.

Much attention has focused on the role of oxidative stress in the various forms of tissue damage in patients with diabetes. Diabetic rats were treated with 1 g/100 g GSH as a dietary supplement. GSH significantly suppressed the diabetes-induced increase in urinary 8-hydroxy-2'-deoxyguanosine, one of the markers of oxidative stress. It also prevented the diabetes-induced increases in albumin and creatinine in urine. The diabetes-induced increase in the tail flick reaction time to thermal stimuli also was normalized by treatment with dietary GSH. GSH treatment can beneficially affect STZ-induced diabetic rats, with preservation of in vivo renal and neural function. That suggested a potential usefulness of dietary GSH treatment to reduce diabetic (Ueno *et al.*, 2002). Chun-Ching *et al.* (2002) have worked on the antihyperglycaemic and anti-oxidant properties of *Anoectochilus formosanus* in diabetic rats. In their study, they investigated aqueous extracts of *Anoectochilus formosanus* (AFE) for antihyperglycaemic and anti-oxidant effects in diabetic rats induced by streptozotocin (STZ). Diabetic rats were randomly divided into groups and treated orally by gavage with vehicle (distilled water) or AFE (1 and 2 g/kg), once a day for 21 days. 3. At the end of the 21 day period, AFE (2 g/kg) significantly reduced fasting blood glucose, serum fructosamine, triglycerides and total cholesterol compared with vehicle-treated diabetic rats. In vehicle-treated diabetic rats, levels of renal lipid peroxidation were increased, whereas glutathione concentrations were not affected. Renal lipid peroxidation levels were significantly lower and renal reduced glutathione (GSH) concentrations were significantly higher in AFE-treated diabetic rats compared with vehicle-treated diabetic rats. The diabetic kidney in the vehicle-treated group showed a decrease in catalase, but the activity of glutathione peroxidase (GSH-Px) was increased. 4. The activity of catalase, but not GSH-Px, was significantly reversed by

AFE treatment. These results indicate that AFE (1 and 2 g/kg) not only possesses an antihyperglycemic effect, but that it might also reduced oxidative stress in diabetic rats.

Wadood *et al.* (2003) have investigated the effect of *Ficus religiosa* on blood glucose and total lipid levels of normal and alloxan diabetic rabbits. Their study was planned to observe the hypoglycemic effect of the *'Ficus relegiosa'*, a traditional medicinal plant. Normal rabbits were divided into 5 groups (1-5) of six animals each. Group I served as control and received 15 ml of water only. Group 2 received tolbutamide 500-mg/Kg body weight. Group 3-5 received the extract of *Ficus relegiosa* dissolved in 15 ml of water in doses of 200 mg/Kg. 250 mg/Kg and 300 mg/Kg body weight respectively. The diabetic rabbits were also divided in 5 groups on the same pattern. The blood glucose and total lipid levels were estimated before and 1, 2, 3 and 4 hours after the administration of the extract. The extract exerted a significant ($P < 0.05$) hypoglycemic effect in normal rabbits, which was however short lived. The hypoglycemic effect was not significant ($P > 0.05$) in alloxan treated rabbits. The extract had no significant effect ($P > 0.05$) on total lipid levels in normal as well as in alloxan-treated diabetic rabbits. The doses used did not show acute toxicity or result in behavioral changes. From their study it might be concluded that the extract acts by initiating the release of insulin by pancreatic β cells of normal rabbits. Civelek *et al.*, (2004) have made an evaluation of the effects of vitamin E supplementation on oxidative stress in streptozotocin induced diabetic rats. Their experimental study was designed to investigate the effects of vitamin E supplementation, especially on lipid peroxidation and antioxidant status elements 3/4 namely, glutathione (GSH), CuZn superoxide dismutase (CuZn SOD), and glutathione peroxidase (GSH Px), both in blood and liver tissues of streptozotocin (STZ) diabetic rats. The extent to which blood can be used to reflect the oxidative stress of the liver is also investigated. In diabetic rats, plasma lipid peroxide values were not significantly different, from control, whereas erythrocyte CuZn SOD ($p<0.01$), GSH Px ($p<0.001$) activities and plasma vitamin E levels ($p<0.001$), were significantly more elevated than controls. Vitamin E supplementation caused significant decreases of erythrocyte GSH level ($p<0.01$) in control rats and of erythrocyte GSH Px activity ($p<0.05$) in diabetic rats. Liver findings revealed significantly higher lipid peroxide ($p<0.001$) and vitamin E ($p<0.01$) levels and lower GSH ($p<0.001$), CuZn SOD ($p<0.001$) and GSH Px ($p<0.01$) levels in diabetic rats. A decreased hepatic lipid peroxide level ($p<0.01$) and increased vitamin E/lipid peroxide ratio ($p<0.001$) were observed in vitamin E supplemented, diabetic rats. A vitamin E supplementation level which did not cause any increase in the concentration of the vitamin in

the liver or blood was sufficient to lower lipid peroxidation in the liver. Vitamin E/lipid peroxide ratio was suggested as an appropriate index to evaluate the efficiency of vitamin E activity, independent of tissue lipid values. Further, the antioxidant components GSH, GSH Px and CuZn SOD and the relationships among them were affected differently in the liver and blood by diabetes or vitamin E supplementation.

Hyponidd was a herbomineral formulation with antihyperglycaemic and antioxidant potential composed of the extracts of ten medicinal plants (*Momordica charantia, Melia azadirachta, Pterocarpus marsupium, Tinospora cordifolia , Gymnema sylvestre, Enicostemma littorale, Emblica officinalis, Eugenia jambolana, Cassia auriculata* and *Curcuma longa*) . Babu *et al.*, 2004 have investigated hyponidd for its possible antihyperglycaemic and antioxidant effect in diabetic rats. Rats were rendered diabetic by streptozotocin (STZ) (45 mg kg (-1) body weight). Oral administration of hyponidd (100 mg kg (-1) and 200 mg kg (-1)) for 45 days resulted in significant lowered levels of blood glucose and significant increased levels of hepatic glycogen and total haemoglobin. An oral glucose tolerance test was also performed in experimental diabetic rats in which there was a significant improvement in blood glucose tolerance in the rats treated with hyponidd. Hyponidd administration also decreased levels of glycosylated haemoglobin, plasma thiobarbituric acid reactive substances, hydroperoxides, ceruloplasmin and alpha-tocopherol in diabetic rats. Plasma reduced glutathione and vitamin C were significantly elevated by oral administration of hyponidd. The effect of hyponidd at a dose of 200 mg kg (-1) was more effective than glibenclamide (600 microg kg (0^{-1})) in restoring the values to near normal. The results showed that hyponidd exhibits antihyperglycaemic and antioxidant activity in STZ-induced diabetic rats.

Viswanathan *et al.* (2004) reported that the levels of blood glucose, thiobarbituric acid reactive substances (TBARS), hydroperoxides and free fatty acids (FFA) increased in the liver of diabetic animals. The activities of glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) decreased in the liver. Histopathology of pancreas also shows shrunken islets. Supplementation of FA to the diabetic rats resulted in a decrease in the levels of glucose, TBARS, hydroperoxides, FFA and an increase in reduced glutathione (GSH). FA also resulted in increased activities of SOD, CAT, GPx and expansion of pancreatic islets. The effect was much pronounced with lower dose treatment. Thus their study showed that administration of ferulic acid helps in enhancing the antioxidant capacity

of these diabetic animals by neutralizing the free radicals formed thereby reducing the intensity of diabetes.

The antihyperglycaemic and antioxidant activity of *Brassica oleracea* in Streptozotocin diabetic rats have done by Vijaykumar *et al.* (2006). Streptozotocin (STZ 65 mg/kg, i.p) induced diabetic rats were treated with *Brassica oleracea* petroleum ether extract for 60 days. Glucose level was measured in blood and antioxidant enzymes levels viz. superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSHPxase) were measured in erythrocytes. Concentration of glutathione (GSH) and lipid peroxidation product viz. malondialdehyde (MDA) were also measured in blood. Petroleum ether extract of *Brassica oleracea* var. *gongylodes* significantly ($P<0.001$) lowered the STZ induced hyperglycemia. It also produced a significant ($P<0.001$) decrease in peroxidation product viz. MDA. The activity of antioxidant enzymes such as SOD, CAT and GSHPxase were found to be increased in the erythrocytes of diabetic animals treated with the *Brassica oleracea* extract. That confirmed the antihyperglycaemic and antioxidant activity of *Brassica oleracea* var. *gongylodes* in streptozotocin induced diabetic rats.

Chang *et al.* (2006) have investigated the effects of herb extracts, *Rhus verniciflua*, *Agrimonia pilosa*, *Sophora japonica*, and *Paeonia suffruticosa*, on the lowering of blood glucose levels and thiobarbituric acid reactive substances (TBARS) in streptozotocin (STZ)-induced diabetic rats. After 4 weeks, oral administration of *Rhus verniciflua* extract (50 mg/kg) exhibited a significant decrease in blood glucose levels in diabetic rats ($P<0.05$). Blood TBARS concentrations, the products of glucose oxidation in blood, were also lowered by *Rhus verniciflua* extract supplementation. In addition, *Sophora japonica* and *Paeonia suffruticosa* extracts significantly reduced TBARS levels *versus* diabetic controls. Serum concentrations of liver-function marker enzymes, GOT and GPT were also restored by *Rhus verniciflua* (50 mg/kg) supplementation in diabetic rats.

The antidiabetic potential of the alcoholic stem extract of *Coscinium fenestratum* Colebr. (Menispermaceae), a medicinal plant widely used in the traditional Ayurveda and Siddha systems of medicine for the treatment of diabetes mellitus was evaluated in the STZ-nicotinamide induced type 2 diabetic model (Shirwaikar *et al.*, 2005). Graded doses of the alcoholic stem extract were administered to normal and experimental diabetic rats for 12 days. Significant ($p < 0.05$) reduction in fasting blood glucose levels were observed in the normal as well as in the treated diabetic animals. Serum insulin levels were not stimulated in

the animals treated with the extract. In addition, changes in body weight, serum lipid profiles, thiobarbituric acid reactive substance levels, glycosylated hemoglobin and liver glycogen levels assessed in the extract treated diabetic rats were compared with diabetic control and normal animals. Significant results were observed in the estimated parameters, thereby justifying the use of the plant in the indigenous system of medicine.

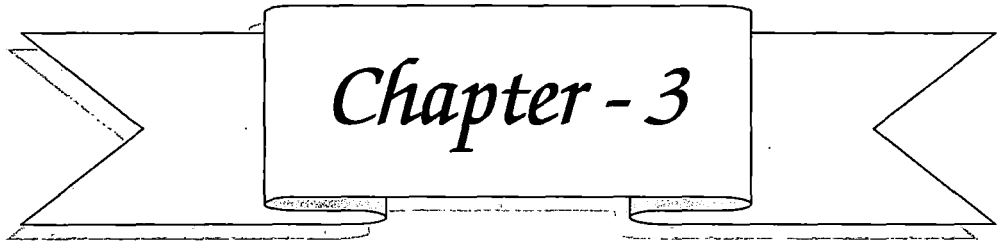
Eun-Mi and Jae-Kwan (2005) studied the effect of some medicinal plants on plasma antioxidant system and lipid levels in rats. Several inflammatory diseases were thought to be related to oxidative injury and free oxygen radicals have been proposed as important causative agents of heart disease and aging. To investigate the effects of daily intake of medicinal plants on antioxidant enzymes, lipid peroxidation and lipid profiles in rat, 28 rats were randomly divided into four groups and administered with three plant extracts (0.2 g/kg body weight): *Piper cubeba* (fruit), *Physalis angulata* (flower), *Rosa hybrida* (flower) and with saline as a control. After 3 weeks, superoxide dismutase (SOD), catalase, thiobarbituric acid reactive substance (TBARS), triglyceride (TG) and cholesterol levels in plasma were measured. The SOD activity of the *Piper cubeba* group and the catalase activity of the *Piper cubeba* and *Rosa hybrida* groups were significantly increased compared with the control group, while the SOD and catalase activities of the *Physalis angulata* group were not significantly changed ($p < 0.05$). TBARS, a marker of lipid peroxidation, was significantly lower in all experimental groups compeered with the control group. No significant changes occurred in the TG, total- and LDL-cholesterol of all groups, but the HDL-cholesterol of the *Physalis angulata* group was significantly increased. That study showed that the intake of medicinal plants in rats results in an increased in antioxidant enzyme activity and HDL-cholesterol, and a decreased in malondialdehyde, which might reduced the risk of inflammatory and heart disease.

Evaluations on the insulin regulation of glutathione and contractile phenotype in diabetic rat ventricular myocytes have done by Shumin *et al.* (2007). Cardiovascular complications of diabetes mellitus involved oxidative stress and profound changes in reduced glutathione (GSH), an essential tripeptide that controls many redox-sensitive cell functions. Their study examined regulation of GSH by insulin to identify mechanisms controlling

cardiac redox state and to define the functional impact of GSH depletion. GSH was measured by fluorescence microscopy in ventricular myocytes isolated from Sprague-Dawley rats made diabetic by streptozotocin, and video and confocal microscopy were used to measure mechanical properties and Ca^{2+} transients, respectively. Spectrophotometric assays of tissue extracts were also done to measure the activities of enzymes that control GSH levels. Four weeks after injection of streptozotocin, mean GSH concentration ([GSH]) in isolated diabetic rat myocytes was 36% less than in control, correlating with decreased activities of two major enzymes regulating GSH levels: glutathione reductase and gamma-glutamylcysteine synthetase. Treatment of diabetic rat myocytes with insulin normalized [GSH] after a delay of 3–4 h. A more rapid but transient upregulation of [GSH] occurred in myocytes treated with dichloroacetate, an activator of pyruvate dehydrogenase. Inhibitor experiments indicated that insulin normalized [GSH] via the pentose pathway and γ-glutamylcysteine synthetase, although the basal activity of glucose-6-phosphate dehydrogenase was not different between diabetic and control hearts. Diabetic rat myocytes were characterized by significant mechanical dysfunction that correlated with diminished and prolonged Ca^{2+} transients. This phenotype was reversed by in vitro treatment with insulin and also by exogenous GSH or *N*-acetylcysteine, a precursor of GSH. Their data suggested that insulin regulates GSH through pathways involving de novo GSH synthesis and reduction of its oxidized form. It is proposed that a key function of glucose metabolism in heart is to supply reducing equivalents required to maintain adequate GSH levels for the redox control of Ca^{2+} handling proteins and contraction.

In a study by Yazdanparast *et al.* (2007), they examined possible protective effect of *Achillea santolina* L. (Compositae) against pancreatic damage in streptozotocin (STZ)-treated diabetic rats. *Achillea santolina* extract (ASE) was used by the traditional healers in many part of Iraq, as a hypoglycaemic agent. They evaluated the effect of ASE on blood glucose level, serum nitric oxide (NO) concentration and the oxidative stress status in rat pancreatic tissue. STZ was injected intraperitonealy at a single dose of 40 mg kg^{-1} to induce diabetes. ASE (0.1 g/kg day) was orally administered to a group of diabetic rats for 30 consecutive days. Results showed significant reduction in the activities of superoxide dismutase (SOD),

catalase (CAT) and pancreatic glutathione (GSH) levels in the diabetic rats compared to the control subjects. On the other hand, blood glucose level, serum NO, malondialdehyde (MDA), a marker of lipid peroxidation, protein oxidation indices including protein carbonyl (PCO) and advanced oxidation protein products (AOPP) were significantly elevated in pancreas of the diabetic group. Treatment with ASE reduced blood glucose level, serum NO, pancreatic MDA, PCO and AOPP. In addition, the content of GSH was restored to the normal level of the control group. Furthermore, ASE significantly increased CAT and SOD activities in ASE-treated rats. Based on their data, it could be concluded that *Achillea santolina* had a high hypoglycaemic activity and that might be attributed to its antioxidative potential.



MATERIALS AND METHODS

3.1. Survey

3.1.1. Study area

The study was conducted in 48 randomly selected villages of 4 blocks of Dakshin Dinajpur district in West Bengal (Plate I). The study area falls within latitude $25^0 10' 5''$N and longitude $89^0 0' 30''$E. It is surrounded by the Malda and Uttar Dinajpur districts of West Bengal from the West and by the neighboring country Bangladesh from the north, south, and east. There are six rivers viz. Atreye, Punorbhaba, Tanjan, Jamuna, Icchhamati, Bramhani and Shree in the district. Total area of the district is 2219 sq km. The villagers of the district are mostly poor, under-developed and neglected.

3.1.2. Procedure

A detailed ethno botanical survey was undertaken following the method of Jain and Goel (1995) in the selected villages of Dakshin Dinajpur, West Bengal during 2005-2006 (Plate II). While collecting information on ethno medicinal plants, information have been gathered from the village chiefs, medicine men (Kabiraj), and even local man and women and cultivators (Plate III). The data were collected through repeated interactions, participatory rural appraisal and semi-structured interviews with the help of elder peoples especially tribal, using a structured questionnaire. According to the interviews from traditional healers and elders living in 48 villages in four blocks of Dakshin Dinajpur the medicinal plants used by tribal were listed with local names, scientific names, family, parts of used, mode of preparations and medicinal uses.

The medicinal plants used by tribals were collected and herbarium specimens were prepared using conventional techniques. The specimens were identified with the help of literature and comparison with authentic specimens at Central National Herbarium (Cal) and North Bengal University Herbarium and the specimens were deposited to the North Bengal University herbarium.

3.1.3. Selection of test plants

From the large list of plants showing some form of medicinal value as obtained from the locals 4 were selected for this study. Out of these four, one was used as vegetable, one as spice and the other two were weeds.

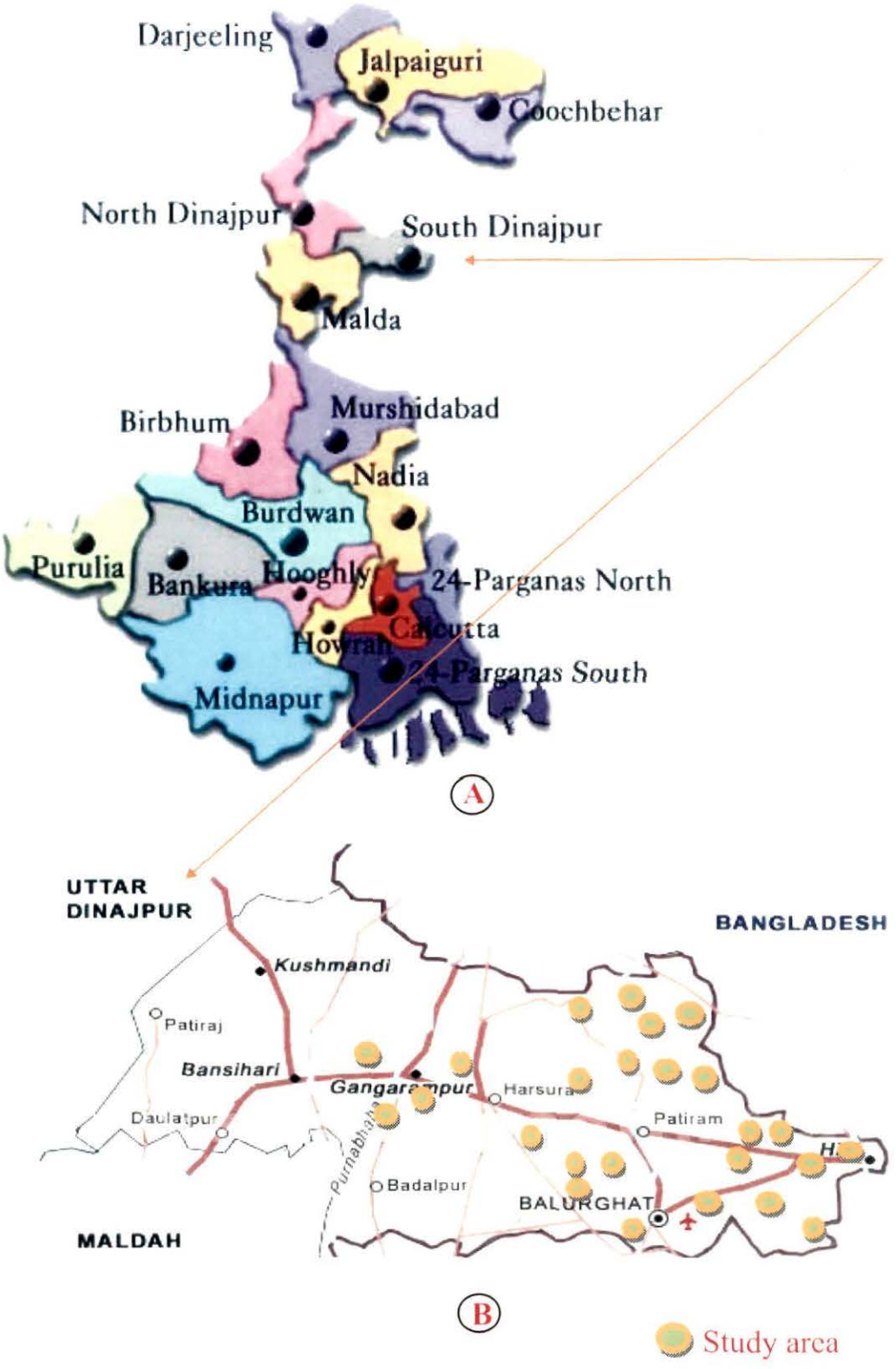


PLATE-I : A: Map of West Bengal; B: Map of Dakshin Dinajpur district (Highlighted portions were study area)

Plate-II : A, B, C, D showing some locality of the study area dominated with medicinal plants

PLATE-III - A: Kabiraj seated in market place for selling of medicinal plants;B and C : Conversation with local people, D: Collection of plant specimens with the help of villager

3.2. Biochemical analysis of test plants

3.2.1. Qualitative analysis

3.2.1.1. Extraction

Leaves of plants were air-dried and ground into uniform powder (Plate IV). The aqueous extract of each sample was prepared by soaking 100g of dried powder samples in 200 ml of distilled water for 12hour. The extracts were filtered using Whatman filter paper No 42 (125mm).

Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

3.2.1.1.1 Alkaloid

Analysis of alkaloids was done by the methods of Kokate *et al* (1995). The extracts were dissolved separately in dilute hydrochloric acid and filtered. The filtrate was tested carefully with Mayer's reagent. Appearance of cream precipitate in response to the reagent indicates the presence of alkaloid.

3.2.1.1.2. Flavonoid

Three methods were used to determine the presence of flavonoids in the plant sample (Sofowara, 1993; Harborne, 1973). 5ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2SO_4. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow colouration was observed indicating the presence of flavonoids.

A portion of the powdered plant sample was in each case heated with 10ml of ethyl acetate over a steam bath for 3min. The mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

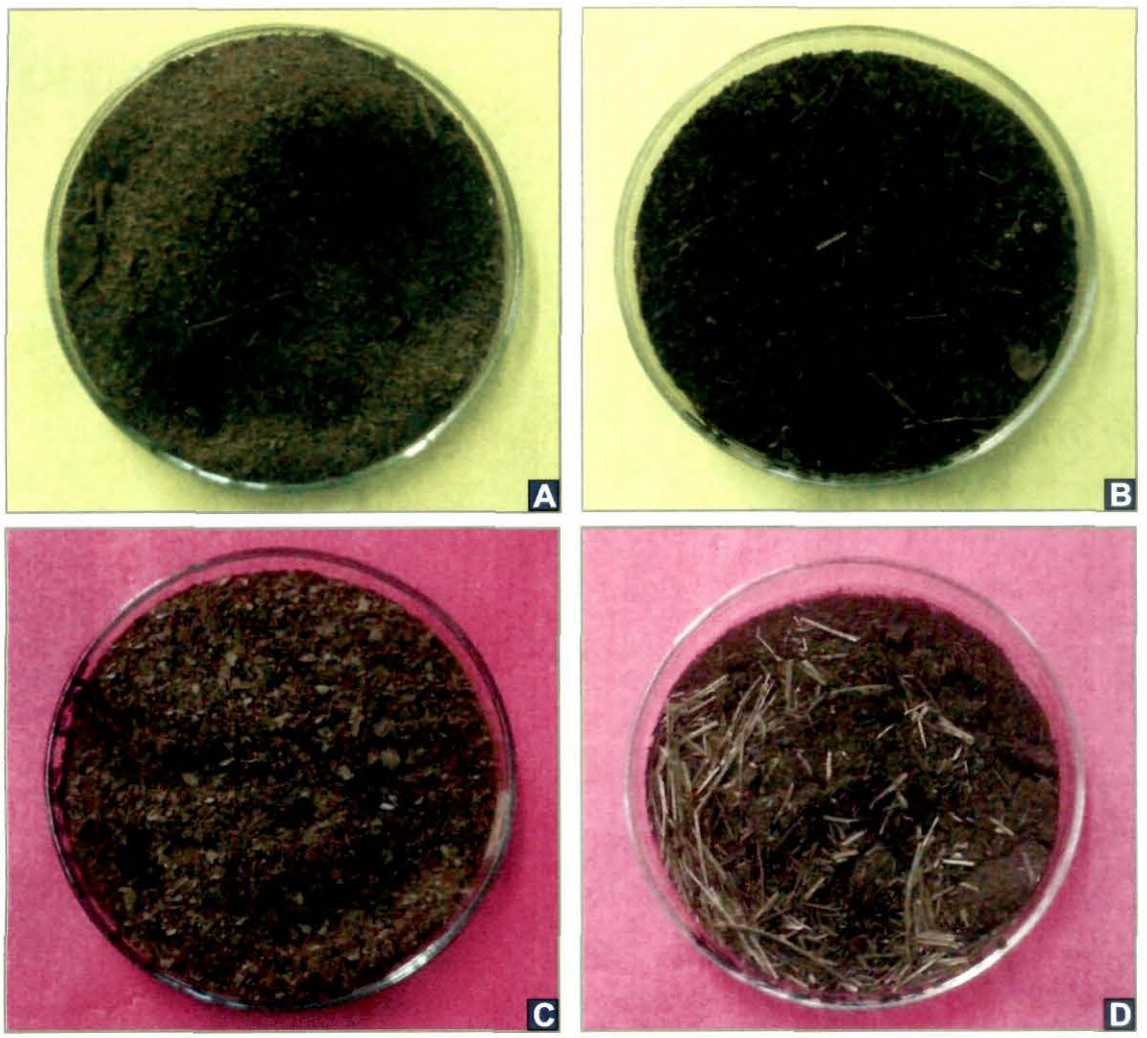


PLATE-IV : Dried leaves of different study plants: A: *Clerodendrum viscosum*, B: *Moringa oleifera*, C: *Cinnamomum tamala*, D: *Scoparia dulcis*

3.2.1.1. 3. Terpenoid (Salkowski test)

Five ml of each extract was mixed in 2ml of chloroform, and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

3.2.1.1.4. Cardiac glycosides (Keller-Killani test)

Five ml of each extracts was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear bellow the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

3.2.1.2. Steroid

Two ml of acetic anhydride was added to 0.5g ethanolic extract of each sample with 2ml H_2SO_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

3.2.1.3. Tannin

About 0.5 g of each dried powdered samples were boiled in 20 ml of water separately in test tubes and filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

3.2.1.4. Saponin

About 2 g of the each four powdered sample was boiled in 20ml of distilled water separately bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for stable persistent froth. The froth was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

3.2.2. Quantitative analysis

3.2.2.1. Alkaloids

Quantification of alkaloid was carried out by the method of Harborne (1973) as follows:

Two g of the leaves of the test plants were defatted with 100ml of diethyl ether using a soxhlet apparatus for 2h. Five g of the each sample was weighed into a 250 ml beaker and allowed to stand for 4h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid, which was dried and weighed.

3.2.2.2. Proteins

3.2.2.2.1. Extraction

Soluble proteins were extracted following the method of Chakraborty *et al.* (1995). Leaf tissues (1g) were homogenized with 0.05M sodium phosphate buffer (pH- 6.8) containing 10mM $Na_2S_2O_5$, 0.5mM $MgCl_2$, 2 mM soluble polyvinyl pyrrolidone (PVPP 10,000 M) and 2 mM Poly methyl Sulphonyl fluoride (PMSF) in mortar with pestle at 4^0C with sea sand and insoluble PVPP. The homogenate was centrifuged at 4^0C for 20 min. at 10,000 r.p.m and the supernatant was used as crude protein extracts and immediately stored at -20^0C for further use.

3.2.2.2.2. Estimation

Protein estimation was done following the method of Lowry *et al.* (1951). To 1 ml of test solution (crude extract at 10^{-2} and different concentrations of standard BSA solution), 5 ml of alkaline reagent was mixed thoroughly. The mixtures were allowed to stand for 15 min at room temperature. Addition of 0.5 ml of Folin-Ciocalteau diluted 1:1 with water was done rapidly. Reaction mixtures were allowed to stand for a further 20 min after mixing well. O.D. values were measured in a colorimeter at 720 nm.

3.2.2.3. Carbohydrates

3.2.2.3.1. Extraction

Carbohydrates were extracted from the fresh plant leaves of test plants following the method of Harbone (1973). Plant parts were weighed and then crushed with 95% ethanol. The alcoholic fractions were evaporated of on a boiling water bath. The aqueous fractions

were centrifuged in a table centrifuge and supernatants were collected. Finally volumes were made up with distilled water.

3.2.2.3.2. Estimation of total sugar

Estimation of total sugar was done by the method of Plummer (1979). To 1ml of each test solution (crude extract at 10^{-2} concentration of glucose), 4ml of anthrone reagent was added and mixed thoroughly. Mixtures were placed in a boiling water bath for 10min.These was then cooled under running tap water. Absorbance was measured at 620nm in a colorimeter and quantification was done using glucose as standard.

3.2.2.3.3. Estimation of reducing sugar

Somogyi Nelson method as described by Plummer (1979) was followed for the estimation of reducing sugar. One ml of alkaline Cu-tartarate solution was added to 1ml of each test solution (crude extract at 10^{-1} concentration or different concentration of standard dextrose). Reaction mixtures were allowed to boil in a water bath for exactly 20 min. These were then cooled under tap water and to each reaction mixtures 1ml of Nelson's Arsenomolybdate reagent were added. Reaction mixtures were diluted to 5ml by adding 2ml of more. Absorbance values were measured in a colorimeter at 540nm using glucose as standard.

3.2.2.4. Phenols

3.2.2.4.1. Extraction

The leaves of the test plants were dried at room temperature and then reduced to coarse powder. In order to prepare the extracts, 20g of the samples were extracted with n-hexane, after stirring for 2 days, and then the extraction solvent was evaporated *in vacuo* at 40^0C (Konyalioglu *et al.*, 2005).

3.2.2.4.2. Estimation

The method used for the determination of total phenols using Folin-Ciocalteau reagent was adapted from McDonald *et al.* (2001). Dried samples and standards were prepared in distilled water. Test solutions (samples and standards) of 0.5 ml were added to 4.0 ml of 1 M Na_2CO_3, 5 ml of Folin-Ciocalteau reagent (1:10, v/v) was added and the solutions allowed to stand at 45^0C for 15 min. Absorbance was measured at 750 nm. The

blank consisted of all reagents and solvents without test compounds or standards. The standard was Gallic acid prepared in concentrations of 50 to 200 mg/L.The phenol concentrations were determined by comparison with the standard calibration curve. Total phenol values were expressed as gallic acid equivalents (mg g^{-1} dry mass).

3.2.2.5. Ascorbic acid

Quantification of Ascorbic Acid was done following the method of Mukherjee and Choudhuri (1983). The leaves of selected plants each were homogenized in a cold mortar placed on ice using 10ml of 6% trichloroacetic acid. To 4.0 ml of the extract, 2.0 ml 2% dinitrophenylhydrazine (in acidic medium) and 1 drop of 10% thiourea (in 70% ethanol) were added. The mixture was then kept in a boiling water bath for 15 min. and after cooling at room temperature 5ml of 80% (v/v) H_2SO_4 was added to the mixture at 0^0C. The absorbance at 530nm was recorded. The concentrations of ascorbate were calculated from a standard curve plotted with known concentration of ascorbic acid.

3.2.2.6. Carotenoid

Carotenoids were extracted and estimated according to the method given by Lichtenthaler (1987). Extractions of carotenoids were done by homogenizing 1g to the plants leaves samples of test plants in methanol. The homogenate were filtered through Whatman No 1 filter paper and the volume was made up. Absorbances of filtrates were determined at 480nm, 645nm and 663nm in a UV-Vis spectrophotometer (DIGISPEC- 200 GL) and the carotenoid content was calculated by using the formula:

Carotenoid = $A_{480} - (0.114 * A_{663}) - 0.638\ (A_{645})$ ug/g fresh weight.

3.2.3. Analysis of protein pattern

Total soluble proteins extracted in 0.05M sodium phosphate buffer were used as crude protein extract for analysis of protein pattern by SDS-PAGE. Analysis was carried out on 10% SDS-PAGE gels following the method of Sambrook *et al.* (1989). Protein samples were loaded on the gel and separated for 3 hour at 200 V and 15-20 mA current. Following electrophoresis the gel was fixed, stained in Coomassie Brilliant Blue (R-250) solution & finally destained in a solution of methanol, glacial acetic acid and water (4.5:4.5:1).

3.2.3.1. Preparation of stock solution

For the preparation of gel the following stock solutions were prepared.

A) Acrylamide and N'N'-methylene bis acrylamide

A stock solution containing 29% acrylamide and 1% biacrylamide was prepared in water. As both of this amide is slowly deaminated to acrylic and bis acrylic acid by alkali and light the pH of the solution was kept below 7.0. The stock solution was then filtered through Whatman No 1 filter paper, kept in brown bottle, stored at 4^0C and used within one month.

B) Sodium Dodecyl Sulphate (SDS)

A 10% stock solution of SDS was prepared in warm water and stored at room temperature.

C) Tris buffer

i) 1.5 M Tris buffer was prepared for resolving gel. The pH of the buffer was adjusted to 8.8 with concentrated HCl and stored at 4^0C for use.

ii) 1.0 M Tris buffer was prepared for use in stacking and loading buffer. The pH of this buffer was adjusted to 6.8 with concentrated HCl and stored at 4^0C for use.

D) Ammonium Persulphate (APS)

Fresh 10% APS solution was prepared with distilled water each time before use.

E) Tris-Glycine electrophoresis buffer

Tris running buffer consists of 25mM Tris base, 250mM Glycine (pH 8.3) and 0.1% SDS. 10 X solution was made by dissolving 3.02g Tris base, 18.8g Glycine and 10ml of 10% SDS in 1L of distilled water.

F) SDS gel loading buffer

Tris buffer contains 50mM Tris-HCl (pH 6.8), 10mM β-mercaptoethanol, 2% SDS, 0.1% bromophenol blue, 10% glycerol. 10X solution was prepared by dissolving 0.5ml of 1M Tris buffer (pH 6.8), 0.5ml of 14.4M β-mercaptoethanol, 2ml of 10% SDS, 10mg bromophenol blue, 1ml glycerol in 6.8ml of distilled water.

3.2.3.2. Preparation of gel

Mini Slab gel (Plate size 8 cm X 10 cm) was prepared for the analysis of protein patterns by SDS-PAGE. For gel preparation two glass plates were thoroughly cleaned with dehydrated alcohol to remove any traces of grease and then dried. Then 1.5 mm thick spacers were placed between the glass plates at three sides and two slides were sealed with high

vacuum grease and clipped tightly to prevent any leakage of the gel solution during pouring. Resolving and stacking gels were prepared by mixing compounds in the following order and poured.

Composition of solutions

10% resolving gel

Solutions	Amount (ml)
Distilled water	2.85
30% acrylamide	2.55
1.5 M Tris (pH 8.8)	1.95
10% SDS	0.075
10% APS	0.075
TEMED	0.003

5% stacking gel

Solutions	Amount (ml)
Distilled water	2.10
30% acrylamide	0.50
1.5 M Tris (pH 6.8)	0.38
10% SDS	0.030
10% APS	0.030
TEMED	0.003

After pouring the resolving gel solution, it was immediately over layered with isobutanol and kept for polymerization for 2 hrs. After polymerization of the resolving gel was complete, overlay was poured off and washed with water to remove any unpolymerized acrylamide, stacking gel solution was poured over the resolving gel, and the comb was inserted immediately and over layered with water. Finally the gel was kept for polymerization for 30-45 min. After polymerization of the stacking gel the comb was removed and washed thoroughly. The gel was then finally mounted in the electrophoresis apparatus. Tris-Glycine buffer was added sufficiently in both upper and lower reservoir. Any bubble trapped at the bottom of the gel was removed carefully with a bent syringe.

3.2.3.3. Sample preparation

Sample (50µl) was prepared by mixing the sample protein (35 µl) with1X SDS gel loading buffer (15 µl) in cyclomixer. All the samples were floated in boiling water bath for 3 min to denature the protein sample. The samples were immediately loaded in a pre-determined order into the bottom of the wells with a micro liter syringe. Along with the samples, protein markers consisting of a mixture of Phosphorylase b, Bovine Serum Albumin, Ovalbumin, Carbonic Anhydrase, Soyabean Trypsin inhibitor and Lysozyme proteins ranging from high to low molecular masses (97400, 68000, 43000, 29000, 20000, 14300 Dalton) was treated as the other samples and loaded in separate well.

3.2.3.4. Electrophoresis

Electrophoresis was performed at a constant 15 mA current for a period of three hours until the dye front reached the bottom of the gel.

3.2.3.5. Fixing and staining

After electrophoresis the gel was removed carefully from the glass plates and then the stacking gel was cut off from the resolving gel and finally fixed in glacial acetic acid: methanol: water (10:20:70) for overnight. The gel was removed from the fixer and stained in coomassie blue stain for 4 h at 37^0C with constant shaking at low speed. The staining solution was prepared by dissolving 250 mg of coomassie brilliant blue (Sigma R 250) in 45 ml of methanol. After the stain was completely dissolved, 45 ml of water and 10 ml of glacial acetic acid were added. The prepared stain was filtered through Whatman No 1 filter paper.

After staining the gel was finally destained with destainig solution containing methanol, water and acetic acid (4.5:4.5:1) at 4^0C with constant shaking until the background become clear.

3.2.4. Detection of α-tocopherol

3.2.4.1. Extraction

The leaves of the selected plants were dried at room temperature and then reduced to coarse powder. In order to prepare the extracts, 20g of the samples were extracted with n-hexane, after stirring for 2 days, and then the extraction solvent was evaporated *in vacuo* at 40^0C (Konyalioglu *et al.*, 2005).

3.2.4.2. TLC

Silica gel plates (5715) plates were activated at 100^0C for 10 minutes. The extract and the pure standard dissolved in methanol were subjected to TLC using a mixture of cyclohexane: diethyl ether (4:1) as mobile phase. The mobile phase was allowed to run a distance of 100mm in the saturated tank. The developed plate was left to dry at room temperature and oven dried for 15 min at 100^0C. The plate was sprayed with 10% $CuSo_4$-phosphoric acid followed by charring at 190^0C for 10min. α-tocopherol gave a black spot. α-tocopherol was identified in the extracts by comparison of the Rf (0.53) value with that of corresponding pure standard (Konyalioglu *et al.*, 2005).

3.2.5. Antioxidant activity

3.2.5.1. Extraction

The leaves of the test plants were dried at room temperature and then reduced to coarse powder. In order to prepare the extracts, 20g of the samples were extracted with n-hexane, after stirring for 2 days, and then the extraction solvent was evaporated *in vacuo* at 40^0C (Konyalioglu *et al.*, 2005).

3.2.5.2. Estimation

The spectrophotometric assay for the quantitative determination of antioxidant capacity (TAC) was carried out essentially as described by Prieto *et al.* (1999). The assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate/ Mo (V) complex at acidic pH. An aliquot of 0.1 ml of extracts (1mg/ml) was combined in an Ependroff tube with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95^0C for 90 minutes. After the samples had cooled to room temperature, the absorbance of the aqueous solution was measured at 695 nm against a blank. TAC was determined by comparison with the α-tocopherol acetate standard calibration curve. The amount of TAC was expressed for extract samples in mM α-tocopherol acetate equivalent/ g dry mass.

3.2.6. Partial characterization of active principles

3.2.6.1 Solvent extraction

All the leaves were separately oven dried at 60°C and powdered. For solvent extraction, 1g each of the powdered leaves was extracted separately with 4 solvents of increasing polarity- methanol, ethyl acetate, diethyl ether and petroleum ether. After extraction for 3 times, extracts were filtered, filtrates were evaporated to dryness and finally dissolved in 5 ml of methanol. These were then used for UV-Spectrophotometric analysis and bioassays.

3.2.6.2. UV-Spectrophotometry

The antifungal compounds were analyzed in UV-Spectrophotometer (Systronics) at a range of 200 to 400 nm and maximum absorption was determined.

3.3. Bio-assay of extracts

3.3.1. Spore germination test

Spore germination bioassay was performed following the method of Trivedi and Sinha (1976). A drop (10 µl) of crude extract was placed at the two ends of each clean grease free slide and the extract was allowed to dry. A drop of spore suspension of the test fungus was added to the crude extract drop. Similarly, two other slides were prepared for the control: one with sterile distilled water and other in methanol. The latter was air dried as the crude extract and a drop of spore suspension of the same test fungus was added to them. The slides were kept on glass rods in moist sterilized petridishes and incubated for 24 hour. Little quantity (5ml) of sterile distilled water was poured in each petridish to avoid drying out spots on the glass slides. Both germinated and ungerminated spores were stained with cotton blue in lacto phenol after 24 hour of incubation and observed under microscope. Percentage of germination was calculated.

3.3.2. Agar cup bio-assay

The medium (PDA, 20 ml/petridish) were seeded with 1 ml of spore suspension of the test pathogen. After solidification the plates were chilled for 30 minutes, and then bored with sterile cork-borer (8 mm diameter). Test solution (0.1 ml) was added to each cup and incubated at 30^0C for desired period. Diameters of inhibition zones were recorded.

3.4. Anti- hyperglycemic studies

3.4.1. Preparation of plant extracts

Leaves of test plants (each 500g) were extracted separately with 1.5 L of water by the method of continuous hot extraction at 60^0C for 6 h and evaporated. The residual extract was dissolved in water and used in the study (Jain, 1968).

3.4.2. Drugs and chemicals

All the drugs and biochemical used in this experiment were purchased from the Himedia Laboratory, Mumbai, India and the chemicals were of analytical grade.

3.4.3. Animals

Male Wister albino rats (180-200g) were obtained from Ghosh Enterprise, Kolkata. The animals were grouped and housed in polypropylene cages (Plate V) and maintained under standard laboratory conditions (temperature 25±2^{0}C) with a 12-h/12-h dark and light cycle (Niyonzima and Vllietinck, 1993) (Plate VI). All animals were maintained on a standard laboratory diet and tap water and had free access to food and water (Plate VII).

3.4.3.1. Permission

All procedures described were reviewed and approved by the University Animals Ethical Committee (NBU).

3.4.4. Induction of experimental diabetes

A freshly prepared solution of Streptozotocin (55mg/kg, i.p.) in 0.1M citrate buffer, pH 4.5, was injected intra-peritoneally in the rats in a volume of 1ml/kg (Siddique *et al.*, 1987) (Plate VIII). After 48 h of streptozotocin administration, rats with moderate diabetes having glycosuria and hyperglycemia (i.e., with blood glucose of 200-300mg/dl) were taken for the experiment.

3.4.5. Treatment

In the experiment, a total of 36 rats (28 diabetic surviving rats, 8 normal rats) were used. The rats were divided into 9 groups of 4 rats each. Group-1 and Group-2 treated as control, Group-3 treated as diabetic control. After 48 h of STZ induction diabetic rats treated with the extracts of the leaves of study plants were grouped into Group-4 to Group-9 (Plate IX)

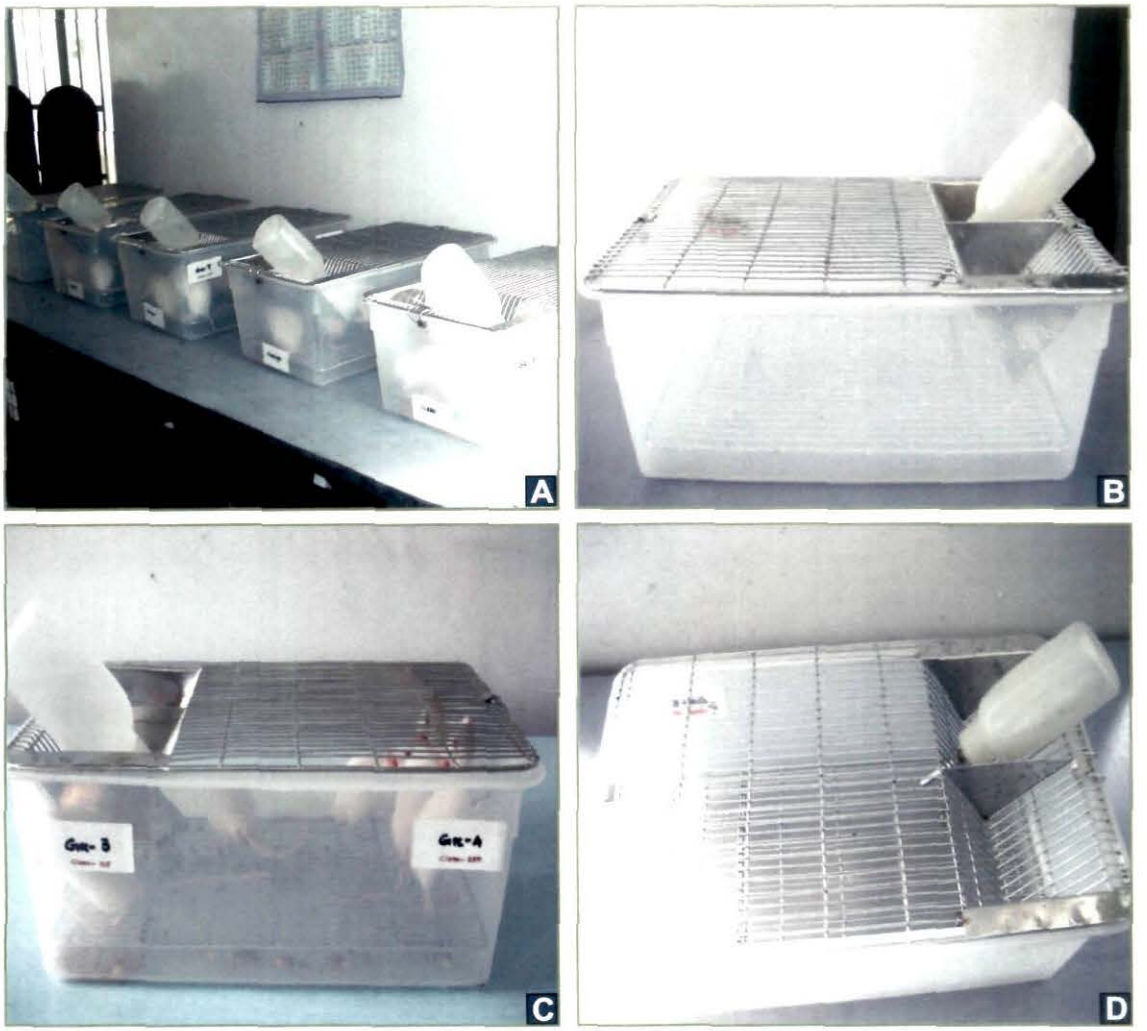


PLATE-V : A, B, C, and D: Polypropylene cages for housing of rats with drinking water facilities (The animals were grouped and housed in polypropylene cages and maintained under standard laboratory conditions (temperature 25±2^{0}C) with a 12-h/12-h dark and light c)

PLATE- VI : A, B, C: Grouping of animals for different treatment, B and D: Marking of animal(s)

PLATE-VII : A and B: Feeding of proper diet, C and D: Animals drinking water.

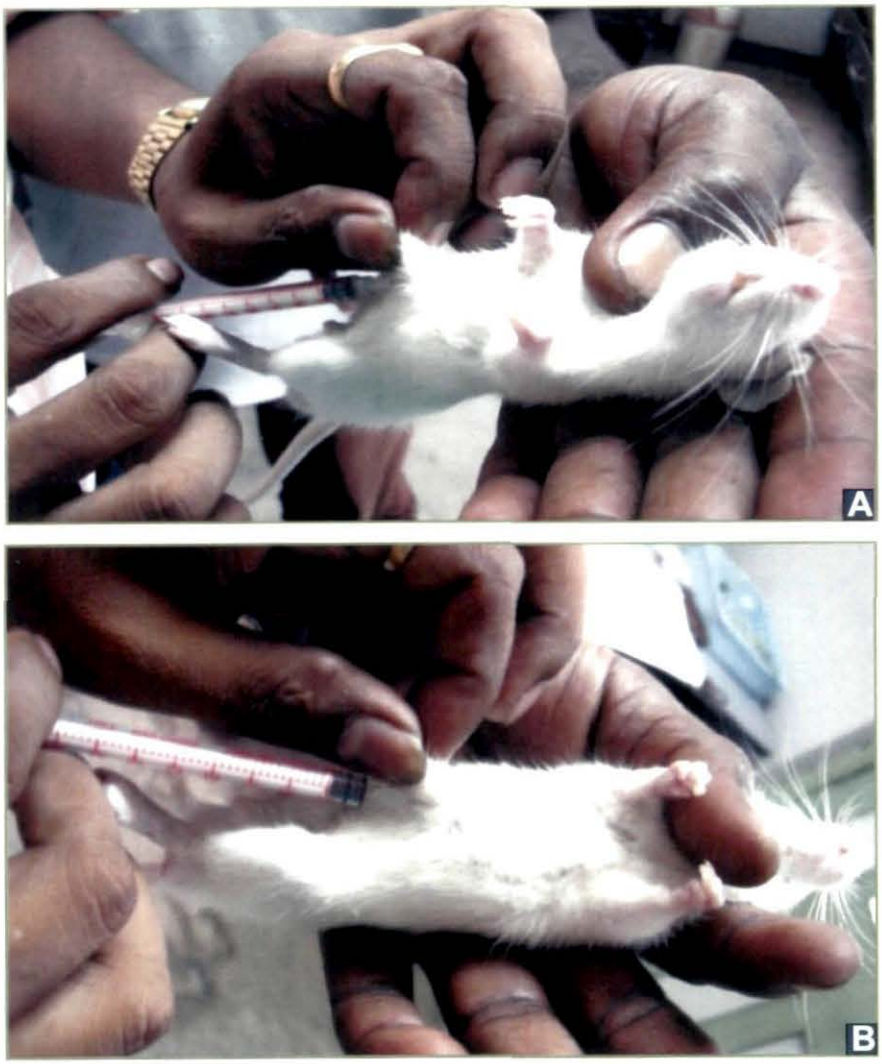


PLATE-VIII: A and B: Induction of STZ to animal (A freshly prepared solution of Streptozotocin (55mg/kg, i.p.) in 0.1M citrate buffer, pH 4.5, were injected intraperitoneally in a volume of 1ml/kg)

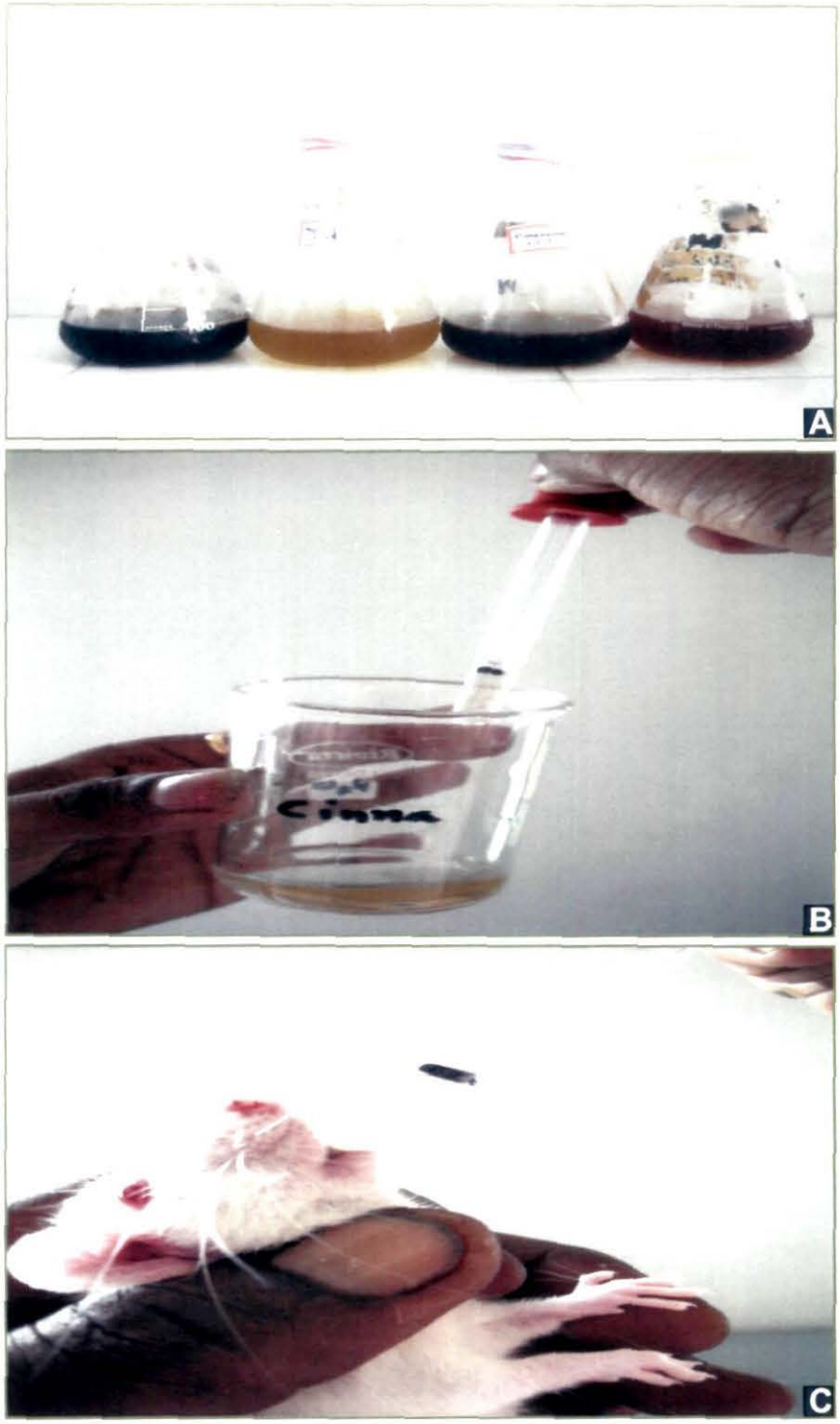


PLATE-IX : A and B: Different extracts of fresh leaves (Leaves of study plants (each 500g) were extracted with 1500 ml of water by the method of continuous hot extraction at 60^{0}C for 6 h and evaporated. The residual extract was dissolved in water and used in the study), C: Feeding of extract

Group-1: Control untreated rats receiving distilled water.

Group-2: Control rats receiving 0.1M citrate buffer (pH 4.5).

Group-3: STZ treated diabetic rats.

Group-4: STZ treated diabetic rats treated with *Moringa oleifera* leaf extract (125mg/kg body weight) in dist water using intragastric tube twice a day for 20 days.

Group-5: STZ treated diabetic rats treated with *Moringa oleifera* leaf extract (250mg/kg body weight) in dist water using intragastric tube twice a day for 20 days.

Group-6: STZ treated diabetic rats treated with *Cinnamomum tamala* leaf extract (125mg/kg body weight) in dist water using intragastric tube twice a day for 20 days.

Group-7: STZ treated diabetic rats treated with *Cinnamomum tamala* leaf extract (250mg/kg body weight) in dist water using intragastric tube twice a day for 20 days

Group-8: STZ treated diabetic rats treated with *Scoparia dulcis* leaf extract (125mg/kg body weight) in dist water using intragastric tube twice a day for 20 days.

Group-9: STZ treated diabetic rats treated with *Scoparia dulcis* leaf extract (250mg/kg body weight) in dist water using intragastric tube twice a day for 20 days

The body weight gain, fasting blood glucose and urine sugar of all the rats were determined at regular intervals during experimental period.

After 20 days, all the rats were fasted overnight and sacrificed by cervical decapitation (Plate X). Blood was collected in tubes containing sodium fluoride for the estimation of fasting blood glucose. Livers were removed immediately, rinsed in ice chilled normal saline and patted dry and weighed.

3.4.6. Analytical procedure

3.4.6.1. Measurement of body weight

The body weight of all the rats were taken at regular intervals during the experimental period (1st day and after 2 days, 10 days and 20 days of STZ induction).

3.4.6.2. Collection of urine

Urine was collected from the treated and untreated albino rats after 24 h of fasting. Collection was done in glass tube and brought to laboratory and analyzed.

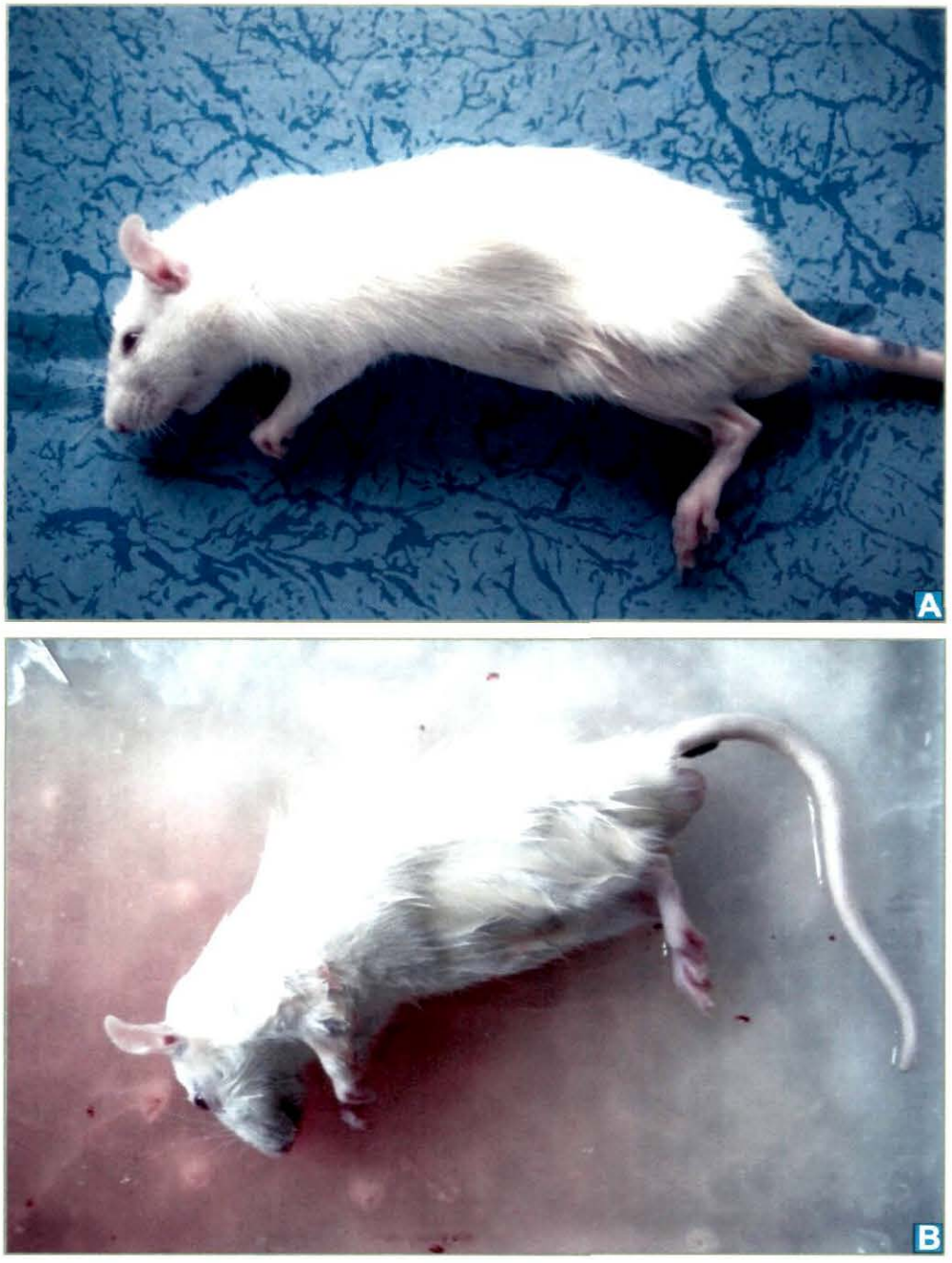


PLATE X - A and B: Decapitation of animals for collection of liver (after fasting overnight).

3.4.6.3. Collection of blood

Blood was collected into glass tubes containing 10mg sodium fluoride per ml blood and was processed immediately after collection (Plate-XI).

3.4.6.4. Collection of liver

The liver was removed quickly, cleaned and washed twice with phosphate buffered saline (PBS, pH 7.4) and immediately processed for the estimation of Lipid Peroxidation (LPO). Another portion of liver immediately put into a weight stopper test tube containing 30% KOH for glycogen estimation (Plate-XII).

3.4.7. Biochemical studies

3.4.7.1. Qualitative determination of urine sugar

Glucose was detected in the urine by the method of Benedict *et al.*, 1908. Benedict's reagent (5ml) was taken in a test tube and 8 drops of urine was added to it. Tubes were boiled for 1-2 min and then cooled slowly. The solutions were filled with greenish/yellow/red and no precipitate depending upon the quantity of glucose present. Greenish precipitate would indicate very small amount of glucose. The solution remained clear where no glucose was there.

3.4.7.2. Estimation of blood glucose

Fasting blood glucose was quantified by the method of Nelson and Somogyi (1944, 1945). Into a test tube containing 3.5 ml water was introduced 0.1 ml blood and mixed well. To the tube 0.2 ml of 0.3N barium hydroxide added. Mixture turned brown and then 0.2 ml zinc sulphate was added and mixed. After 15 minutes mixture was filtered through Whatman No 1 filter paper. Into two separate test tubes 1 ml of aliquot of the filtrate was transferred and 1 ml alkaline copper reagent was added. The tubes were covered with a glass marble and placed in water bath for 20 min. Tubes were cooled under running water. 1 ml of arsenomolybdate reagent was added. Solution diluted to 25 ml. Standard and reagent blanks were prepared similarly. Absorbances were read at 500 nm.

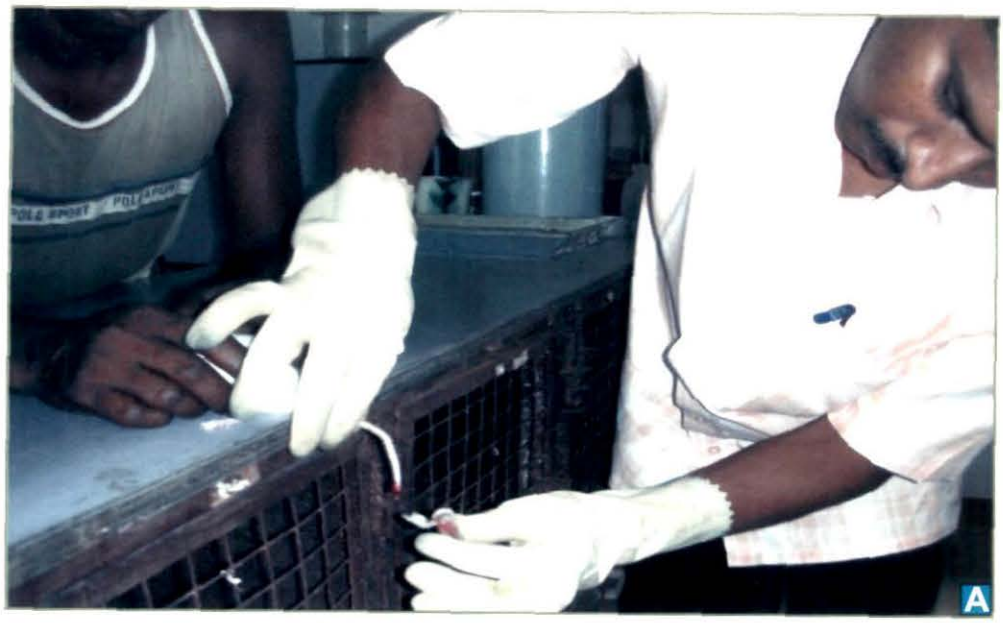


PLATE XI - A and B: *Collection of Blood (Blood was collected into* tubes containing 10 mg sodium fluoride per ml blood at regular intervals).

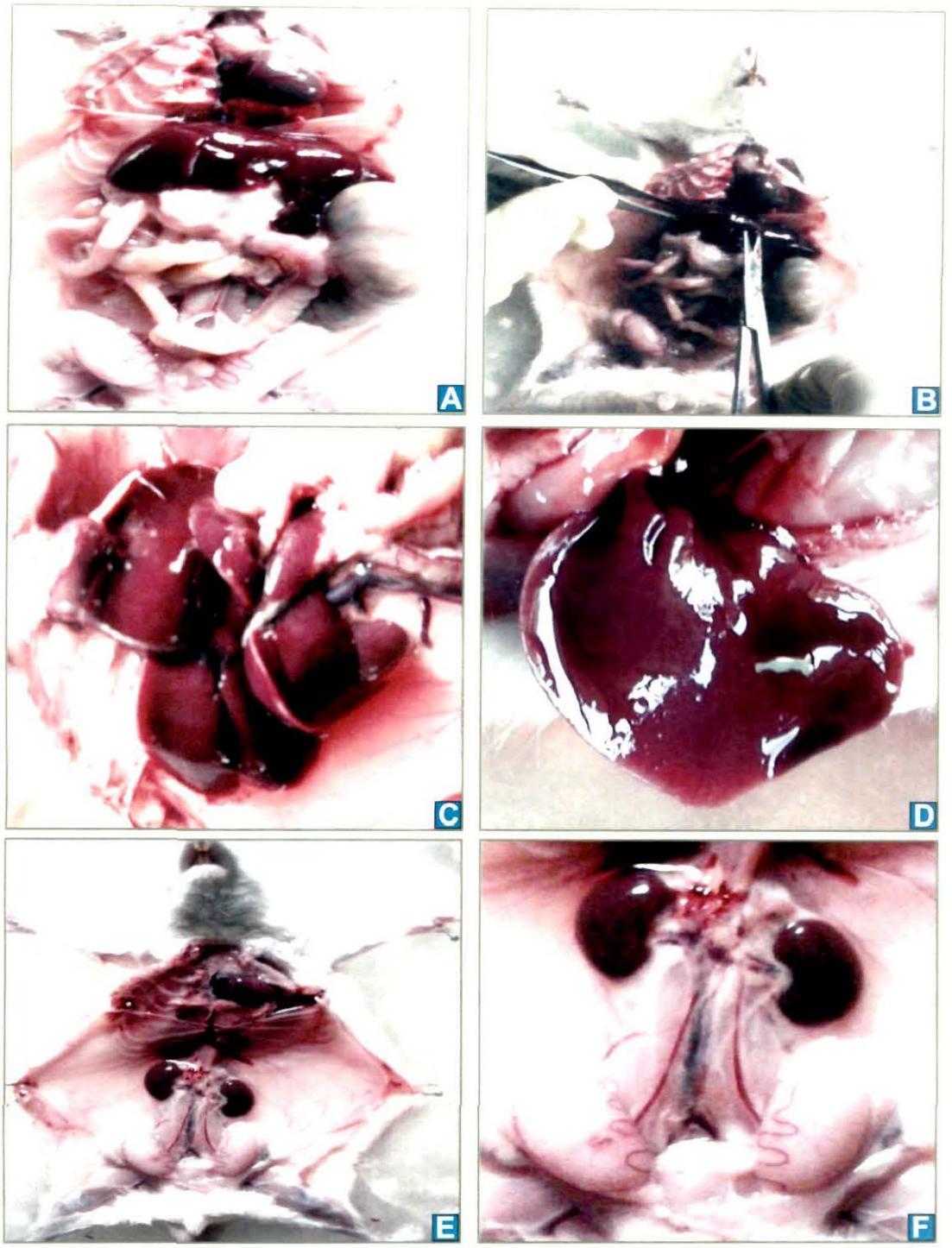


PLATE XII : Dissection of animals: A: Elementary system, B: Collection of liver (After 20 days, all the rats were fasted overnight and sacrificed by cervical decapitation. The liver was removed with phosphate buffered saline (PBS, pH 7.4) and immediately processed for the estimation of Lipid Peroxidation (LPO). Another portion of liver immediately put into a weight stopper test tube containing 30% KOH for glycogen estimation.), C and D: Enlarged liver of plant extract treated animals, E: Renal system, F: Enlarge kidney of plant extract treated animals.

3.4.7.3. Quantitative estimation of glycogen

Glycogen was hydrolyzed to glucose by the method of Raghuramula *et al.* (2003) and the glucose thus formed was estimated by Nelson and Somogyi's method (1944, 1945).

The liver was taken out rapidly from the animal and the excess blood removed by blotting between folds and filter paper and immediately put into a weight stopper test tube containing 30% KOH and weight again. The amount of alkali was then adjusted to get 2ml per g of liver. The tissue was digested in a boiling water bath for 1 hr. The filtrate was cooled in ice cold water. Two volumes of 95% ethanol were then added and the mixture heated just to boiling. Spurting was avoided. This was left to stand overnight in the cold. The tubes were centrifuged and the precipitate dissolved in 5-10 ml warm water. The glycogen was re-precipitated with 2 volumes of 95% ethanol. The precipitate was centrifuged and washed several times with 60% ethanol. Two ml of 2 N H_2SO_4 per g of initial liver weights was added and hydrolyzed in a boiling water bath for 3-4 h. The solution was neutralized with NaOH using Phenol red as indicator. Volume was noted and filtered. Glucose was determined in that aliquot. The factor 0.93 used to convert glucose to glycogen.

3.4.7.4. Determination of Thiobarbituric acid reactive sub-stances (TBARS)

TBARS in tissues was estimated by the method of Ohkawa *et al.* (1979). After collection of blood samples the rats were killed and livers were excised, rinsed in ice cold normal saline, followed by 0.15 M Tris-HCl (pH 7.4) blotted dry and weight. A 10% w/v of homogenate was prepared in 0.15 M Tris-HCl buffer and processed for the estimation of lipid peroxidation.

To 0.5ml tissue homogenate, 0.5ml saline and 1.0ml 10% TCA were added, mixed well and centrifuged at 3000 rpm for 20 min. To 1.0 ml of the protein free supernatant, 0.25 ml of thiobarbituric acid (TBA) reagent was added; the contents were mixed and heated for 1h at 95^0C. The tubes were cooled to room temperature under running water and absorbance measured at 532 nm. The levels of lipid peroxides were expressed as no spaces moles of thiobarbatituric acid reactive substances (TBARS)/mg protein.

3.4.7.5. Determination of reduced glutathione (GSH)

GSH in tissues was estimated by the method of Ellman *et al.* (1959). After killing the rats livers were excised, rinsed in ice cold normal saline, followed by 0.15 M Tris-HCl (pH

7.4) blotted dry and weighed. A part of homogenate after precipitating proteins with Trichloro no space acetic acid (TCA) was used for estimation of glutathione.

An aliquot of 50 ul sample was mixed with 1.7 ml of disodium hydrogen phosphate solution (0.3 M). The final volume of 2 ml was made by adding 250 µl of DTNB reagent [4 mg, 5, 5- dithiobis (2-nitrobenzoic acid) in 10 ml of 1% (w/v) sodium citrate]. The absorbance of the sample was measured at 412 nm. GSH solution of known concentrations (10-50 ug) was simultaneously processed to prepare a standard curve. The amount of GSH in the sample was calculated using the standard curve generated from known GSH.

3.5. Statistical analysis

The collected data were subjected to statistical analysis by standard procedures of Standard Error, CD, Student's 't' test and Pearson's correlation.

EXPERIMENTAL

4.1. Survey of Dakshin Dinjapur district for information on uses of plants in local medicine

The present investigation has been undertaken to know the traditional knowledge about the uses of medicinal plants, generally predominant in the selected areas of Dakshin Dinajpur district. The investigation has carried out on the 48 villages of 4 blocks of Dakshin Dinajpur district. The villagers of Dakshin Dinajpur district depends mainly on cultivation. The information on scientific name, local name of the plant part used to cure and method of dosage has been provided (Table 1). The herbarium specimens of the plants with voucher specimen number have been deposited in the North Bengal University herbarium.

The data on medicinal plants, which was collected from inhabitants in and around the villages in the Dakshin Dinajpur district, were pooled and analyzed. The present course of investigations has revealed the usage of 107 medicinal plant species used by the villagers, especially Santal, Munda tribes from the Dakshin Dinajpur district of West Bengal. The investigation revealed the medicinal plants of 107 species and 96 genera belonging to 48 families, which are commonly used for the various diseases by various tribes (Santal, Munda, etc.) of the selected area (Table 2, Fig I and plates XIII to XVI).

According to the information gained from villagers, the medicinal plants used by them for kidney and other urinary problems are *Kalanchoe pinnata, Tribulus terretris, Saraca indica, Abroma augusta.* Jaundice is treated with the plants, *Achyranthes aspera, Andrographis paniculata, Swertia chirata., Cajanus indicus, Saccharum officinarum, Piper betle* etc.

For the treatment of dysentry the plants used by the villagers are *Coccinia cordifolia, Enhydra fluctuans, Clerodendrum viscosum, Punica granatum, Syzygium cumini, Tamarindus indica, Aegle marmelos, Kalanchoe pinnata, Acacia arabica, Centratherum anthelminticum., Cynodon dactylon., Piper betle, Psidium guyava, Centela asiatica, Lannea grandis, Murraya paniculata, Pterocarpus santalinus, Basella alba, Averrhoe carambola, Paederia foetida., Glycyrrhiza glabra* etc.

Table 1: Plants used for the treatment of various diseases in the villages of Dakshin Dinajpur, West Bengal

Sl. No.	Scientific name	Local name	Parts used	Uses
1	*Acacia arabica.* Wild. Leguminaceae	Babla	Fruit, bark, gum	Dysentery, stomach/ liver problem, gallbladder infection, reduces urine sugar, mail sterility, mumps, ulcer
2	*Achyranthes bidentata Blume* Amaranthaceae	Apang, Chatchatia	Root, leaf, plant body	Joining of bones, rheumatism, reduces urine sugar, mail sterility, piles, periodic problem, hum, urinary track infection
3	*Adhatoda vasica* Acantheceae	Basak	Leaf, root, flower, stem, bark	Cold-cough, fever, asthma
4	*Aegle marmelos* Rutaceae	Bel	Leaf, root, bark, fruit,	Reduces blood sugar, dysentery, stomach/ liver problem
5	*Allium sativum. Linn.* Liliaceae	Rasun	Bulbils, clove	Rheumatism,
6	*Aloe Barbadensis* Mill.(=*Aloe vera* Linn.) Burm.f Liliaceae	Ghritokumari, Kumari	Leaf	Headache, dysentery, reduces urine sugar, mail sterility
7	*Alstonia scholaris.* R.Br. Apocynaceae	Chhatim	Bark	Reduces urine sugar, mail sterility,
8	*Andrographis paniculata* Nees. Acanthaceae	Kalmegh	Leaf, whole plant	Fever, worm, stomach/ liver problem
9	*Asparagus racemosus.* Wild. Liliaceae	Satamuli/ Satavari	Root, leaf	Gallbladder infection, blood purifier, reduces urine sugar, mail sterility
10	*Averrhoe carambola* Linn. Oxalidaceae	Kamranga	Root, leaf, fruit, Bark	Diarrhea, fever, impotency, asthma
11	*Bacopa monniera* (Linn) Pennell. Scrophulariaceae	Brahmi	Whole plant, root, leaf	Memory increases, nervous disorder,
12	*Basella alba* Linn. Basellaceae	Pui	Leaf, stem	Tumor, white dysentery, blood dysentery
13	*Blumea lacera.* De. Asteraceae	Kukursoka	Whole plant	Stomach/ liver problem, loss of appetite
14	*Boerhavia difusa.* Linn. Ficoidaceae	Purnanaba	Leaf	Rheumatism, urinary track infection

Sl. No.	Scientific name	Local name	Parts used	Uses
15	*Borassus flabellifer.* Linn. Palmae	Tal	Leaf bud, fruit, endosperm	Insomnia, gonorrhea, leucorrhea, acidity, piles,
16	*Butea monosperma* (L) Tanb *B. Frondosa* Koen ex Roxb Pappilionaceae	Palash	Leaf, bark, flower, gum, seed	Skin care, urinary problem, worm, piles
17	*Cajanus cajan* (L.) Huth. Leguminaceae	Arhar	Leaf, root, seed	Reduces blood sugar, jaundice, dyspepsia, cough
18	*Calotropies procera* Asclepedaceae	Akanda	Leaf, flower, root, dried bark and gum	Cold-cough, rheumatism, piles, snake bite, asthma, headache
19	*Cariandrum sativum.* Linn. Apiaceae	Dhane	Plant body, leaf, fruit	Gallbladder infection, bile
20	*Cassia sophera* Ceasalpinaceae	Kalkasunde	Leaf, entire plant	Stomach/ liver problem, mail sexual problem
21	*Catheranthus roseus* Apocynaceae	Nayantara	Leaf	Reduces blood sugar, cancer, leukemia, hypertensive, antispasmodic.
22	*Hydrocotyle asiatica* (Linn) Urban. Apiaceae	Thankuni	Pod, leaf	Dysentery, anti-inflammatory, jaundice, diuretic, diarrhea.
23	*Centratherum anthelminticum.* Wild. Asteraceae	Somraj	Leaf, seed	Dysentery, stomach/ liver problem, Cold-cough, worm,
25	*Cinnamomum tamala* (Hamilton) Nees and Ebermaier. Lauraceae	Tejpata	Leaf, bark	Reduces blood sugar, cold-cough, memory decrease, quench thirst, skin care,
26	*Cissus quadrangularis.* Linn. Vitaceae	Harjora	Whole plant, leaf	Joining of bones, rheumatism, piles, periodic problem, worm
27	*Clerodendrum viscosum* Vent. Verbenaceae	Bhati, Ghetu	Leaf, bud, root, Whole plant	Malarial fever, any fever, worm, dysentery, stomach/ liver problem, piles
28	*Clitoria ternatea* Linn. Papilionaceae	Aparajita	Root	Epilepsy, headache, memory increase, migraine, eye problem
29	*Coccinia cordifolia* Cogn. Cucurbitaceae	Telakutcha	Leaf	Reduces blood sugar, dysentery, stomach/ liver problem, cold-cough, contraception, cardiac problem, reduce blood pressure

Sl. No.	Scientific name	Local name	Parts used	Uses
30	*Cucurbita maxima* Duchesue. Cucurbitaceae	Kumra	Whole plant, flower, fruit, seed	Gallbladder infection, loss of appetite, mail sterility, increase of milk in breast
31	*Curcuma amada* Roxb. Zingiberaceae	Aam ada	Whole plant	Cold-cough, fever, piles, rheumatism
32	*Curcuma longa* Linn. Zingiberaceae	Halud, Haridra	Rhizome	Bacterial infection, skin protection, liver problem, worm, filaria, allergy, asthma, ulcer
33	*Cynodon dactylon.* (Linn.) Pers. Gramineae	Durba	Grass	Dysentery, blood purifier, piles, periodic problem, leucorrhoea
34	*Cyperus rotundus* Linn Cypreraceae	Motha	Plant body, bulbous root	Dysentery, poison bite
35	*Datura metel* Linn. Solanaceae	Dhutura	root, Leaf,	Hydrophobia, mental handicap, rheumatism,
36	*Eclipta alba* Asteraceae	Bhimraj, Vringraj	Leaf, seed	Stomach/ liver problem, anti-inflammatory, digestive, hair tonic.
37	*Enhydra fluctuans* Asteraceae	Helencha	Plant body	Reduces blood sugar, dysentery, stomach/ liver problem, blood purifier
38	*Erythrina variegate* Linn. Leguminaceae	Kantamother/ Mother	Root, root bark, leaf	Rheumatism, mail sexual problem, rickets, worm, periodic problem
39	*Ficus bengalensis.* Linn. Moraceae	Bat	Gum, bark	Reduces urine sugar, mail sterility
40	*Ficus carica* Moraceae	Dumur	Fruit, leaf	Reduces blood sugar, anemia
41	*Ficus religiosa.* Linn. Moraceae	Aswatha	Bark	Gallbladder infection, blood purifier, sexual disease- female
42	*Foeniculum vulgare.* Mill. Apiaceae	Mouri	Leaf, root	Periodic problem
43	*Glinus oppositifolia* Linn. Aizoaceae	Geema	Leaf, whole plant	Blood purifier
44	*Glycyrrhiza glabra* Linn. Leguminaceae	Jastimadhu	Roots and runner	Cold-cough, fever, diarrhea, allergy in respiratory track
45	*Heliotropium indicum* Linn. Boraginaceae	Hatisur	Leaf	Rheumatism, fever, typhoid, cold-cough
46	*Hemidesmus indicus.* R.Br. Asclepiadaceae	Anantamul	Root	Stomach/ liver problem, menstrual problem, piles, asthma,

Sl. No.	Scientific name	Local name	Parts used	Uses
47	*Hibiscus rosa sinensis* Linn. Malvaceae	Jaba	Leaf	Vomiting, menstrual problem, periodic problem
48	*Holarrhena antidycenterica* Well. Apocynaceae	Kurchi	Bark, seed	Scabies, antipyretic, amoebic dysentery
49	*Hygrophylla spinosa.* Nees. Acantheceae	Kulekhara	Leaf, branch	Blood purifier, rheumatism, anemia, liver disease, mail sexual problem
50	*Ipomoea reptans* (Linn.) Poir. Convolvulaceae	Kalmi	Whole plant, leaf, flowering twig	Ulcer, insomnia
51	*Justicia gendarussa* Burm. Acantheceae	Bishalyakarani	Leaf	Stop bleeding, ulcer
52	*Kalanchoe pinnata* Pers. Crassulaceae	Patharkutchi	Leaf	Dysentery, stomach/ liver problem, kidney/ urinary infection, gallbladder infection, piles, cardiac problem
53	*Lannea grandis* Dennst. Anacardaceae	Ziga (Ziol)	Leaf, bark, Gum	Dysentery, diarrhea, cardiac problem
54	*Leucas indica* (L.) Vatke. Lamiaceae	Kesta, Swetdrone	Leaf, flower	Jaundice, fever, cough, worm
55	*Linum usitatissimum* Linn. Linaceae	Atashi, Tishi	Leaf, flower, seed, seed oil	Reduces blood sugar, urinary infection, headache
56	*Marsilea minuta* Linn. Marsileaceae	Sushni	Leaf, whole plant	Asthma, memory increases, blood pressure, nervous disorder
57	*Melia azadirecta* Linn. Meliaceae	Neem	Leaf, bark, root bark, flower, fruit, seed	Reduces blood sugar, fever, contraception
58	*Mimosa pudica* linn. Mimosoidae	Lajjabati	Root	Reduces blood sugar, stomach/ liver problem, reduces urine sugar, mail sterility, sexual disease- female, periodic problem
59.	*Modhuca longifolia* (Koenig) *Macbrwar latifolia* A. Chevb. *Bassia longifolia* Sapotaceae	Mahua	flower	Piles, nervous disorder
60	*Momordica charanta* Cucurbitaceae	Karola	Fruit, seed	Reduces blood sugar, stomach/ liver problem, gallbladder infection, blood purifier
61	*Moringa oleifera.* Lamk Moringaceae	Sagina	Leaf, fruit	Reduces blood sugar, rheumatism, cardiac problem
62	*Mucuna prurita* Hook. Leguminaceae	Alkushi	Root, seed	Reduces urine sugar, mail sterility, mail sexual problem, sexual disease-female, rheumatism

Sl. No.	Scientific name	Local name	Parts used	Uses
63	*Murraya koenigi* Rutaceae	Currypata	Leaf	Reduces blood sugar
64	*Murraya paniculata (L.)* Jack. Rutaceae	Kamini	Leaf	Dysentery, inflammation of ear, black fever
65	*Musa paradisiaca* Musaceae	Kala	Fruit, seed, flowering twig	Reduces blood sugar, reduces urine sugar, mail sterility, cholera,
66	*Nyctanthes arbortristis.* Linn. Nyctanthaceae	Sewli	Leaf, seed	Fever, worm, rheumatism, decrease sexuality, irregular stool
67	*Occimum sanctm* Lamiaceae	Tulshi	Leaf, root	Cold-cough, malaria general debility, reduces urine sugar, mail sterility
68	*Oxalis corniculata.* Linn. Oxalidaceae	Amrul	Leaf, whole plant	Dysentery, cold-cough
69	*Paederia foetida.* Linn. Rubiaceae	Gandal	Leaf	Stomach/ liver problem, dysentery, spermatorrhoea, paralysis, rheumatism
70	*Phyllanthus emblica* Linn Euphorbiaceae	Amlaki	Dried fruit, seed	Acidity, blood sugar, purging, leucorrhoea, biliary colic, insomnia,
71	*Piper betle* Linn. Piperaceae	Pan	Leaf, root	Stomach problem, dysentery, gallbladder infection, contraception
72	*Piper longum* Linn. Piperaceae	Pipul	Root, stem, fruit	Reduces blood sugar, periodic pain, colic pain, cold-cough
73	*Pongamia pinnata* Vent. Leguminaceae	Karanj	Bark, seed, leaf	Worm, cough, reduces blood sugar
74	*Psidium guyava* Linn. Myrtaceae	Piara	Leaf, root, fruit, bark	Dysentery, sexual disease- female, loss of appetite, pyorrhea,
75	*Pterocarpus santalinus* Linn. Leguminaceae	Chandan	Bark, stem	Reduces urine sugar, mail sterility
76	*Punica granatum* Linn. Punicaceae	Dalim	Fruit, flower, leaf, root	Reduces blood sugar, dysentery, blood purifier, cough
77	*Ricinus communis.* Linn. Euphorbiaceae	Reri (Erond)	Root, root bark, leaf, seed oil	Stomach/ liver problem, rheumatism, eye problem, headache, biliary colic
78	*Rowolfia serpentina* Apocynaceae	Sarpagandha	Bark	Cardiac problem, mentally handicap, hyper tension, insomnia.

Sl. No.	Scientific name	Local name	Parts used	Uses
79	*Saccharum officinarum* Linn. Gramineae	Aankh	Stem	Jaundice, liver problem
80	*Bombax ceiba* Linn. Schoott and Endl. Bombaceae	Shimul	Bark, gum, leaf, flower, fruit, root	Cold-cough, reduces urine sugar, mail sterility, rheumatism, semen increase
81	*Saraca indica.* Linn. Leguminaceae	Ashok	Leaf, bark, seed	Stomach/ liver problem, kidney/ urinary infection, sexual disease-female
82	*Sida cordata* (Burm.f.)Borss =*Sida veronicufolia* Lamk. Malvaceae	Berala/ Bala	Leaf	Rheumatism, reduces urine sugar, mail sterility
83	*Solanum nigram* Solanaceae	Got begun, Makoi (H) Kakamachi	Root	Rheumatism, dropsy, general debility, diuretic, anti dysenteric
84	*Solanum virginianum* Linn. *S. surattense* Burm. f, *S xanthocarpum* Sch. and Wendle. Solanaceae	Kantikari	Root	Cold-cough, rheumatism, fever, influenza, enlargement of liver and spleen
85	*Stephania hernandifolia Walp.* Menispermaceae	Aagnati, Aagnadi	Root, whole plant	Cholera, fever, dysentery, cough, stomachache, contraception, irregular stool, leucorrhea
86	*Swertia chirata.* Ham. Gentianaceae	Chirata	Leaf, whole plant	Stomach/ liver problem, spermatorrhoea, worm, asthma, influenza,
87	*Syzygium cumini* Myrtaceae	Jam	Bark	Reduces blood sugar, dysentery, rheumatism
88	*Tamarindus indica* Linn. Leguminaceae	Tetul	Leaf, bark, fruit, seed	Reduces blood sugar, dysentery, stomach/ liver problem, pox, rheumatism
89	*Termanalia arjuna* (Roxb.ex Dc.) Wight. and Arn. Combretaceae	Arjun	Bark, leaf, fruit	Blood pressure, spermatorrhoea, blood dysenteries, joining of bones
90	*Terminalia belerica.* Retz. Combretaceae	Bahera	Fruit (ripe and unripe)	Cold-cough, insomnia, dropsy, vomiting, ulcer, trifala

Sl. No.	Scientific name	Local name	Parts used	Uses
91	*Terminalia chebula*.Retz. Combretaceae	Haritaki	Fruit, seed	Trifala, wound ulcer, leprosy, inflammation, cough. piles, fever
92	*Tinospora cordifolia* (Willd.) Hook.f. and Thoms. Menispermaceae	Gulancha	Branch	Stomach/ liver problem, jaundice, loss of appetite, fever, rheumatism, gallbladder infection, blood purifier
93	*Tragia involucrate* Linn. Euphorbiaceae	Bichhutika	Root, fruit	Stomach/ liver problem, rheumatism, irregular stool, asthma
94	*Tribulus terretris.* Linn. Zygophyllaceae	Gokhur	Whole plant, fruit, spine	kidney/ urinary track infection, rheumatism, sexual disease-men, nervous disorder
95	*Trichosanthes dioica* Roxb. Cucurbitaceae	Patal	Leaf, fruit	Urinary track infection, liver disease, bile complain
96	*Trigonella foenum-graceum* Linn. Leguminaceae	Methi	Leaf, seed, whole plant	Gynecological problem, pox, loss of appetite, sexual problem
97	*Vanda roxburghi* R.BR. Orchidaceae	Rasna	Root	Rheumatism
98	*Vitex nigandu* Linn. Verbenaceae	Niscinda/ Narsingha	Root, fruit, flower, leaf and bark	Cold-cough, fever, rheumatism, memory increases
99	*Scoparia dulcis* L. Scrophulariaceae	Vassourinha, sweet broom	Leaf, plant body	Reduce blood sugar, anti inflammatory, sore, cough
100	*Abroma augusta* Sterculaceae	Ulatkamal	Leaf, bark	Reduces blood sugar, kidney/ urinary infection, reduces urine sugar, mail sterility, sexual disease- female
101	*Litsea glutinosa* Lauraceae	Malibabla	Leaf, bark	Dysentery, reduces urine sugar, mail sterility, sexual disease- female
102	*Amaranthus spinosus* Amaranthaceae	Katakhura	Whole plant, leaf	Reduces urine sugar, mail sterility
103	*Jatropha curcas* L. Euphorbiaceae	Bharenda	Stem, root	Dysentery, bad teeth, , amoebic dysentery, blood dysentery
104	*Clerodendrum indicum* Verbenaceae	Bamunhati/ Brahmajasti	Leaf, stem	Asthma, rheumatism, anthelmintic
105	*Coffea arabica* Rubiaceae	Coffee	Seed	Migraine, headache, fever, cardiac trouble
106	*Withania somnifera* Solanaceae	Aswagandha	Root	Nervous disorder, mail sterility, sexual disease- female
107	*Opuntia dilleni* L. Cactaceae	Fanimanasa	Leaf	General debility, reduces urine sugar, mail sterility

Table 2: Number of genera for the treatment of various diseases

Sl. No.	Family	Total No. of genera	Sl. No.	Family	Total No. of genera
1.	Acantheceae	4	25.	Linaceae	1
2.	Amaranthaceae	2	26.	Malvaceae	2
3.	Anacardiaceae	1	27.	Meliaceae	1
4.	Apiaceae	4	28.	Menispermaceae	2
5.	Apocynaceae	4	29.	Moraceae	1
6.	Asclepediaceae	2	30.	Moringaceae	1
7.	Asteraceae	4	31.	Musaceae	1
8.	Basellaceae	1	32.	Myrtaceae	2
9.	Bombaceae	1	33.	Nyctanthaceae	1
10.	Boraginaceae	1	34.	Orchidaceae	1
11.	Cactaceae	1	35.	Oxalidaceae	2
12.	Combretaceae	1	36.	Palmae	1
13.	Convolvulaceae	1	37.	Piperaceae	1
14.	Crassulaceae	1	38.	Punicaceae	1
15.	Cucurbitaceae	4	39.	Rubiaceae	2
16.	Cypreraceae	1	40.	Rutaceae	3
17.	Euphorbiaceae	4	41.	Sapotaceae	1
18.	Ficoideae	2	42.	Scrophulariaceae	2
19.	Gentianaceae	1	43.	Solanaceae	2
20.	Gramineae	2	44.	Sterculaceae	1
21.	Lamiaceae	2	45.	Verbenaceae	2
22.	Lauraceae	2	46.	Vitaceae	1
23.	Leguminaceae	14	47.	Zingiberaceae	1
24.	Liliaceae	3	48.	Zygophyllaceae	1

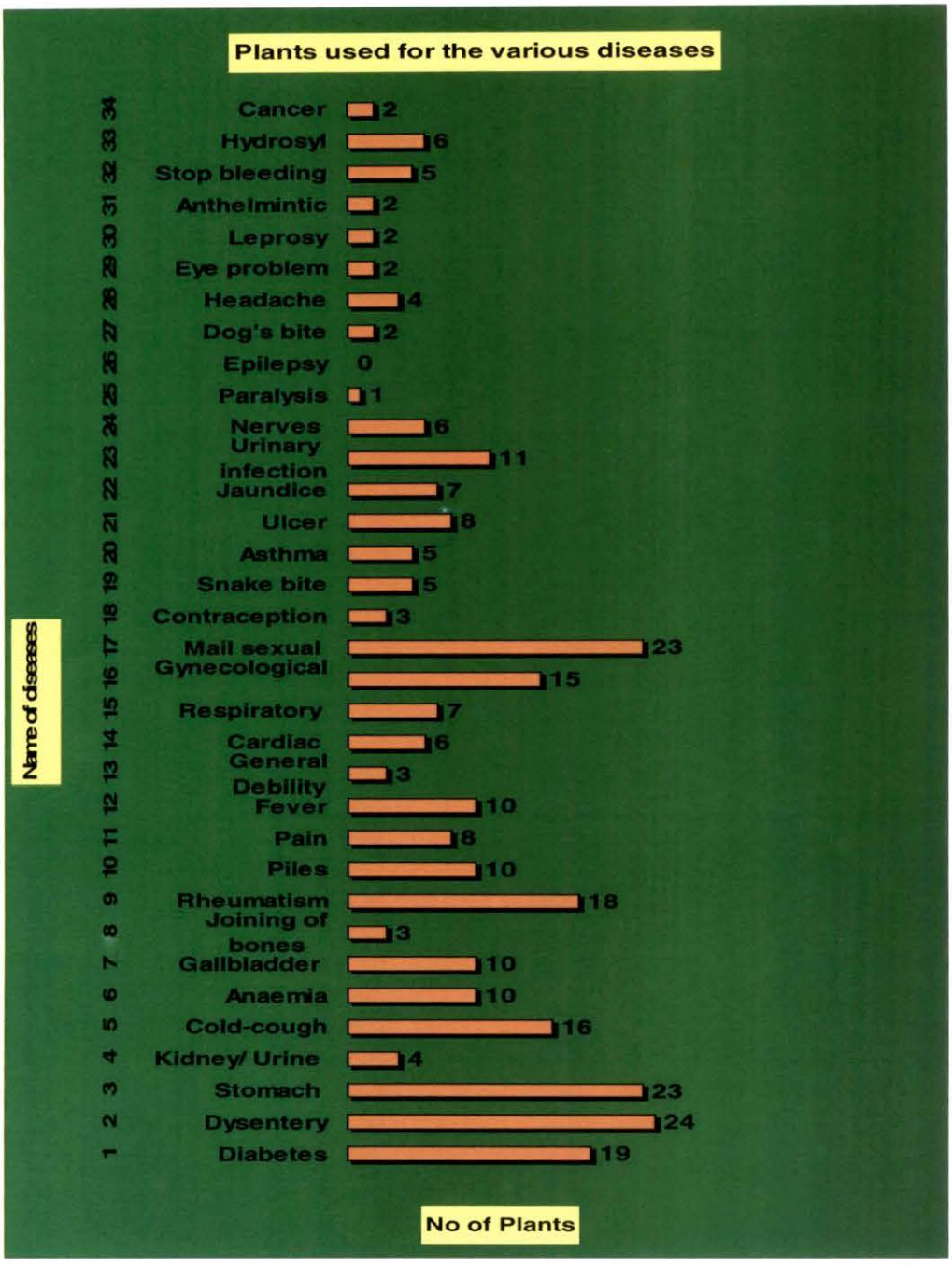


Fig-I: Number of plants for the treatment of various diseases

Abroma augusta

Acacia arabica

Achyranthes aspera

Adhatoda vasica

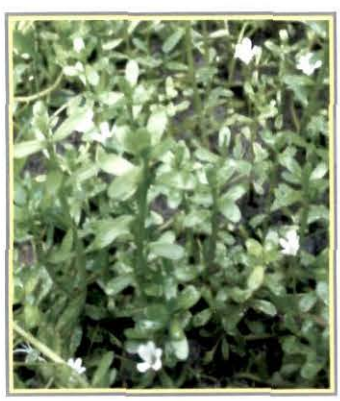
Aegle marmelos

Aloe barbadensis

Alstonia scholaris

Amaranthus spinosus

Andrographis paniculata



Bacopa monniera

Butea monosperma

Cajanus indicus

PLATE-XIII : Medicinal plants of study area

Coffea arabica

Cynodon dactylon

Datura metel

Enhydra fluctuans

Erythrina variegate

Ficus bengalensis

Ficus carica

Ficus religiosa

Heliotropium indicum

Hydrocotyle asiatica

Hygrophylla spinosa

Ipomoea reptans

PLATE-XIV : Medicinal plants of study area.

Jatropha curcas

Justicia gendarussa

Kalanchoe pinnata

Lannea grandis

Leucas cephalotes

Litsea glutinosa

Marsilea minuta

Melia azadirecta

Mimosa pudica

Modhuca longifolia

Momordica charanta

Moringa oleifera

PLATE-XV : Medicinal plants of study area

Pterocarpus santalinus

Punica granatum

Ricinus communis

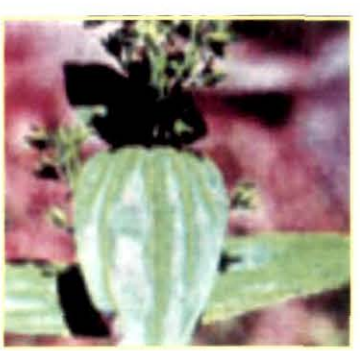
Rowolfia serpentina

Saccharum officinarum

Sida cordata

Solanum virginianum

Stephania hernandifolia



Swertia chirata

Syzigium cumini

Scoparia dulcis

Tamarindus indica

PLATE-XVI : Medicinal plants of study area

Medicinal plants used for the treatment of male sexual problems are *Aloe barbadensis, Mimosa pudica, Musa paradisiaca, Acacia arabica., Occimum sanctum, Salmalia malabaricum, Asparagus racemosus, Sida cordifolia, Mucuna prurita, Ficus bengalensis, Pterocarpus santalinus, Alstonia scholaris, Achyranthes aspera, Cassia sophera, Aegle marmelos, Clitoria ternatea, Piper betle, Coccinia cordifolia*, etc.

Diabetes is treated with the plants- *Abroma augusta, Aegle marmelos, Cajanus cajan, Catheranthus roseus, Cinnamomum tamala, Coccinia cordifolia, Enhydra fluctuans, Ficus carica, Melia azadirecta, Mimosa pudica, Momordica charanta, Moringa oleifera, Murraya koenigii, Musa paradisiaca, Piper longum, Punica granatum, Scoparia dulcis, Syzygium cumini, Tamarindus indica* etc (Table 3).

After analysis of investigated plants in the present study *Clerodendrum viscosum, Cinnamomum tamala, Moringa oleifera* and *Scoparia dulcis* were selected for detailed investigations (Table 4).

4.2. Description of the selected plants

4.2.1. *Clerodendrum viscosum*

A tall shrub; stem 4-angled; leaves simple, ovate, often form a cordate base, entire or dentate, blade 5-9, petiole 1-6 in. long. Flower white, tinged with pink, in an ample terminal trichotomous corymbiform thyrsus. Calyx cleft to near the base, segments lanceolate. Corolla-tube longer than calyx-segments. Stamens 4, didynamous, long exserted. Ovary globose, 4-celled, 4-ovuled, style filiform, stigma bifid. Fruits drupe fleshy (Plate XVII).

It is propagated by seeds and vegetative method.

4.2.2. *Moringa oleifera*

A small or medium sized, deciduous, perennial tree. Trunk straight, bark thick, corky; Leaves feathery, pale green, alternate, decompound, usually tripinnate, leaflets on short slender petiolules, ovate or obovate, obtuse, green above, paler beneath; Flower strongly honey-scented, white or creamy-white in axillary or terminal panicles; bracts linear, shorter than pedicels; Calyx 5- partite, segments petaloid, unequal; Petals 5 free, linear- spathulate, unequal; Stamens yellow 10, in two series; Ovary tricarpellary, ovary and base of filaments

Table 3 : Medicinal plants for the treatment of Diabetes

Sl. No.	Scientific name	Local name	Part(s) used
1	*Abroma augusta* Sterculaceae	Ulatkamal	Leaf, bark
2	*Aegle marmelos*, Rutaceae	Bel	Leaf, root, bark, fruit,
3	*Cajanus cajan* Leguminaceae	Arhar	Leaf, root, seed
4	*Catheranthus roseus* Apocynaceae	Nayantara	Leaf
5	*Cinnamonum tamala* Lauraceae	Tejpata	Leaf, bark
6	*Coccinia cordifolia* Cucurbitaceae	Telakutcha	Leaf
7	*Enhydra fluctuans* Asteraceae	Helencha	Plant body
8	*Ficus carica* Moraceae	Dumur	Fruit, leaf
9	*Melia azadirecta* Meliaceae	Neem	Leaf, bark, root bark, flower, fruit, seed
10	*Mimosa pudica* Mimosoidae	Lajjabati	Root
11	*Momordica charanta* Cucurbitaceae	Karola	Fruit, seed
12	*Moringa oleifera* Moringaceae	Sagina	Leaf, fruit
13	*Murraya koenigii* , Rutaceae	Currypata	Leaf
14	*Musa paradisiaca* Musaceae	Kala	Fruit, seed, flowering twig
15	*Piper longum* Piperaceae	Pipul	Root, stem, fruit
16	*Punica granatum* Punicaceae	Dalim	Fruit, flower, leaf, root
17	*Scoparia dulcis* Scrophulariaceae	Vassourinha, sweet broom	Leaf, plant body
18	*Syzygium cumini* Myrtaceae	Jam	Bark
19	*Tamarindus indica* Leguminaceae	Tetul	Leaf, Bark, fruit, seed

Table 4 : Ethnobotanical uses of the selected plants

Sl. No.	Scientific name	Local name	Part(s) used	Reported Uses
1.	*Cinnamomum tamala* (Hamilton) Nees and Ebermaier. Acc. No. 9490 Lauraceae	Tejpata	Leaf, bark	Reduces blood sugar, cold-cough, memory decrease, quench thirst, skin care,
2.	*Clerodendrum viscosum* Vent. Acc. No. 9489 Verbenaceae	Bhati, Ghetu	Leaf, bud, root, whole plant	Malarial fever, any fever, worm, dysentery, stomach/ liver problem, piles
3.	*Moringa oleifera* Lamk. Acc. No. 9492 Moringaceae	Sagina	Leaf, fruit	Reduces blood sugar, rheumatism, cardiac problem
4.	*Scoparia dulcis* L. Acc. No. 9491 Scrophulariaceae	Vassourinha, sweet broom	Leaf, plant body	Reduce blood sugar, anti inflammatory, sore, cough

PLATE-XVII : A: *Clerodendrum viscosum*; B: Leaves of *C. viscosum;* C and D: Flowering stage of *C. viscosum.*

hairy, stipulate with numerous ovules; Fruit an elongate, pendulous, cylindrical longitudinally ribbed, loculicidal capsule; Seeds rounded- trigonous with broad wings at the angles (Plate XVIII).

In India, the plant is propagated by planting limb cuttings 1–2 m long, from June to August, preferably. The plant starts bearing pods 6–8 months after planting but regular bearing commenced after the second year. The tree bears for several years and flowers at January- February.

4.2.3. *Scoparia dulcis*

Annual herbaceous plant, Stem woody at the base, much branched, quadrangular when young, leaves small 3-verticilate or opposite, coarsely toothed-serrate. Flower white, crowded in the axils of the leaves. Capsule small, globosely, many seeded (Plate XIX).

It is propagated by seeds and vegetative method.

4.2.4. *Cinnamomum tamala*

A moderate sized evergreen tree 7.5 m in height with dark brown or blackish rough bark and pinkish or reddish brown blaze; leaves simple, opposite, sub-opposite or alternate, ovate-lanceolate or ovate-oblong, acuminate, coriaceous, glabrous, 3-nerved from base to apex; flowers pale yellowish in axillary and terminal panicles; fruits ovoid, fleshy, black drupe, supported by enlarged perianth tube (Plate XX).

It is propagated by seeds only.

4.3. Qualitative analysis of the phytochemical compounds of the selected plants

Leaves of *Cinnamomum tamala, Moringa oleifera, Clerodendrum viscosum and Scoparia dulcis* were subjected to screen phytochemical characters and to evaluate phytochemical constituents. Results presented in Table 5 showed that the various phytochemical constituents on various solvent of *Cinnamomum tamala, Moringa oleifera, Clerodendrum viscosum* were rich in alkaloid, tannin, saponin, steroid, terpenoid, flavonoid and cardiac glycoside. Alkaloid, tannin, saponin, terpenoid, flavonoid, and cardiac glycoside were present in *Scoparia dulcis* but steroid was absent in *Scoparia dulcis.*

PLATE-XVIII : A: Plant of Moringa oleifera, B and C: Flowering stage of Moringa oleifera, D: Fruits of Moringa oleifera

PLATE-XIX : A: Plant of *Scoparia dulcis*, B: Flowering stage of *Scoparia dulcis*, C: Leaves of *Scoparia dulcis*, D: Flower of *Scoparia dulcis*.

PLATE-XX : A: Plant of *Cinnamomum tamala,* B: Leaves of *Cinnamomum tamala,* C and D: Flowering stage of *Cinnamomum tamala.*

Table 5 : Qualitative analysis of the phytochemical compounds of the selected plants

Plants	Alkaloids	Tannin	Saponin	Steroid	Terpenoid	Flavonoid	Cardic Glycoside
CV	+	+	+	+	+	+	+
MO	+	+	+	+	+	+	+
CT	+	+	+	+	+	+	+
SD	+	+	+	-	+	+	+

(CV = *Clerodendrum viscosum*, MO= *Moringa oleifera*, CT = *Cinnamomum tamala*, SD = *Scoparia dulcis*; '+' = Present, '-'= Absent)

4.4. Quantitative analysis of the biochemical constituents of the selected plants

4.4.1. Alkaloids

Quantitative analysis of the percentage crude alkaloids in *Cinnamomum tamala, Moringa oleifera, Clerodendrum viscosum and Scoparia dulcis* leaves extracts were done. Results (Table 6) revealed that *Cinnamomum tamala* contained the highest percentage crude yield of alkaloid (4.92%) while *Scoparia dulcis* contained the lowest yield of alkaloid (0.84%). *Moringa oleifera* also contained the moderate percentage crude yield of alkaloid followed by *Clerodendrum viscosum.*

Table 6: Alkaloid contents of leaves of different study plants

Sample	Content (% of alkaloid/g leaf tissue)
Clerodendron viscosum	1.76 ± 0.08
Moringa oleifera	2.56 ± 0.25
Cinnamomum tamala	4.92 ± 0.04
Scoparia dulcis	0.84 ± 0.02

Each value represents mean ± SE

4.4.2. Proteins

Estimation of contents of proteins extracted from the *Cinnamomum tamala, Moringa oleifera, Clerodendrum viscosum, Scoparia dulcis* leaves were done and results (Table 7) revealed that *Moringa oleifera* contained the highest amount of protein (55 mg/g tissue)

among the four studied leaves extracts and *Clerodendrum viscosum* contained the least amount (7.5 mg/g tissue). *Cinnamomum tamala* and *Scoparia dulcis* contained the moderate amount of protein in their tissue.

Table 7 : Protein Contents of leaves of different study plants

Sample	Protein content (mg/g tissue)
Clerodendron viscosum	10.83 ± 2.20
Moringa oleifera	59.17 ± 2.20
Cinnamomum tamala	23.33 ± 1.67
Scoparia dulcis	19.50 ± 0.29

Each value represents mean; ± =SE

4.4.3. Carbohydrates

4.4.3.1. Total sugar

Carbohydrates were extracted from the leaves of *Cinnamomum tamala, Moringa oleifera, Clerodendrum viscosum, Scoparia dulcis* and estimations of total and reducing sugar contents were done. Results revealed that *Moringa oleifera* contained the highest amount of total sugar (40.5mg/g tissue) while *Clerodendrum viscosum* contained the least amount (5mg/g tissue) of total sugar. Another two studied plants *Cinnamomum tamala, Scoparia dulcis* also contained the moderate amount (29mg/g tissue and 31mg/g tissue respectively) (Fig II).

4.4.3.2. Reducing sugar

Results for reducing sugar revealed the same trend ie., leaves of *Moringa oleifera* contained maximum reducing sugar (15mg/g tissue), *Cinnamomum tamala, Scoparia dulcis* also contained the moderate amount (11.5mg/g and 10.5mg/g tissue respectively) but *Clerodendrum viscosum* contained the least amount (4mg/g tissue) (Fig II).

4.4.4. Phenols

Quantitative estimation of total phenol from leaves of *Cinnamomum tamala, Moringa oleifera, Clerodendrum viscosum, Scoparia dulcis* were done. The spectrophotometric assay for the quantitative determination of phenol revealed that *Cinnamomum tamala* contained the

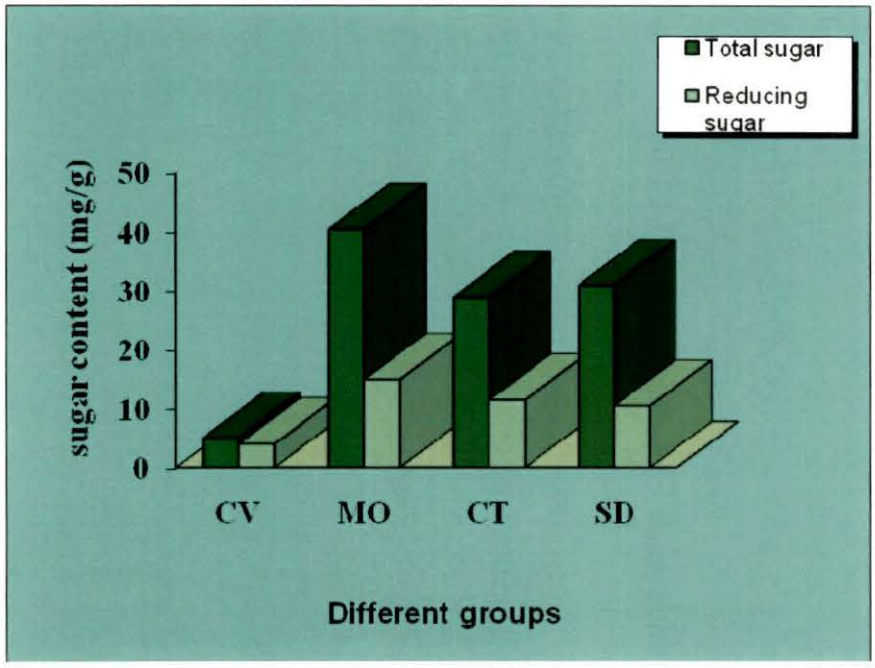


Fig-II : Sugar content of study plants (both total sugar and reducing sugar content; CV= *Clerodendrum viscosum*, MO= *Moringa oleifera*, CT= *Cinnamomum tamala*, SD= *Scoparia dulcis*.

highest amount (20.83mg/g tissue) of phenol than other three and *Clerodendrum viscosum* contained the least amount (2.32mg/g tissue). *Moringa oleifera* and *Scoparia dulcis* contained 6.25mg/g tissue and 4.31mg/g tissue respectively (Table 9).

Table 9: Total phenol content of leaves of the study plants

Sample	Total phenol content (mg/g tissue)
Clerodendron viscosum	2.33 ± 0.36
Moringa oleifera	6.25 ± 0.07
Cinnamomum tamala	20.83 ± 0.11
Scoparia dulcis	4.31 ± 0.11

Each value represents mean; ± =SE

4.4.5. Ascorbic acid

Quantification of ascorbic acid of *Cinnamomum tamala, Moringa oleifera, Clerodendrum viscosum, Scoparia dulcis* leaves was carried out and results revealed that *Cinnamomum tamala* contained the highest amount (22.3mg/g tissue) of ascorbic acid followed by *Moringa oleifera, Scoparia dulcis* (15.45mg/g tissue, 8.52mg/g tissue). The least amount of ascorbic acid found in the *Clerodendrum viscosum* (7.21mg/g tissue) (Table 10).

Table 10 : Ascorbic acid content of leaves of the study plants

Sample	Ascorbic acid content (mg/g tissue)
Clerodendron viscosum	7.22 ± 0.15
Moringa oleifera	15.46 ± 0.14
Cinnamomum tamala	22.30 ± 0.21
Scoparia dulcis	8.53 ± 0.11

Each value represents mean; ± = SE

4.4.6. Carotenoids

Results of quantitative estimation of carotenoids of *Cinnamomum tamala, Moringa oleifera, Clerodendrum viscosum, Scoparia dulcis* leaves have been depicted in the Table 11. *Cinnamomum tamala* contained the higher amounts of carotenoids (1.85µg/g tissue) followed by *Moringa oleifera* and *Scoparia dulcis* (0.82 µg/g tissue and 0.41µg/g tissue).

Clerodendrum viscosum contained the least amount of carotenoids (0.082 μg/g tissue) among the four studied plants.

Table 11 : Carotenoid content of leaves of the study plants

Sample	Carotenoid content (mg/g tissue)
Clerodendrum viscosum	0.08 ± 0.01
Moringa oleifera	0.41 ± 0.03
Cinnamomum tamala	0.82 ± 0.04
Scoparia dulcis	1.85 ± 0.04

Each value represents mean; ±= SE

4.5. Analysis of protein patterns

Analysis of the soluble proteins of the leaves of all selected plants revealed that they contained proteins of different molecular masses ranging from 97,400 Da to 14,300 Da. *Moringa oleifera* contained proteins of different molecular masses viz., 47.77, 38.58, 36.25, 31.71, 29.90, 21.27, 18.72 Kd and 14.79. *Clerodendrum viscosum* contained proteins of 44.10, 29.0 and 17.87 Kd (molecular masses) and *Scoparia dulcis* contained 81.6, 72.68, 42.26, 34.43, 29.0, 19.57, 17.87, and 14.05 KD and in *Cinnamomum tamala* 51.45, 40.42, 34.43, 19.14, 13.56 Kd (Table 12 and Plate XXI).

Table 12 : Analysis of the soluble proteins of the leaves of study plants

Sl No	Sample	Molecular masses (Kd) of proteins
1	*Clerodendron viscosum*	44.10, 29.0, 17.87
2	*Moringa oleifera*	47.77, 38.58, 36.25, 31.71, 29.90, 21.27, 18.72, 14.79
3	*Cinnamomum tamala*	51.45, 40.42, 34.43, 19.14, 13.56
4	*Scoparia dulcis*	81.6, 72.68, 42.26, 34.43, 29.0, 19.57, 17.87, 14.05

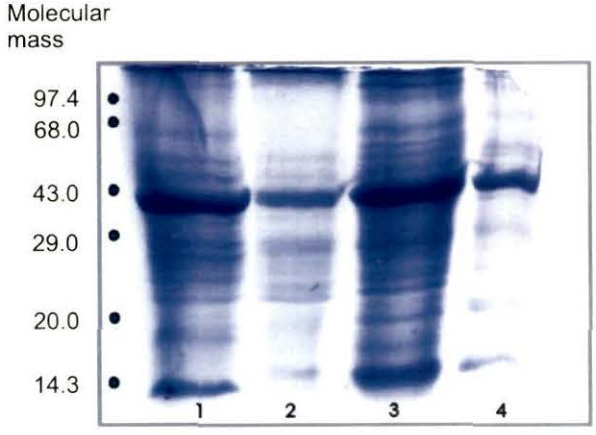


PLATE XXI: SDS-PAGE analysis of proteins from leaves of study plants; Lane 1: *Moringa oleifera*, Lane 2: *Clerodendrum viscosum*, Lane 3: *Scoparia dulcis*,Lane 4: *Cinnamomum tamala*.

4.6. TLC analysis of α-tocopherol

Tocopherols extracted from the different leaves samples were analyzed by TLC as described in Materials and Methods. Rf values were calculated and have been depicted in Fig III.

4.7. Antioxidant capacity of different plant extracts

The antioxidant capacities of *Cinnamomum tamala, Moringa oleifera, Clerodendrum viscosum, Scoparia dulcis* leaf extracts have been depicted in Fig IV. The results, expressed as mg α-tocopherol acetate/ g dry mass were 11.27 in *Cinnamomum tamala*, which was the highest among the other three plants which were 7.0 in *Moringa oleifera*, 5.92 in *Scoparia dulcis* and 4.58 mg α-tocopherol acetate/ g dry mass in *Clerodendrum viscosum.*

4.8. Partial characterization of solvent extracts

The different solvent extracts were analyzed by UV-spectrophotometry and the results have been depicted in Figs. V-VIII. Methanolic extracts had the highest concentration of compounds. All of the extracts had UV- absorption maxima in the range 200-208 nm, most of them having single peak. Different extracts of *S.dulcis* had 2 or more broad peaks other than the single sharp peak. .

4.9. Bio-assay of solvent extracts

Spore germination of the solvent extracts was carried out against different plant pathogenic fungi- *Poria hypobrunnea* (Fig IX), *Fusarium oxysporum, Curvularia lunata* and *Bipolaris sorokiana.* No inhibitory effects were observed, only very small inhibitory zone was observed against p. *hypobrunnea.* The extracts could not also inhibit growth of bacteria such as *Bacillus megaterium* and *Serratia marcescens* as tested by agar cup bioassay.

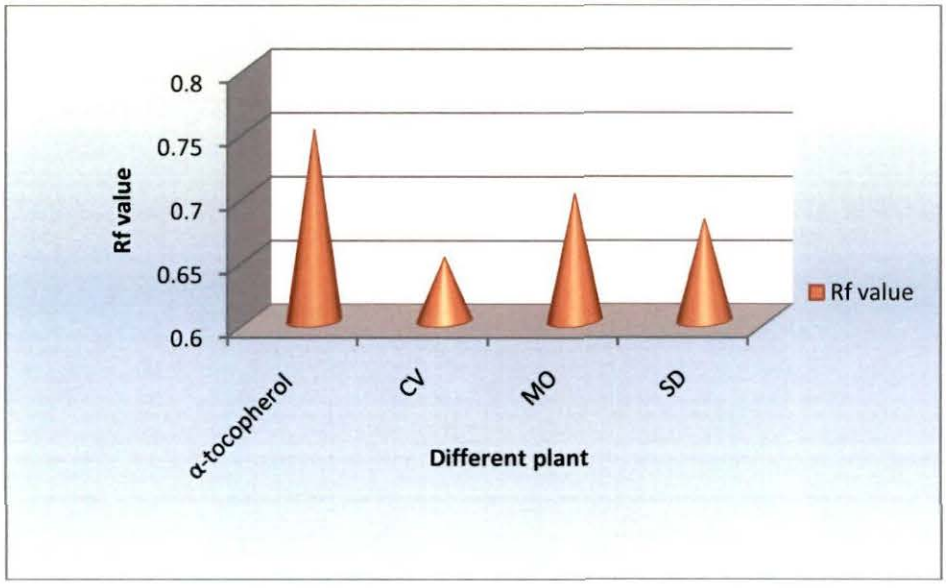


Fig III : Determination of α-tocopherol by TLC. (CV= *Clerodendrum viscosum*, MO= *Moringa oleifera*, SD= *Scoparia dulcis*.

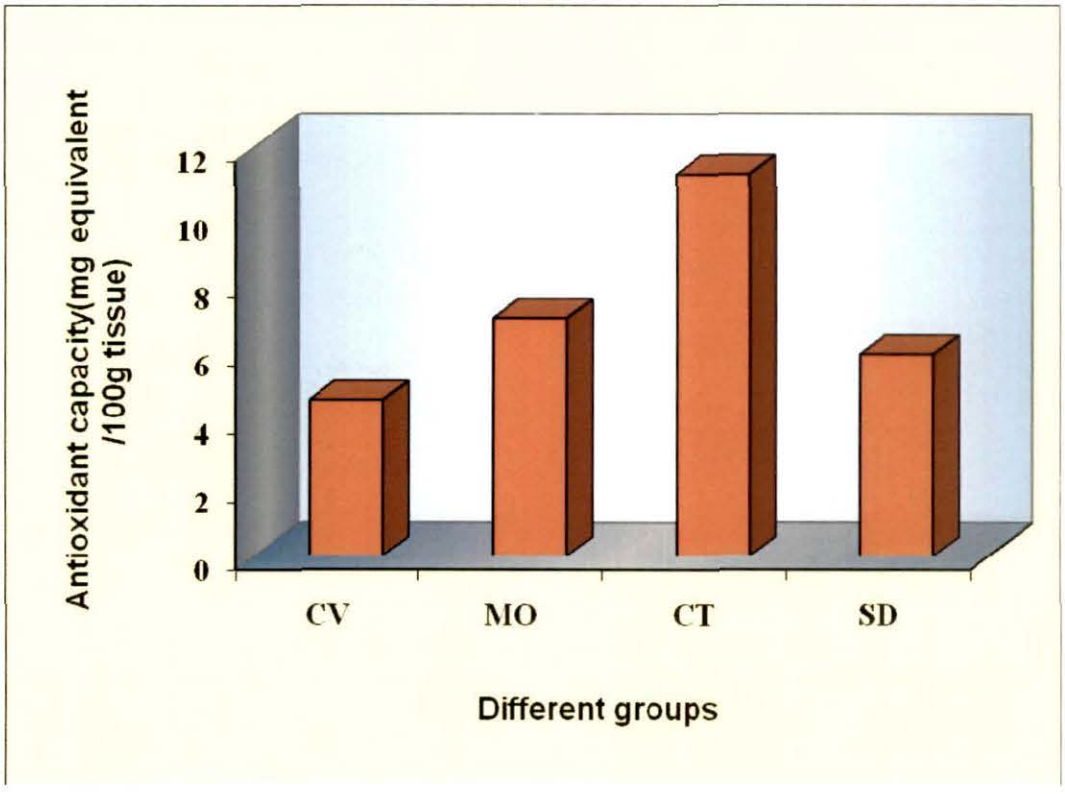


Fig-IV : Antioxidant capacity (CV= *Clerodendrum viscosum*, MO= *Moringa oleifera*, CT= *Cinnamonum tamala*, SD= *Scoparia dulcis*.

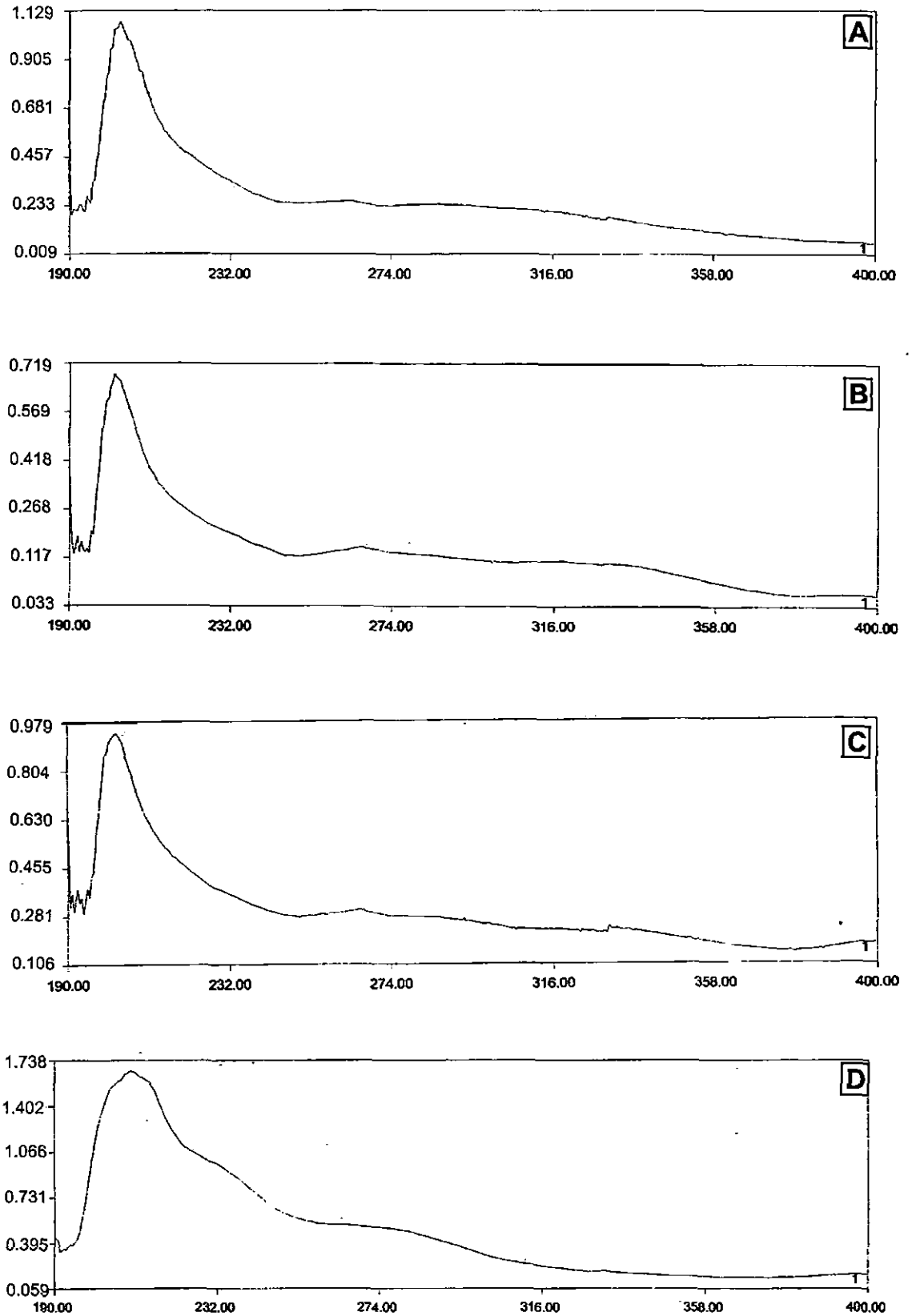


Fig. 5: UV-spectrophotometric analysis of solvent extracts from *Clerodendrum viscosum* (A) Diethyl ether; (B) Methanol; (C) Ethyl acetate and (D) Petrolium ether.

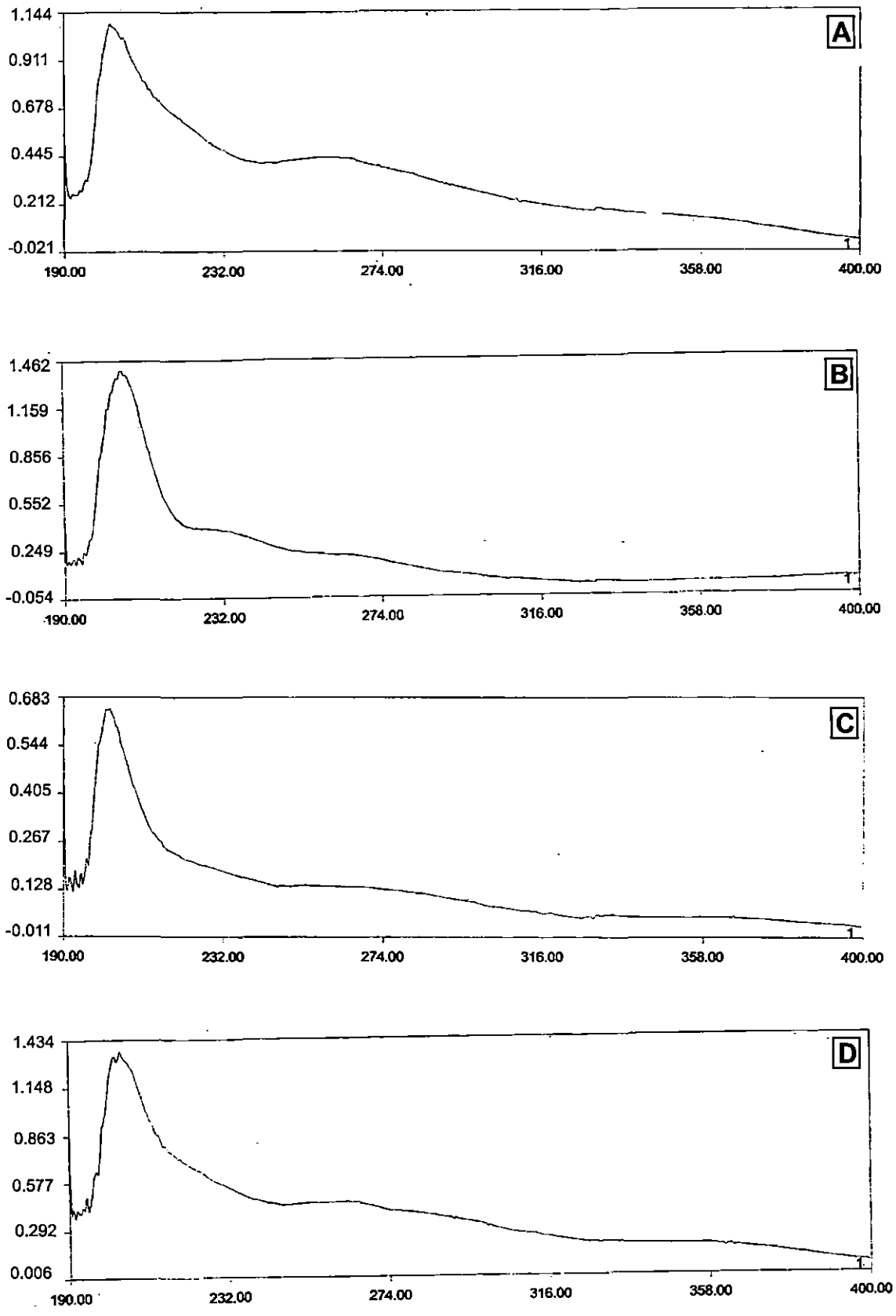


Fig.6: UV-spectrophotometric analysis of solvent extracts from *Moringa oleifera* (A)Methanol; (B) Petrolium ether; (C) Diethyl ether and (D) Ethyl acetate.

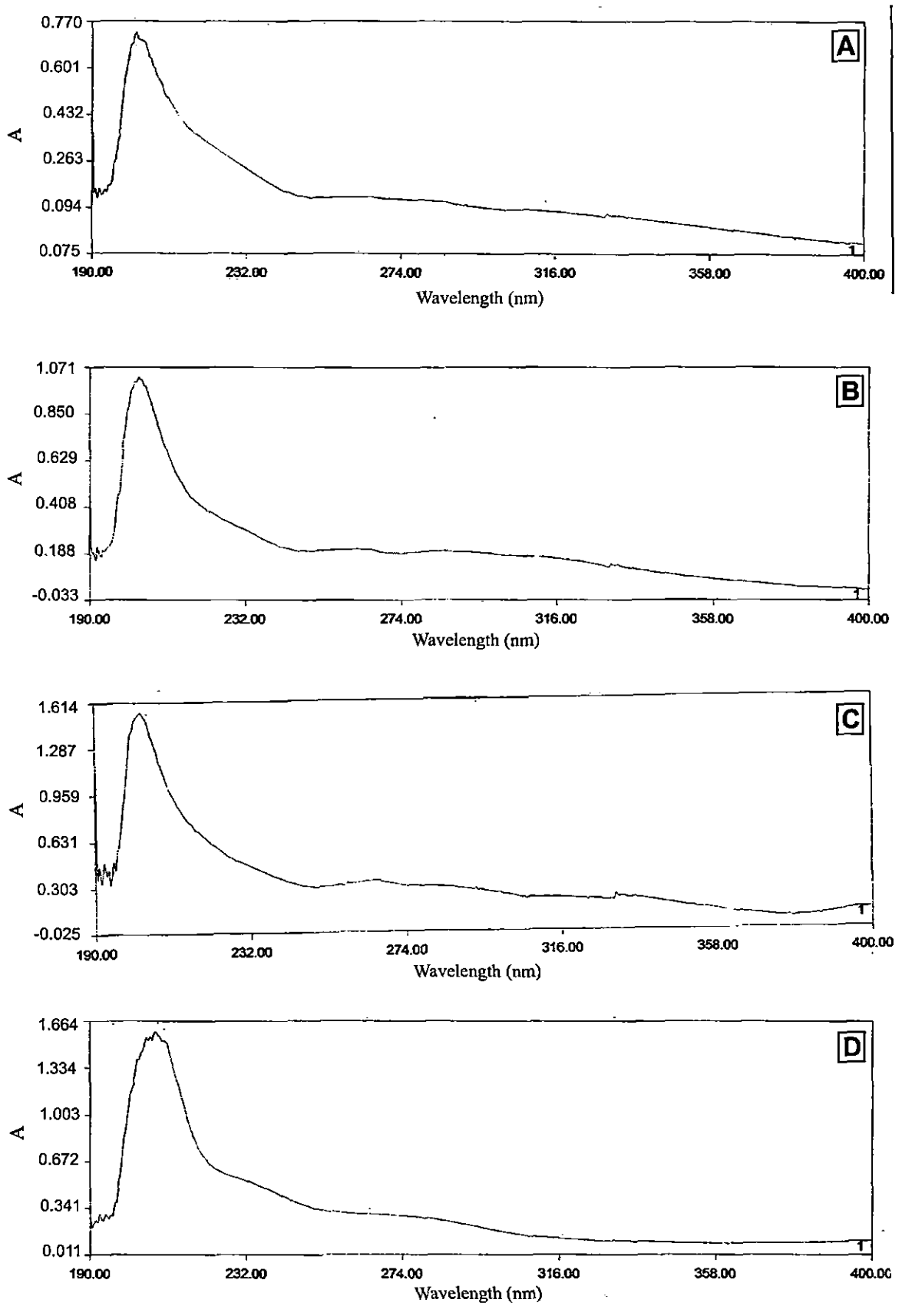


Fig. 7: UV-spectrophotometric analysis of solvent extracts from *Cinnamomum tamala* (A) Methanol; (B) Diethyl ether; (C) Ethyl acetate; and (D) Petrolium ether.

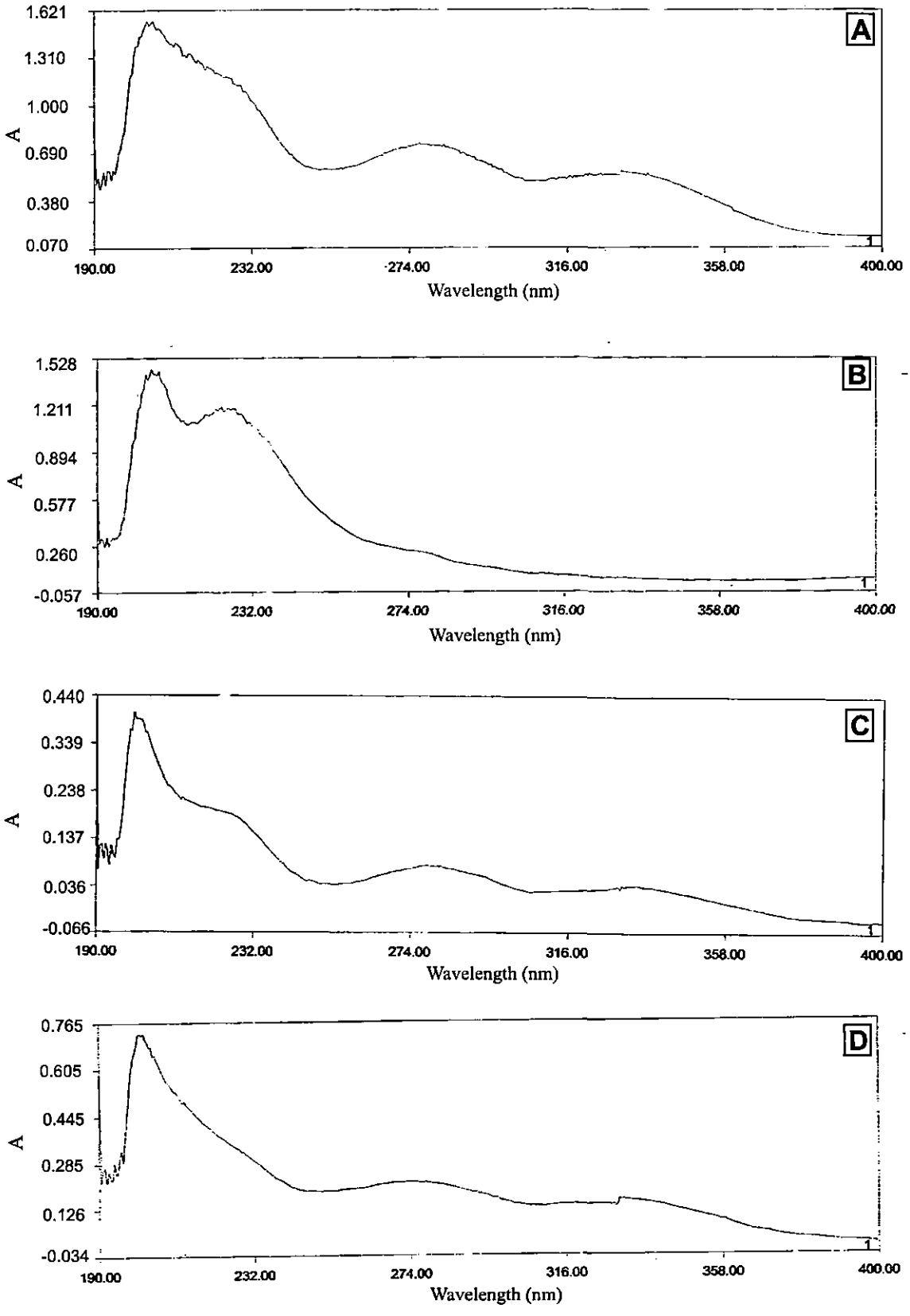


Fig.8 : UV-spectrophotometric analysis of solvent extracts from *Scoparia dulcis* (A) Ethyl acetate; (B) Petrolium ether; (C) Diethyl ethr and (D) Methanol.

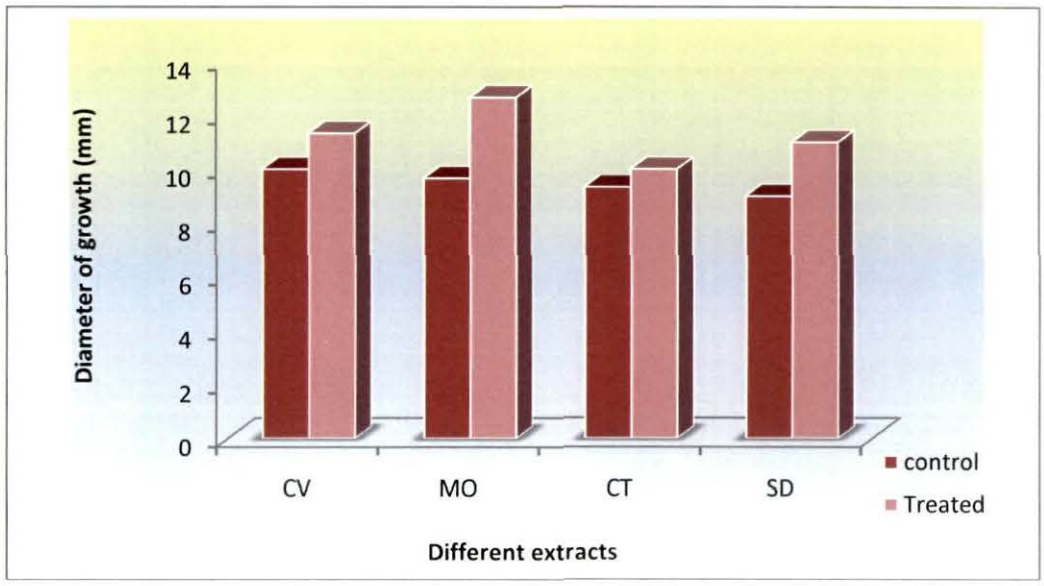


Fig-IX : Effect of different extracts on mycelial growth by Agar cup bioassay of *Poria hypobrunnea.* (CV= *Clerodendrum viscosum*, MO= *Moringa oleifera*, CT= *Cinnamonum tamala*, SD= *Scoparia dulcis*.

4.10. Effect of plant extracts on induced hyperglycemia in rats

Initially, the rats were injected with STZ to induce hyperglycaemia. After 2 days of STZ induction rats were found to be suffering from strong hyperglycemia. There was marked reduction in the body weight of the STZ treated rats, along with an increase in food craving and thirst. Subsequently, the rats were treated with extracts of the test plants and parameters associated with hyperglycaemia such as fasting blood glucose, urine sugar, glycogen content in liver tissue, TBARS and GSH of liver tissue, were tested. After 20 days of treatment with test plants most of the rats recovered from hyperglycemia and became healthy (Plate XXII).

4.10.1. Effect of different extracts on changes in body weight in normal and experimental rats

Changes in the body weight on the treatment of diabetic and normal rats with *Cinnamomum tamala, Moringa oleifera, Scoparia dulcis* leaves extracts have been demonstrated in Tables 14 and 15. The body weight of the diabetic rats decreased from 171g to 147g after the 20 days treatment with STZ (Fig X). The body weight of *Cinnamomum tamala* treated group (125mg/kg and 250mg/kg respectively) increased from 172.5g and 173 g to 190.5g and 200.5g respectively after the 20^{th} day's treatment. Following *Cinnamomum tamala* treatment, the body weight increased and reached almost upto the level of control rats. The body weight of *Moringa oleifera* treated group (125mg/kg and 250mg/kg respectively) increased from 175g and 171.5g to 191.5g and 194.5g respectively after the 20^{th} day's treatment. The body weight gain of diabetic rats by the treatment of *Moringa oleifera* was significant when compared with normal rats. The body weight of *Scoparia dulcis* treated group (125mg/kg and 250mg/kg respectively) increased from 169.5g and 172.5g to 186.5g and 196.5g respectively after the 20 days of treatment that was significantly increased ($p < 0.01$). Thus, it was observed that the body weight of rats, which had abruptly fallen due to induction of hyperglycaemia by STZ, increased following treatment of all test extracts and became almost similar to that of control.

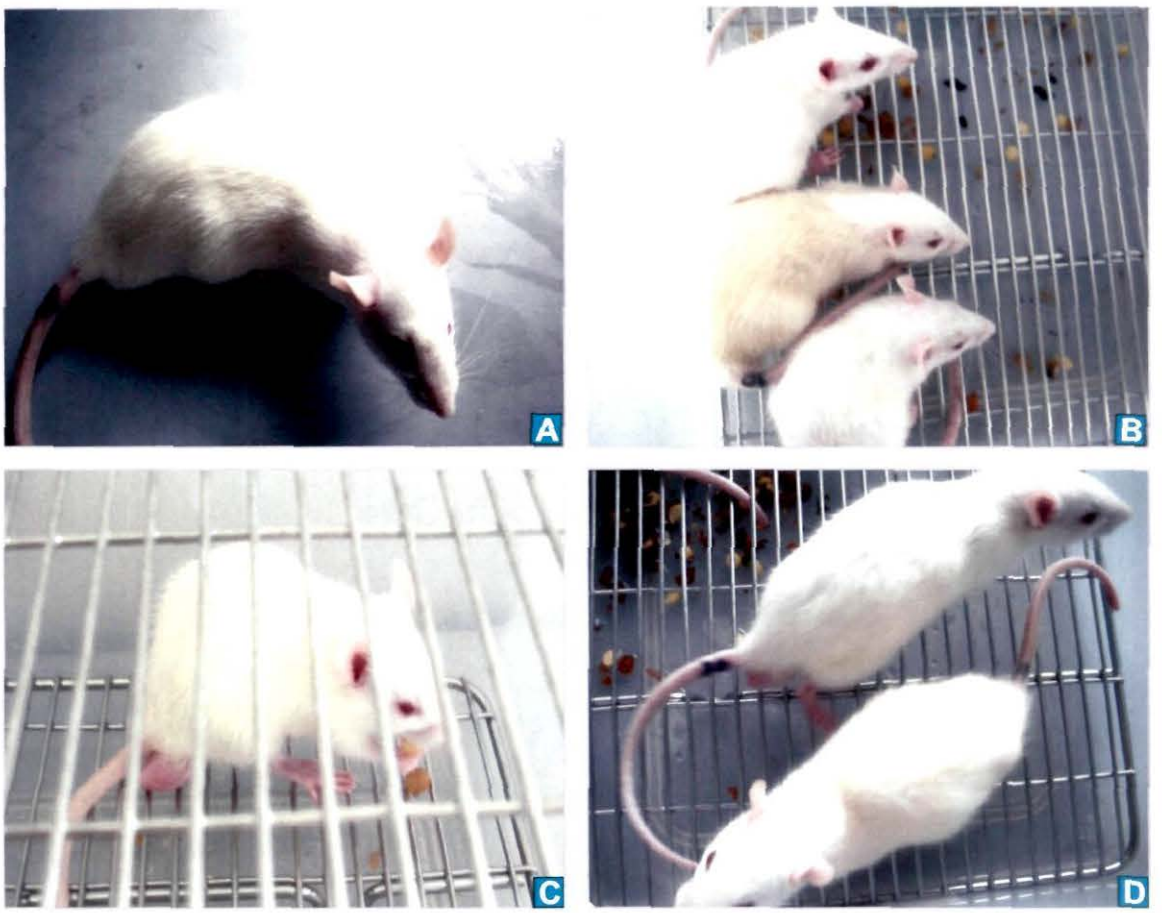


PLATE-XXII: A, B, C, D: Plant extracts treated healthy rats recovering hyperglycemia after 20 days treatment of different plant extracts.

Table 14 : Effect of different extracts on changes in body weight in normal and experimental rats

Group	Treatments	Body Weight (g)			
		First day	After 2 days of STZ induction	Days after plant extract treatment 10	20
I	Normal (only vehicle distilled water)	173.5 ± 4.73	175.0 ± 4.83	188.7 ± 5.54	202.5 ± 5.86
II	Citrate buffer treated	166.0 ± 4.99	167.0 ± 5.00	179.5 ± 4.50	191.5 ± 3.50
III	STZ (diabetic Control)	171.0 ± 1.15	167.6 ± 0.66	156.66 ± 0.88	147.0 ± 0.57
IV	STZ + MO (125mg/kg)	175.0 ± 2.00	177.5 ± 1.50	184.5 ± 1.50	191.5 ± 1.22
V	STZ +MO (250 mg/kg)	171.5 ± 0.50	172.5 ± 0.50	182.5 ± 2.50	194.5 ± 4.70
VI	STZ +CT (125mg/kg)	172.5 ± 4.50	174.0 ± 5.00	182.5 ± 5.50	190.0 ± 2.00
VII	STZ +CT (250mg/kg)	173.0 ± 4.00	176.5 ± 6.50	183.0 ± 5.00	201.0 ± 8.00
VIII	STZ +SD (125mg/kg)	169.5 ± 4.50	171.0 ± 4.00	178.0 ± 5.65	186.5 ± 3.50
IX	STZ +SD (250mg/kg)	172.0 ± 1.00	173.0 ± 2.00	185.0 ± 2.00	195.5 ± 2.50

Each value represents mean; ± =SE; MO= *Moringa oleifera*, CT= *Cinnamomum tamala*, SD= *Scoparia dulcis*. Values were statistically significant at $p < 0.01$ as compared with diabetic control, $p < 0.01$ as compared with normal.

Table 15 : Effect of different plant extracts on changes in body weight in normal and experimental rats

Groups	Treatment	Body weight (g)	
		Initial	Final
I	Control	173.5 ± 4.73	202.5 ± 5.86
II	Citrate buffer control	166.0 ± 4.99	191.5 ± 3.50
III	STZ (Diabetic control)	171.0 ± 1.15	147.0 ± 0.57
IV	STZ +MO (125mg/kg)	175.0 ± 2.00	191.5 ± 1.22
V	STZ +MO (250 mg/kg)	171.5 ± 0.50	194.5 ± 4.70
VI	STZ +CT (125mg/kg)	172.5 ± 4.50	190.0 ± 2.00
VII	STZ +CT (250mg/kg)	173.0 ± 4.00	201.0 ± 8.00
VIII	STZ +SD (125mg/kg)	169.5 ± 4.50	186.5 ± 3.50
IX	STZ +SD (250mg/kg)	172.0 ± 1.00	195.5 ± 2.50

Each value represents mean; ± = SE. MO = *Moringa oleifera*, CT= *Cinnamomum tamala*, SD= *Scoparia dulcis*. Values were statistically significant at $p < 0.01$ as compared with diabetic control, $p < 0.01$ as compared with control.

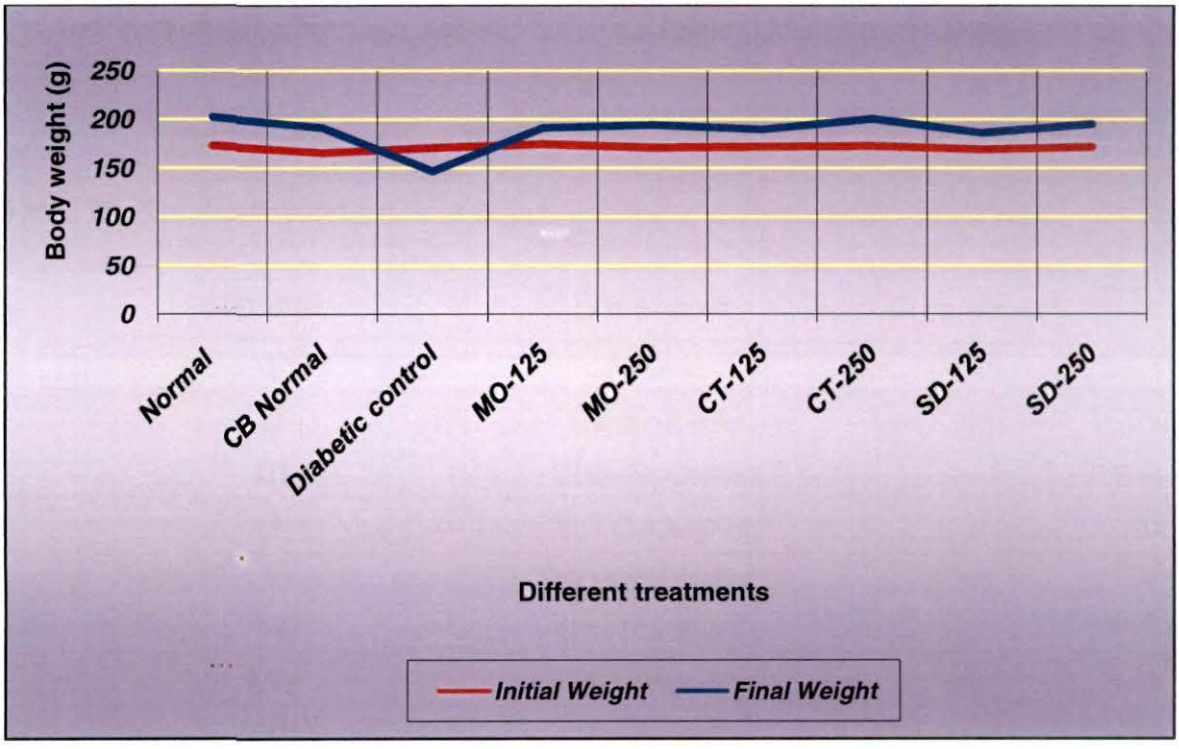


Fig- X : Effect of different extracts on changes in body weight; MO= *Moringa oleifera*, CT= *Cinnamomum tamala*, SD= *Scoparia dulcis.*

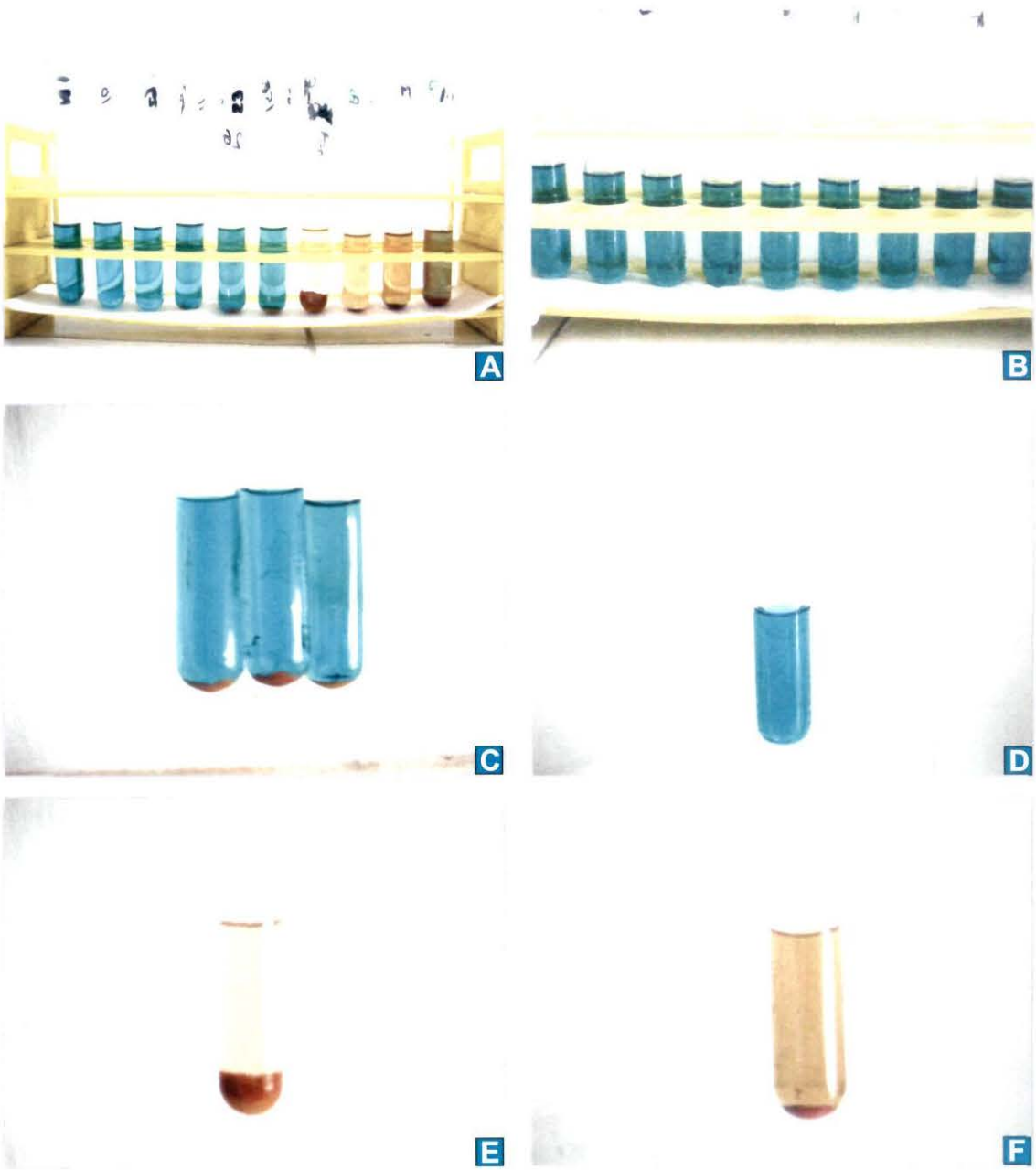


PLATE-XXIII : A: Result of urine test, B: Clear solution showing no sugar in the urine (after treatment with plant extract in the dose of 250 mg/kg, C: Minimum sugar in the urine in the STZ + plant extract (125 mg/ kg dose) treated rats, D: Clear solution showing no sugar in the urine of normal untreated rats, E and F: Maximum sugar in the STZ treated diabetic rats

4.10.3. Effect of different extracts on fasting blood glucose in control and diabetic rats

Tables 17 to 19 demonstrate the levels of blood glucose in normal and experimental rats (Fig XI to XIII). Fasting blood glucose levels in the control rats remained unchanged during the course of the experiment. There was a significant ($p< 0.05$) increase in blood glucose in diabetic rats after two days of STZ administration. The rats having blood glucose level more than 200 were considered for the study. In the study, all the extracts showed significant activity ($p< 0.05$) on the 20th day of treatment. *Cinnamomum tamala* reduced the fasting blood glucose level in the 20th day from STZ-2day by 72.52% (250mg/kg) and 54.20% (125mg/kg) followed by *Moringa oleifera* (250mg/kg- 68.50%, 125mg/kg- 42.93%), *Scoparia dulcis* (250mg/kg- 69.29%, 125mg/kg- 50.77%).

Table- 17 : Effect of different extracts on fasting blood glucose in normal and experimental rats

Group	Treatment	Fasting Blood Glucose (mg/dl) Days of STZ treatment	
		0	2
I	Normal (only vehicle distilled water)	76.10 ± 1.05	76.40 ± 0.25
II	Citrate buffer treated	76.85 ± 0.69	77.15 ± 0.83
III	Diabetic Control	76.40 ± 0.81	266.89 ± 1.38
IV	MO (125mg/kg)	75.96 ± 0.37	241.79 ± 1.98
V	MO (250 mg/kg)	77.49 ± 0.28	260.44 ± 0.69
VI	CT (125mg/kg)	78.20 ± 1.07	249.55 ± 0.92
VII	CT (250mg/kg)	77.52 ± 0.29	266.40 ± 0.28
VIII	SD (125mg/kg)	78.07 ± 0.29	238.09 ± 1.79
IX	SD (250mg/kg)	74.49 ± 0.49	259.43 ± 0.38
	SEM	0.5968	1.0636
	CD	1.9918	3.5498

Each value represents as mean± SE; MO= *Moringa oleifera*, CT= *Cinnamomum tamala*, SD= *Scoparia dulcis*. Values were statistically significant at $p < 0.05$ as compared with diabetic control, $p < 0.05$ as compared with normal.

The effect of administration of *Cinnamomum tamala* (250mg/kg and 125mg/kg) leaves extracts decreased significantly ($p< 0.05$) the level of blood glucose near to normal. After the 20th day in STZ- diabetic control rats blood glucose increased to 337.07mg/dl that was increased by 26.29% from STZ-2day. Administration of *Scoparia dulcis* (250mg/kg and 125mg/kg) also significantly ($p< 0.01$) decreased the level of blood glucose.

Table- 18 : Effect of different extracts on fasting blood glucose in normal and experimental rats

Group	Treatment	Fasting Blood Glucose (mg/dl) Days of STZ treatment	
		10	20
I	Normal (only vehicle distilled water)	75.51 ± 0.52	76.40 ± 0.25
II	Citrate buffer treated	77.45 ± 0.60	77.15 ± 0.83
III	Diabetic Control	290.94 ± 1.69	266.89 ± 1.38
IV	MO (125mg/kg)	195.84± 3.08	241.79 ± 1.98
V	MO (250 mg/kg)	176.40 ± 1.82	260.44 ± 0.69
VI	CT (125mg/kg)	78.20 ± 1.07	249.55 ± 0.92
VII	CT (250mg/kg)	77.52 ± 0.29	266.40 ± 0.28
VIII	SD (125mg/kg)	78.07 ± 0.29	238.09 ± 1.79
IX	SD (250mg/kg)	74.49 ± 0.49	259.43 ± 0.38
	SEM	1.3997	2.1180
	CD	4.6716	7.0689

Each value represents as mean ± SE; Values were statistically significant at $p < 0.001$ as compared with diabetic control, $p < 0.01$ as compared with control. MO= *Moringa oleifera*, CT= *Cinnamomum tamala*, SD= *Scoparia dulcis*.

Treatment with *Moringa oleifera* (250mg/kg and 125mg/kg) significantly ($p< 0.01$) decreased blood glucose and brought them near to normal level. The administration of *Cinnamomum tamala* to diabetic rat decreased the blood glucose to 73.20mg/dl and shown more effective than *Moringa oleifera, Scoparia dulcis.* Fasting blood glucose levels remain unchanged during the treatment.

Table-19 : Effect of different extracts on changes in fasting blood glucose in normal and experimental rats (Initial-final)

Group	Treatments	Initial	Final
I	Normal (only vehicle distilled water)	76.11 ± 1.05	77.75 ± 2.89
II	Citrate buffer treated	76.85 ± 0.68	76.85 ± 1.13
III	Diabetic Control	76.40 ± 0.81	337.07 ± 6.36
IV	MO (125mg/kg)	75.95 ± 0.37	137.97 ± 1.73
V	MO (250 mg/kg)	77.49 ± 0.28	82.02 ± 1.84
VI	CT (125mg/kg)	78.20 ± 1.07	114.26 ± 0.84
VII	CT (250mg/kg)	77.53 ± 0.29	73.20 ± 1.86
VIII	SD (125mg/kg)	78.08 ± 0.29	117.19 ± 1.18
IX	SD (250mg/kg)	74.49 ± 0.49	79.66 ± 0.97
	SEM	0.59	2.11
	CD	1.99	7.06

MO= *Moringa oleifera*, CT= *Cinnamomum tamala*, SD=*Scoparia dulcis*. Values were statistically significant at $p < 0.01$ as compared with diabetic control, $p < 0.01$ as compared with normal.

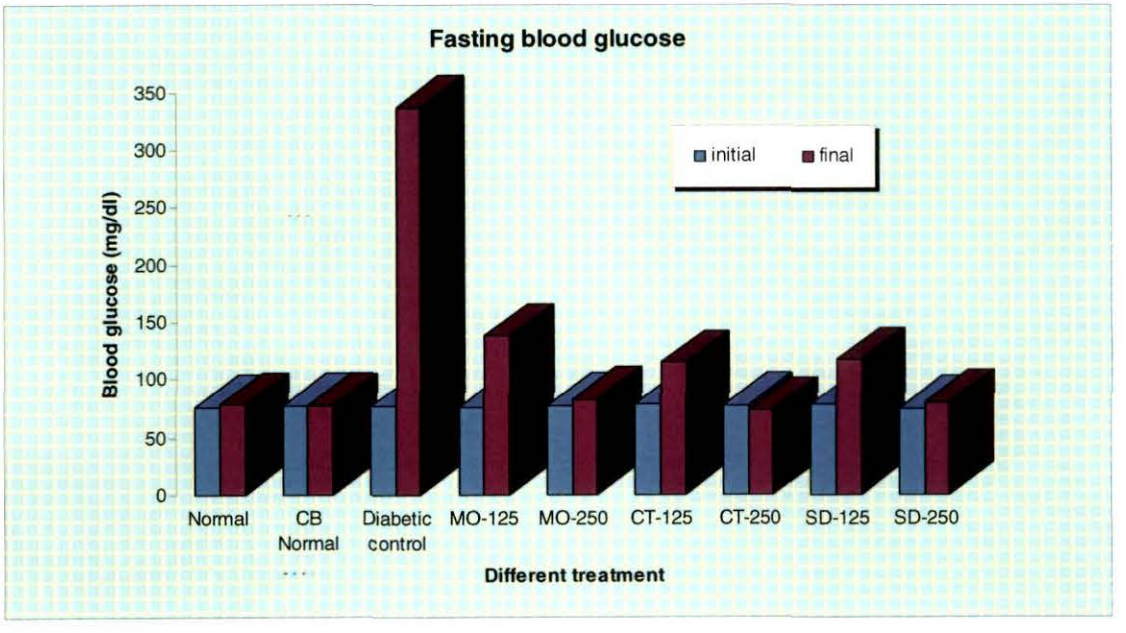


Fig- XI : Effect of different extracts on fasting blood glucose. MO= *Moringa oleifera*, CT= *Cinnamomum tamala*, SD= *Scoparia dulcis*.

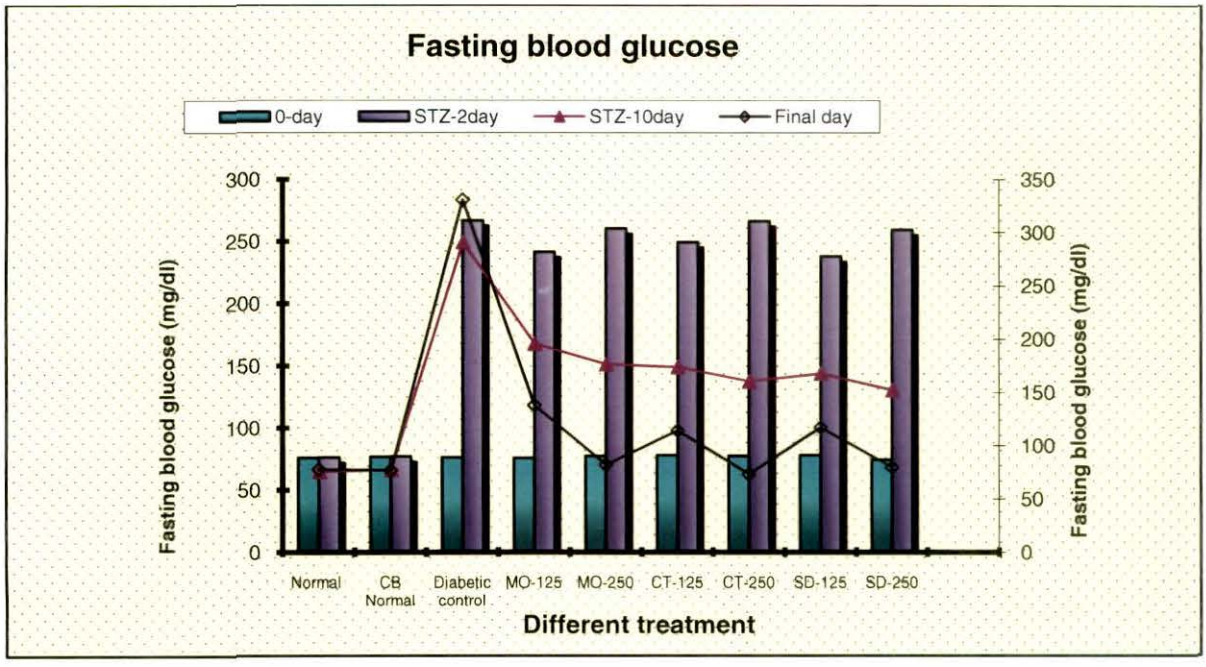


Fig-XII : Effect of different extracts on Fasting Blood glucose levels. MO= *Moringa oleifera*, CT= *Cinnamomum tamala*, SD= *Scoparia dulcis.*

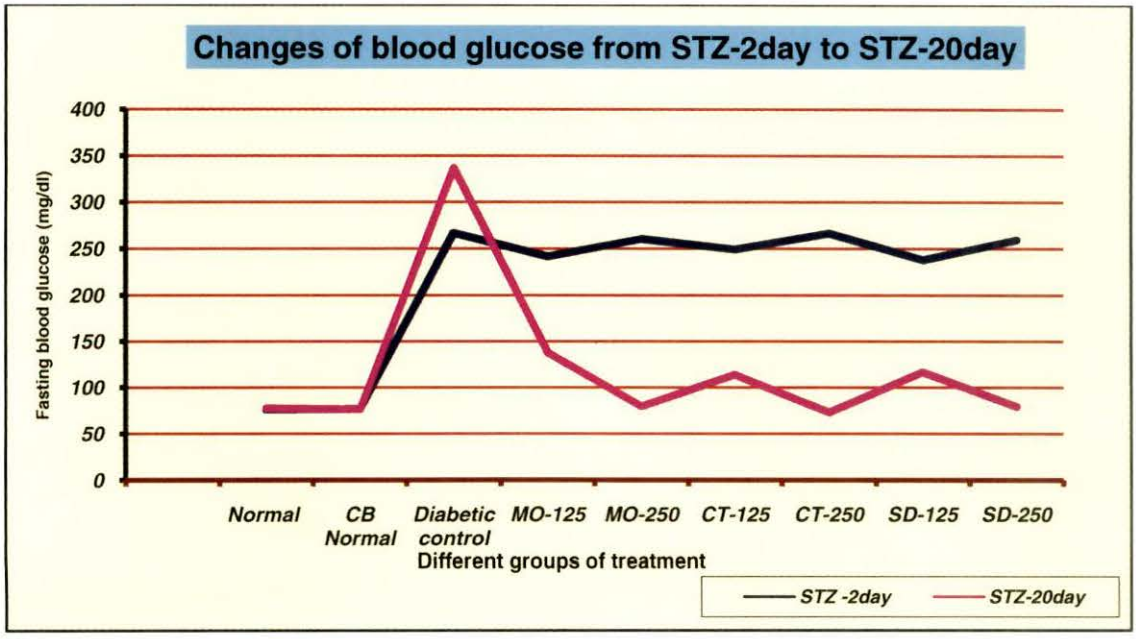


Fig-XIII : Effect of different extracts on fasting blood glucose (STZ-2day- STZ 20day).
MO= *Moringa oleifera*, CT= *Cinnamomum tamala*, SD= *Scoparia dulcis.*

4.10.4. Effect of different extracts on glycogen in control and diabetic rats

The effects of *Cinnamomum tamala, Moringa oleifera, Scoparia dulcis* leaves extracts on Glycogen content are shown in the Table 20 and Fig XIV. The level of glycogen content decreased (21.17 mg/100g) significantly ($p< 0.001$) in the STZ-diabetic rats as compared to control (40.64 mg/100g). Administration of *Scoparia dulcis* (250mg/kg and 125mg/kg) increased the level of glycogen (41.33 and 30.53 mg/100g) in the liver significantly ($p< 0.001$). Treatment with *Cinnamomum tamala* (250mg/kg and 125mg/kg) significantly ($p< 0.001$) increase the glycogen (44.04 mg/100g and 33.41 mg/100g) and brought them near to normal level. Administration of *Moringa oleifera* (250mg/kg and 125mg/kg) increases the levels of glycogen (40.19 mg/100g and 30.16 mg/100g) in the liver ($p< 0.001$) during diabetes.

Table 20 : Effect of different extracts on glycogen in control and diabetic rats

Groups	Treatment	Glycogen (mg/100g)
I	Normal	40.65 ± 2.58
II	Citrate Buffer	41.40 ± 2.77
III	Diabetic Control	21.18 ± 2.66
IV	MO (125mg/kg)	30.16 ± 2.58
V	MO (250 mg/kg)	40.20 ± 1.39
VI	CT (125mg/kg)	33.41 ± 1.24
VII	CT (250mg/kg)	44.05 ± 1.24
VIII	SD (125mg/kg)	30.53 ± 1.46
IX	SD (250mg/kg)	41.33 ± 0.72
	SEM	1.61
	CD	5.38

Each value represents as mean± SE; Values were statistically significant at $p < 0.001$ as compared with diabetic control. MO= *Moringa oleifera*, CT= *Cinnamomum tamala*, SD= *Scoparia dulcis*

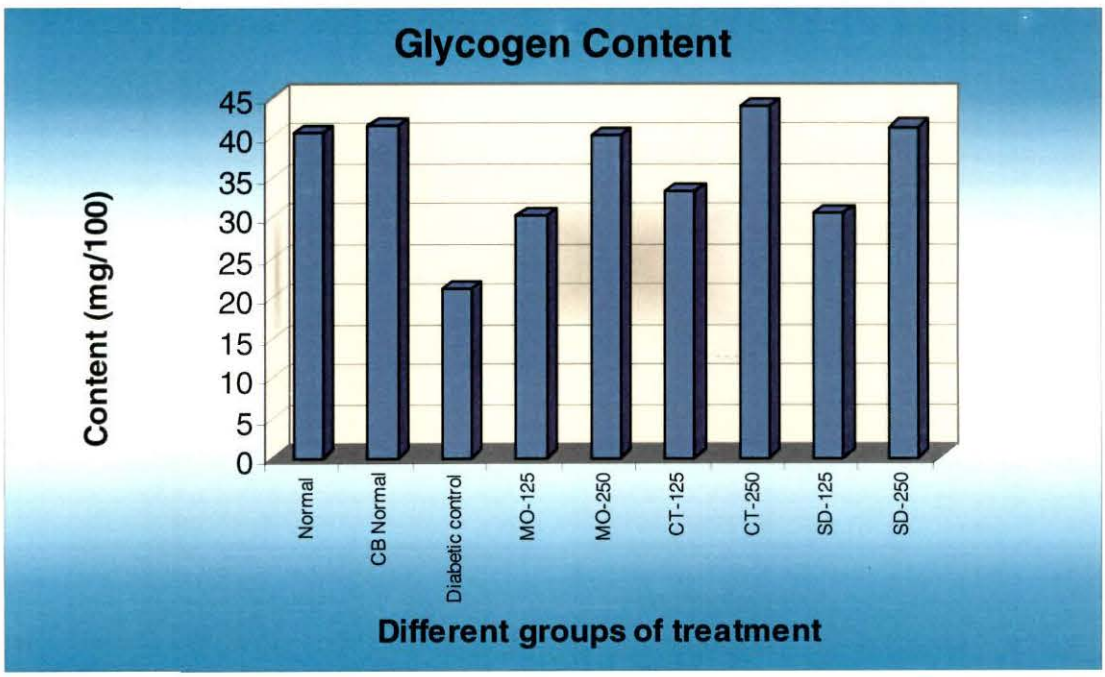


Fig-XIV : Effects of different extracts on glycogen levels. MO = *Moringa oleifera*, CT = *Cinnamonum tamala*, SD = *Scoparia dulcis*.

4.10.5. Effect of different extracts on TBARS level in control and diabetic rats

Changes in the concentration of TBARS in the liver of STZ-diabetic rats on the treatment with *Cinnamomum tamala, Moringa oleifera, Scoparia dulcis* leaves extracts are depicted in the Table 21 and Fig XV. The STZ-diabetic rats showed a significant increase in TBARS (1.84 mM/100g) when compared with normal ((d H_2O-0.81 mM/100g and citrate buffer-0.79 mM/100g) in liver. Thiobarbituric acid reactive substance levels were decreased in the *Cinnamomum tamala, Moringa oleifera, Scoparia dulcis* leaves extracts treated groups when compared with the normal rats. Treatment with *Moringa oleifera* (250mg/kg and 125mg/kg) significantly ($p< 0.001$) prevented the increase (0.90 mM/100g and 1.22 mM/100g) in TBARS levels and brought them near to normal level. Administration of *Scoparia dulcis* (250mg/kg and 125mg/kg) significantly ($p< 0.001$) decreased (0.84 mM/100g and 1.22 mM/100g) the level of TBARS in the liver. There was a significant ($p< 0.001$) reduction (1.00 mM/100g and 1.16 mM/100g) in the activity of TBARS in the liver of rats with the treatment of *Cinnamomum tamala* (250mg/kg and 125mg/kg) when compared with normal rats.

Table-21 : Effect of different extracts on TBARS level in control and diabetic rats

Groups	Treatment	TBARS (mM/100g)
I	Control	0.82 ± 0.03
II	Citrate buffer control	0.79 ± 0.05
III	Diabetic Control	1.84 ± 0.12
IV	MO (125mg/kg)	1.23 ± 0.01
V	MO (250 mg/kg)	0.91 ± 0.05
VI	CT (125mg/kg)	1.16 ± 0.02
VII	CT (250mg/kg)	1.01 ± 0.02
VIII	SD (125mg/kg)	1.22 ± 0.01
IX	SD (250mg/kg)	0.85 ± 0.06
	SEM	0.04
	CD	0.14

Each value represents as mean ± SE, MO= *Moringa oleifera*, CT= *Cinnamomum tamala*, SD= *Scoparia dulcis*. Values were statistically significant at $p < 0.001$ as compared with diabetic control.

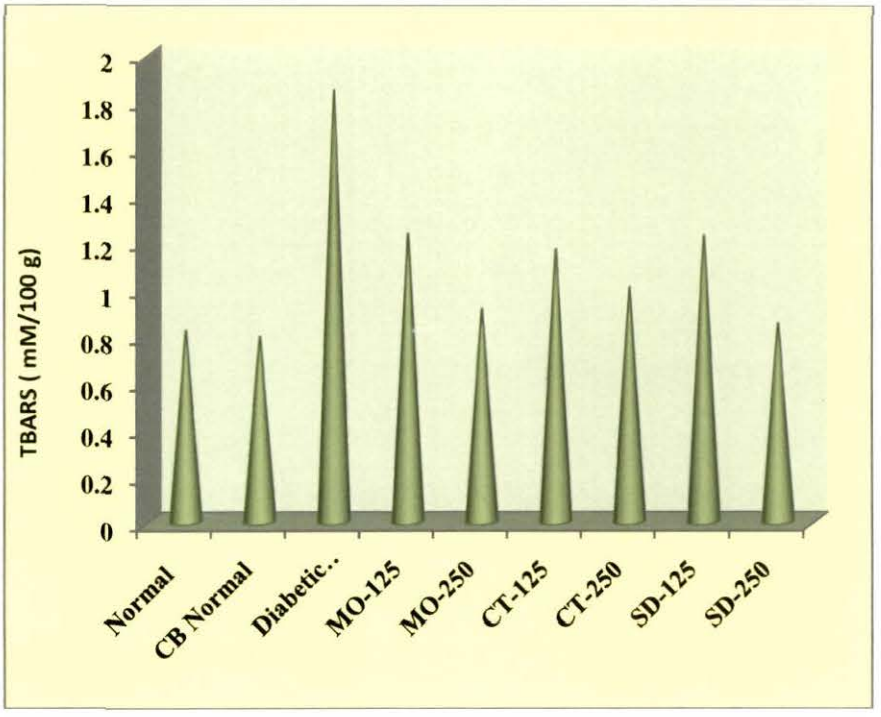


Fig-XV : Effects of Different extracts on TBARS levels
MO= *Moringa oleifera*, CT= *Cinnamomum tamala*, SD= *Scoparia dulcis*)

4.10.6. Effect of different extracts on reduced glutathione (GSH) in control and diabetic rats

The concentrations of GSH in tissues in experimental diabetic rats are shown in Table 22 and Fig XVI. It was seen that the reduced glutathione level in the liver of STZ-diabetic rats were significantly ($p< 0.01$) decreased. Administration of *Cinnamomum tamala* (250mg/kg and 125mg/kg) increased the levels of GSH in the liver ($p< 0.01$) during diabetes. A significant ($p< 0.01$) increased of GSH level was observed in the *Moringa oleifera* (250mg/kg and 125mg/kg) treated group. Administration of *Scoparia dulcis* (250mg/kg and 125mg/kg) also significantly ($p< 0.01$) increased the level of GSH in the liver.

Table-22 : Effect of different extracts on Reduced Glutathione (GSH) in control and diabetic rats

Groups	Treatment	Reduced glutathione (mM/100g tissue)
I	Control (distilled water treated)	45.33 ± 1.76
II	Citrate buffer control	46.00 ± 1.52
III	Diabetic Control	25.66 ± 1.85
IV	MO (125mg/kg)	32.00 ± 1.15
V	MO (250 mg/kg)	39.33 ± 0.87
VI	CT (125mg/kg)	35.66 ± 1.2
VII	CT (250mg/kg)	45.00 ± 0.57
VIII	SD (125mg/kg)	33.33 ± 1.45
IX	SD (250mg/kg)	44.00 ± 1.15

Each value represents as mean ± SE; MO= *Moringa oleifera*, CT= *Cinnamonum tamala*, SD= *Scoparia dulcis*. Values were statistically significant at $p < 0.01$ as compared with diabetic control, $p < 0.01$ as compared with control.

4.10.7. Correlation analysis

Correlation and regression graphs are presented in Figs XVII and XVIII. Since results showed significant trends in body weight as well as other biochemical tests following hypergylcaemia induction and subsequent plant extract treatments, correlation between

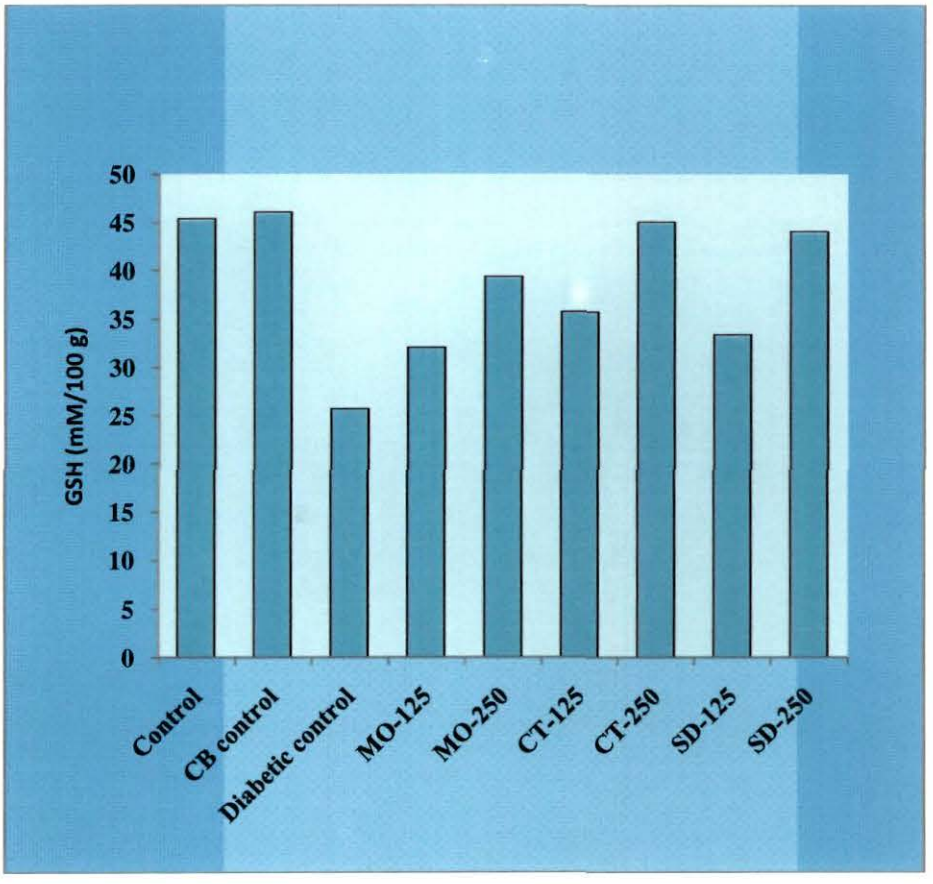
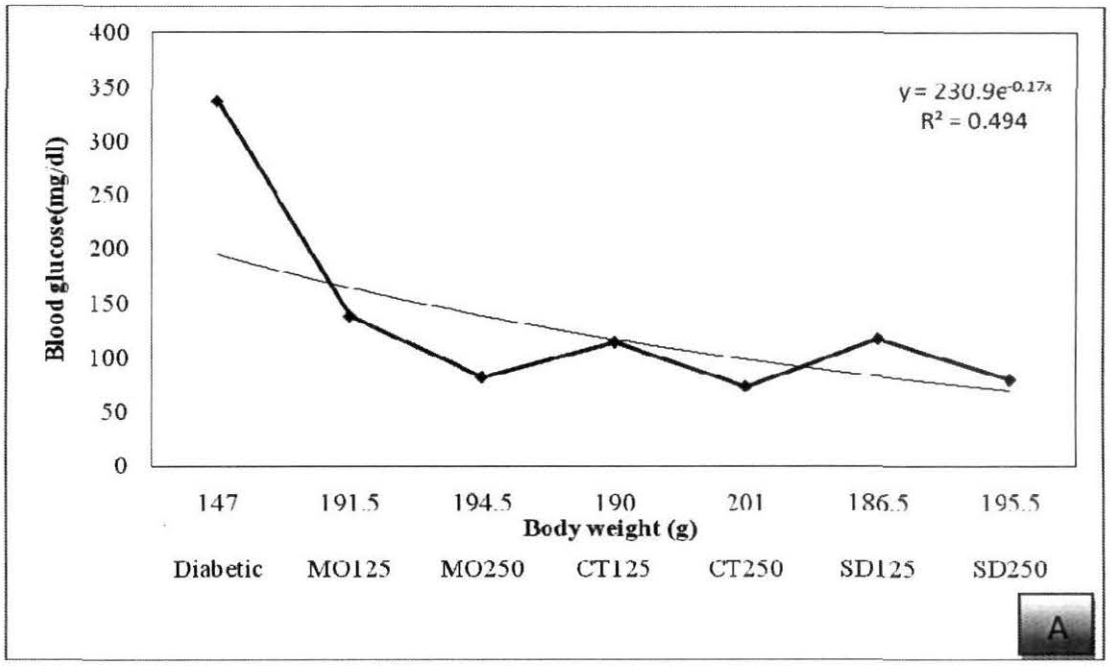


Fig-XVI : Effects of Different extracts on GSH levels
MO= *Moringa oleifera*, CT= *Cinnamomum tamala*, SD= *Scoparia dulcis*)



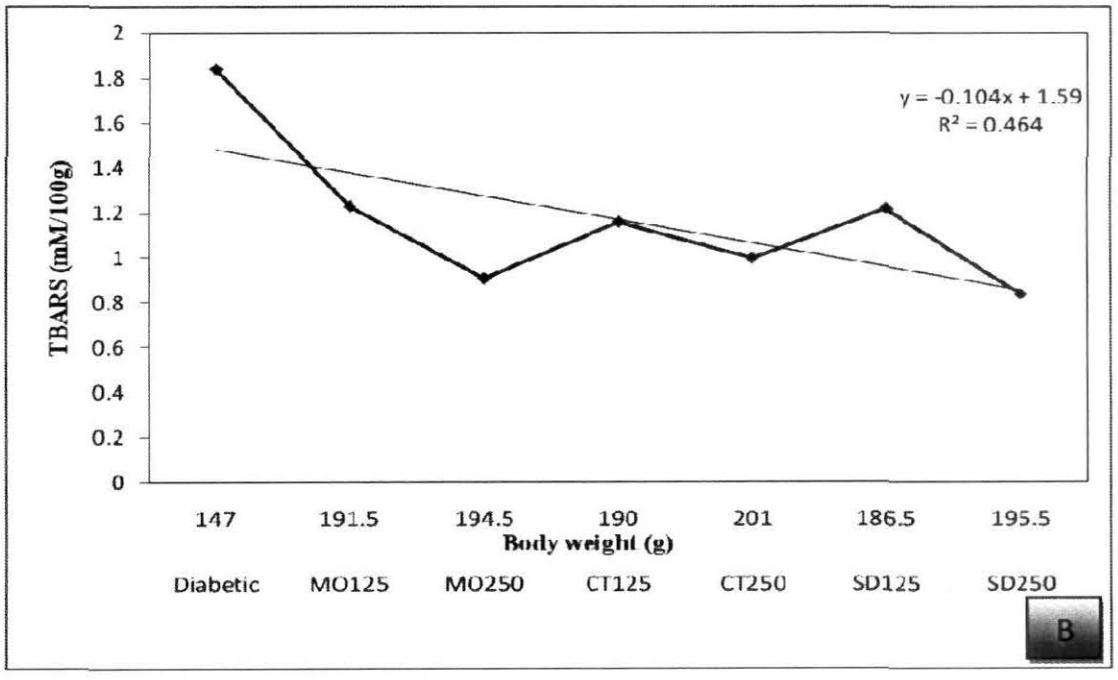
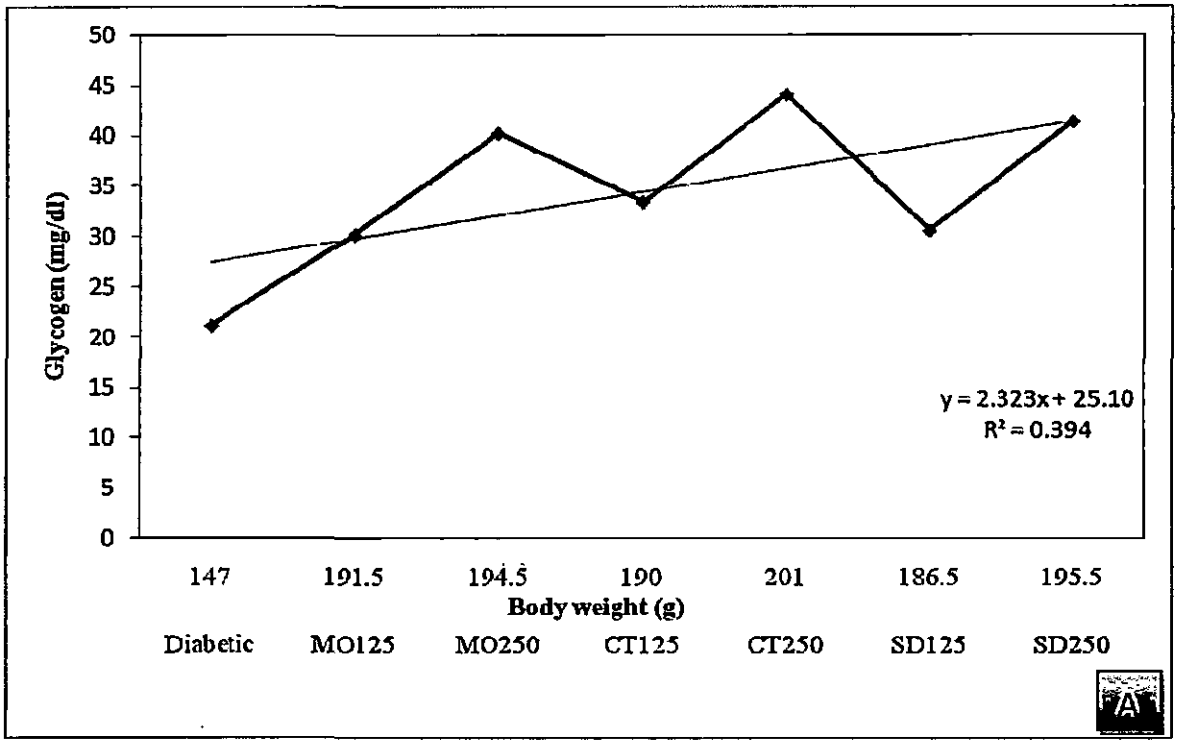


Fig.XVII: Correlation and regression between body weight of diabetic and treated rats and blood glucose content (A) and TBARS (B).



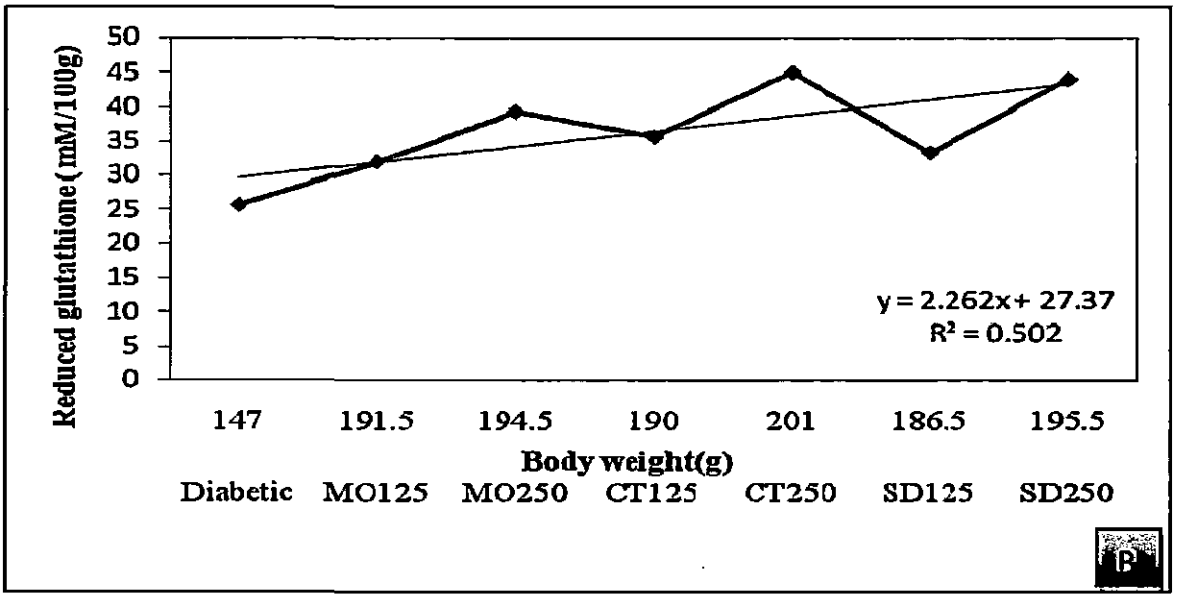


Fig.XVIII: Correlation and regression between body weight of diabetic and treated rats and glycogen (A) and GSH (B).

body weight and blood glucose, TBARS, glycogen and GSH were calculated. Body weight and blood glucose, as well as body weight and TBARS showed high negative correlation (r= -0.985 an -0.939, respectively). On the other hand, correlation between body weight and glycogen, as well as body weight and GSH, was also high, but positive (r= 0.862 and 0.826, respectively). Thus, it is clear that with increase in body weight of diabetic rats due to treatment of plant extracts, there was a decrease in blood glucose and TBARS. On the other hand, glycogen and GSH increased along with increase in body weight.

DISCUSSION

Medicinal plants which constitute a segment of the flora provide raw material for use in all indigenous systems of medicine in India namely Ayurveda, Unani, Siddha and Tibetan Medicine. On account of the fact that the derivatives of medicinal plants are non-narcotic having no side-effects, the demand for these plants is on the increase in both developing and developed countries. Some good works of the preliminary sort have been done several years earlier by Kiritikar and Basu (1935), Nadkarni (1954), Chopra *et al.* (1956) and many others. Higher plants are still "the sleeping giant of drug development", a virtually untapped reservoir of potentially useful sources of drugs (Farnsworth, 1994) that will continue to serve mankind in the 21st century as they have done since the dawn of history.

The present course of investigation has been carried out to know the traditional knowledge about the uses of medicinal plants, generally predominant in the selected areas of Dakshin Dinajpur district and test a few selected plants scientifically to confirm the ethnobotanical reports. The proposed work was divided into two phases. In the first phase the traditional knowledge about medicinal plants of the villagers was collected from the selected areas of the district. In the second phase few of the lesser known ones have been selected and detailed studies have been made on them. Regarding medicinal properties, attempts were made to study the properties like pharmacological, antimicrobial, production of antioxidants and suppression of hyperglycemia.

The present study has revealed that the tribals of Dakshin Dinajpur district are very rich in traditional knowledge. The studies were carried out in 48 villages of Dakshin Dinajpur district. After repeated interactions with the local doctors practicing siddha, Ayurveda and unani (Indian alternative medical systems) as well as villagers it was learnt that these plants listed in the investigation were very much used by them in making various formulations for a variety of ailments. The tribal inherit rich traditional knowledge about the flora investigated and apply this knowledge for making crude phytomedicines to cure infections as simple as cold to as complicated as cancer. These crude herbal medicines were based not only on traditional knowledge but also on rituals and beliefs. The data were gathered with the help of elders and especially tribal villagers. The youngsters are not well versed about the uses as well as not much interested to use the medicinal plants. Due to changing lifestyle and modernization the younger villagers prefer allopathic medicines. Therefore, an ethnobotanical survey is necessary to record this vanishing traditional knowledge.

The information about medicinal plants on scientific name, local name, plant part(s) used and method of dosage has been provided. There are several reports of similar works. During the ethnomedicobotanical survey of Jaunsar-Bawar (Jain and Puri, 1984) a hilly tribal inhabited area in Uttar Pradesh, India, it was observed that about 100 plants were being used by the local Jaunsari tribe for the treatment of various ailments. An alphabetical list of the plants was given along with their family, local name, local uses, and locality and collection number. Jain *et al.* (2005) made another survey on the medicinal plants diversity of Sitamata wildlife sanctuary of Chittorgarh and Udaypur district located in south-west region of Rajasthan. Two hundred forty-three genera belonging to 76 families have been reported which were used by the tribal of about 48 villages around the sanctuary as of means of primary health care to cure various ailments.

The present investigation has revealed the usage of 107 medicinal plants species mentioned by the villagers of Dakshin Dinajpur of West Bengal. These plants belong to the 96 genera and 48 families. Highest number of medicinal plants (14) was found in the family Leguminaceae followed by Acantheceae, Apiaceae, Apocynaceae, Cucurbitaceae, Euphorbiaceae containing each 4 medicinal plants. It was revealed that the ethnomedicinal plants were mostly used to cure the diseases like dysentery, stomach/ liver diseases, cold-cough, rheumatism, skin diseases, urinary track infection etc. An ethnomedicinal survey was carried out by Ayyanar and Ignacimuthu (2005) among the ethnic group (Kani/Kanikaran) in Southern-western Ghat of India. The documented ethnomedicinal plants were mostly used to cure skin diseases, poison bites, wounds and rheumatism. Some indigenous tribes from Northwest Mexico have traditionally used this plant to treat skin ailments by externally applying it on the affected areas (Lopez and Hinojosa, 1988; Xolapa- Molina, 1994). Muthu (2006) also documented medicinal plants for the treatment of stomachache; skin diseases, poison bites and nervous disorders after he made a survey on the uses of medicinal plants in Kancheepuram district of Tamil Nadu. Leporatti *et al.* (1985) while investigated on some new therapeutic used of several medicinal plants in the Province of Terni (Umbria, Central Italy) reported the use of 18 medicinal plants for that therapeutic purpose.

In the present study, more traditional knowledge was also collected about the antidiabetic plants. The plants used to treat diabetes are *Abroma augusta, Aegel marmelos, Cajanus indicus, Catheranthus roseus, Cinnamomum tamala, Coccinia cordifolia, Enhydra fluctuans, Ficus carica, Melia azadirecta, Mimosa pudica, Momordica charanta, Moringa*

oleifera, Murraya koenigi, Musa paradisiacal, Piper longum, Punica granatum, Scoparia dulcis, Syzigium cumini, Tamarindus indica. Chhetri (2005) made a survey on the antidiabetic plants used by Sikkim and Darjeeling Himalayan tribes, India. Harbal medicine was the dominant system of medicine practiced by the local tribes of that region for the treatment of diabetes. During the course of their studies it was found that 37 species of plants belonging to 28 families were used as antidiabetic agents in the folk medicinal practice in the region and 80% of those plants wee hitherto unreported as hypoglycemic agents. Their finding may lead to serious research towards developing new and efficient drugs for diabetes.

Among the 107 plants reported to contain some or other medicinal properties, four have been selected viz., *Clerodendrum viscosum, Cinnamomum tamala, Moringa oleifera* and *Scoparia dulcis* for detailed study. After selection of these plants, the effectiveness of the leaves of *C. viscosum, C. tamala, M. oleifera* and *S. dulcis* by phytochemical, microbiological, antioxidants and suppression of hyperglycemia were investigated in detail.

M. oleifera is the most widely cultivated species of a monogeneric family, the Moringaceae that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. *M. oleifera*, or the horseradish tree, is a pan-tropical species that is known by such regional names as benzolive, drumstick tree, kelor, marango, mlonge, mulangay, nébéday, saijhan, and sajna. Over the past two decades, many reports have appeared describing its nutritional and medicinal properties. In the West, one of the best known uses for *M. oleifera* is the use of powdered seeds to flocculate contaminants and purify drinking water (Berger *et al.*, 1984; Olsen, 1987; Gassenschmidt,1995 but the seeds are also eaten green, roasted, powdered and steeped for tea or used in curries (Gassenschmidt, 1995). This tree has in recent times been advocated as an outstanding indigenous source of highly digestible protein, Ca, Fe, Vitamin C, and carotenoids suitable for utilization in many of the so-called "developing" regions of the world where undernourishment is a major concern.

In the present study, the survey has shown that *M. oleifera* is used to treat various ailments i.e, blood sugar, rheumatism, cardiac problem etc. *M. oleifera* preparations have been cited in the scientific literature as having antibiotic, antitrypanosomal, hypotensive, antispasmodic, antiulcer, anti-inflammatory, hypocholesterolemic, and hypoglycemic activities, as well as having considerable efficacy in water purification by flocculation, sedimentation, antibiosis and even reduction of *Schistosome cercariae* titer. *Moringa*

species have long been recognized by folk medicine practitioners as having value in tumor therapy (Hartwell. 1967-1971; Fahey *et al.,* 2004). The benefits for the treatment or prevention of disease or infection that may accrue from either dietary or topical administration of *Moringa* species preparations (e.g. extracts, decoctions, poultices, creams, oils, emollients, salves, powders, porridges) are not quite so well known (Palada, 1996).

Scoparia dulcis L. (Scrophulariaceae) is a perennial herb widely distributed in tropical and subtropical regions. In these regions, the fresh or dried plant of *Scoparia dulcis* has traditionally been used as one of the remedies for stomach troubles (Satyanarayana, 1969), hypertension (Chow *et al.* 1974), diabetes (Perry, 1980), inflammation (Gonzales, 1986), bronchitis (Farias *et al.* 1993), hemorroids and hepatosis (Satyanarayana, 1969) and as analgesic and antipyretic (Gonzales, 1986). In the present survey, it was found that *Scoparia dulcis* is used by the people for long time for the diseases like blood sugar, anti-inflamatory, sore and cough.

Leaves of *Cinnamomum tamala* are used in colic and diarrheal preparations. *C. tamala* leaf extracts produce a hypoglycaemic effect in experimental rats. Leaves of *Clerodendrum viscosum* used in malarial fever, any fever, worm, dysentery, piles etc.

In the present work, comparative study has been made to determine the effectiveness of phytochemical, microbiological, antioxidative and antidiabetic potential of the leaves of *C. viscosum, C. tamala, M. oleifera* and *S. dulcis.* The plants used for this study was not chosen at random, but on the basis of analysis of survey.

Phytochemical screening of *C. viscosum, C. tamala, M. oleifera* and *S. dulcis* revealed the presence of secondary metabolites including alkaloids, flavonoids, tannins, saponins, cardic glycosides and terpenoids. Saponins have been reported to possess good antihyperglycemic activity in recent studies (Sauvaire *et al.*, 1996; Vats *et al.*, 2003). Glycosides are the plant compounds containing glucose (or a different sugar) combined with other non-sugar molecules, such as glucose + terpene or glucose + phenolic compound. The terms glycoside and glucoside are used interchangeably; however, glucoside is generally used if the sugar component is glucose. Saponins are a group of glycosides (glucosides) found in several plant species, and are characterized by their soap-like property of foaming in a water solution.

Steroid was present in *C. viscosum, C. tamala, M. oleifera* but absent in *S. dulcis.* Some of these compounds were shown to have anti-inflammatory, antibacterial, hepatoprotective, immuno-modulator, cardiovascular, diuretic, protozoocidal, fungicidal, molluscidal, cytotoxic, cytostatic, antitumar activities (Guisalberti, 1998; Bermejo- Benito *et al.* 1998; Emam *et al* 1997; Nishibe, 1994; Lacaille- Duubois and Wagner, 1996). Similar findings have been observed by Shirwaikar *et al.*, 2005. In their work, preliminary screening of the aqueous extract of *Coccinium fenestratum* revealed the presence of saponin, alkaloids, and phenolic substances.

Among the four studied plants highest amount of alkaloid was present in *C.* tamala (4.92% alkaloid/ g tissue), followed by *M. oleifera* (2.56% alkaloid/ g tissue), *C. infortunatum* (1.76% alkaloid/ g tissue) *and S. dulcis* (0.84% alkaloid/ g tissue). Alkaloids are important defense of the plant against pathogenic organisms and herbivores. It is also toxic to insects which further modify the alkaloids and incorporate them into their own defense secretion (Hartmann, 1991).

In the present study, tests revealed that leaves of *M. oleifera* contained more amount of protein than the leaves of other three studied plants and also the total sugar and reducing sugar content were very high in leaves of *M. oleifera* than the leaves of other three studied plants. Ascorbic acid (vitamin C) is an abundant component of plants. Vitamin C protects cells from the damaging oxidation of free radicals. The spetrophotometric analysis of ascorbic acid revealed that *C. tamala* contained the highest amount of ascorbic acid followed by *M. oleifera, S. dulcis.* The least amount of ascorbic acid found in the *C. viscosum.*

The most active form of vitamin E, μ-tocopherol, is a 6-hydroxychroman derivative with methyl groups in position 2, 5, 7, and 8 and a phytyl side chain attached at carbon 2. Vitamin E is important for human and animal health. Many human diseases, such as certain cancers and neurodegenerative and cardiovascular disease, are associated with the insufficient intake of vitamin E. Protective effects of vitamin E as an antioxidant in diabetes were studied extensively (Baydas *et al.*, 2002; Celik *et al.*, 2002). The presence of μ-tocopherol in the n-hexane extract of *Ficus carica* leaves was determined by thin-layer chromatography (TLC) by Konyalioglu *et al.*, 2005). The amount of μ-tocopherol extracted for 100g of dried leaves of *Ficus carica* was 57mg. In comparison with previous studies on the μ-tocopherol content of plant leaves, the amount of μ-tocopherol in dried leaves of *Myrtus communis, Rhamnus alternus* and *Phillyrea angustifoia* were found to be 846, 627,

480 and 442 ppm (Chevolleau *et al.*1993). The major industrial source of μ-tocopherol is a residue obtained from the distillation of soya bean oil (Slover, 1983). In the present investigation also, the presence of μ-tocopherol in the n-hexane extract of *C. viscosum, C. tamala, M. oleifera* and *S. dulcis* leaves were determined by thin-layer chromatography (TLC). Lako *et al.*, (2007) have investigated the phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. A number of herbs exhibited high antioxidant capacity: *Ipomoea batatas* (sweet potato) leaves have the highest TAC (650 mg/100 g) and are rich in TPP (270 mg/100 g), quercetin (90 mg/100 g) and β-carotene (13 mg/100 g). *Moringa oleifera* (drumstick) leaves were rich in TPP (260 mg/100 g), quercetin (100 mg/100 g), kaempferol (34 mg/100 g) and β-carotene (34 mg/100 g). *Curcuma longa* (turmeric ginger) had a high TAC (360 mg/100 g), TPP (320 mg/100 g) and was rich in fisetin (64 mg/100 g), quercetin (41 mg/100 g) and myricetin (17 mg/100 g). *Zingiber officinate* (white ginger) also had a high TAC (320 mg/100 g) and TPP (200 mg/100 g). *Zingiber zerumbet* (wild ginger), a widely used herb taken before meals was the richest source of kaempferol (240 mg/100 g).

The phytochemical screening and quantitative estimation of the percentage crude yields of chemical constituents of the plants studied showed that the leaves and stems were rich in alkaloids, flavonoids, tannins and saponins. They were known to show medicinal activity as well as exhibiting physiological activity (Sofowara, 1993). Edeoga *et al.*, (2005) made a study on ten medicinal plants for the analysis of alkaloids, tannins, saponins, steroids, terpenoids, phlobatannin and cardiac glycoside.

Plant derived natural products such as flavonoids, terpenoids and steroids etc. have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and hepatoprotective activity (DeFeudis *et al.*, 2003, Takeoka and Dao, 2003, Banskota *et al.*, 2000).There has been a growing interest in the analysis of certain flavonoids, triterpenoids and steroids stimulated by intense research into their potential benefits to human health. One of their main properties in this regard is their antioxidant activity. Antioxidants play an important role in inhibiting and scavenging radicals, thus providing protection to humans against infection and degenerative diseases.

In recent time much attention has been focused on the protective biochemical function of naturally occurring antioxidants in biological systems and on the mechanism of their action. The TAC method, based on the reduction of Mo (VI) to Mo (V) by sample analyze,

was used to measure the amount of antioxidant capacity. The spectrometric assay for the quantitative determination of antioxidant capacity was previously determined for *Hypericum* species. The results expressed as nM μ-tocopherol acetate/g dry mass were in the range 4.615-5.483 (Merel, 2003). The antioxidant capacity for *Ficus carica* leaves ranged from 14.0 to 23.5 mM μ-tocopherol acetate/g dry mass with the water extract having the highest activity (Konyalioglu *et al.*, 2005). The results of their study showed that water extract also has the highest total phenol and flavonoid content. There was a correlation between the amount of total phenol and flavonoid contents and the antioxidant capacity.

The spectrophotometric assay for the quantitative determination of phenol revealed that *C. tamala* contained the highest amount of phenol, *C. viscosum* contained the least amount, *M. oleifera* and *S. dulcis* contained significant amount respectively. The spectrophotometric results expressed as mg α-tocopherol acetate/ g dry mass were 11.2 in *C. tamala* that is highest quantity among the other three plants in *M. oleifera* 7.0, in *S. dulcis* 5.9 and in *C. viscosum* 4.5 mg α-tocopherol acetate/ g dry mass. Thus it was found that all the studied plant leaves posses substances having high antioxidant activity. Despite much interest in the antioxidant activity of the studied leaves, it is uncertain which of the phenols and μ-tocopherol exhibit the greatest antioxidant effect.

In order to partially characterize the extracts, the leaves were extracted with different solvents and analysed by UV-spectrophotometer. Absorption maxima were in the range of 202-208 nm in most cases. Interestingly, except *Poria hypobrumea* all the aqueous extracts, as well as solvent extracts had no antimicrobial activity, though there are several reports of plant extracts showing anti-microbial activity. Anti-microbial activity of higher plants was well documented (Saxena and Vyas, 1986; Ahmed *et al.*, 1995; Ahmed *et al.*, 1998; David, 1997; Perumalsamy *et al.*, 1988). A number of studies on anti-microbial activity of plants have been carried out in different parts of world (Belachew, 1993; Lajubuter *et al.*, 1995; Didry *et al.*, 1998; Yadava and Barsainya, 1998). The antimicrobial and antifungal effects of different concentrations of chloroform/methanol fractions of *Scoparia dulcis* were investigated by Latha *et al.*, (2006). The isolated fractions were tested against different bacteria like *Salmonella typhii*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Proteus vulgaris* and fungal strains such as *Alternaria macrospora*, *Candida albicans*, *Aspergillus niger*, and *Fusarium oxysporum*.

Though the present investigation was started with four plants, further work on anti hyperglycaemic activity was carried out only with *M. oleifera*, *S. dulcis* and *C. tamala*, but not with *C. viscosum* as no such ethnomedicinal claim has been reported for this plant.

Traditional preparations for the treatment of diabetes mellitus continue to receive wide spread use in many countries (Said, 1969). Several such plant preparations have shown to posses hypoglycemic properties in animal models of diabetes and in human non- insulin dependant diabetes mellitus patients (Hale *et al.*, 1989; Ruvin Kumar, 1998). The use of local plants for the treatment of diabetes mellitus is quite common in Asia and Middle Eastern countries. More than 400 local plants treatments for diabetes mellitus have been recommended by traditional health care providers (Ajaonkar, 1979; Bailey and Day, 1989; Ivorra *et al.*, 1989). In Indian folk medicine, many plants are used in the treatment of diabetes mellitus e.g., *Syzigium cumini, Musa sapientum* etc. (Prince *et al.*, 1998; Pari and Maheswari, 2000).

For testing the antidiabetic property, *C. tamala, M. oleifera* and *S. dulcis* were selected. For detecting the hyperglycemia in rats the parameters tested were fasting blood glucose, urine sugar, and glycogen, TBARS and GSH of liver tissues. Diabetes was induced in male rats via a single intraperitoneal (i.p.) injection of 55mg/kg body weight streptozotocin in 0.1M citrate buffer, pH 4.5, in a volume of 1ml/kg (Siddique *et al.*, 1987). After 48 h of streptozotocin administration, rats with moderate diabetes having glycosuria and hyperglycemia (i.e., with blood glucose of 200-300mg/dl) were taken for the experiment.

Decrease in bodyweight due to derangement of metabolic pathways is a common feature in diabetes (Al-Shamaony *et al.*, 1994). Administration of *Coccinia indica* leaves extract (Venkateswaran and Pari, 2002) to diabetic rats significantly reversed the loss in body weight, apparently due to its ability to reduce hyperglycemia. Treatment with 2.5 g/kg body weight/day of methanol extract of *Enicostemma littorale* to alloxan-induced diabetic rats up to 20 days showed a 50% reduction in blood glucose levels (Maroo *et al.*, 2003). Oral administration of the methanol fraction of *Salacia reticulata* twice daily to the diabetic animals gained the body weight (Rubin Kumara *et al.*, 2005).

In the present study the body weight of the diabetic rats decreased significantly after the treatment with STZ. The body weights of *C. tamala, M. oleifera* and *S. dulcis* treated

groups were increased significantly after the 20th day compared with control and diabetic control.

Increased urea production in diabetes may be accounted due to enhanced catabolism of both liver and plasma proteins (Morris and Leon, 1960). Qualitative estimation of Urine was a conventional method for the determination of sugar level in urine. The results of the test were positive (+++) to the diabetic rats and amount of sugar were nil to the *C. tamala, M. oleifera* and *S. dulcis* (250mg/kg) treated groups. Least amount (+) of sugar were found in the *C. tamala, M. oleifera* and *S. dulcis* (125mg/kg) treated groups.

Venkateswaran and Pari (2002) found that the diabetic rats showed a significant increase in blood glucose. In their experiment it was observed that the administration of *Coccinia indica* leaves extract and glibenclamide in diabetic rats restored the level of blood glucose to near normal levels. Diabetic rats treated with *Salacia reticulata* showed a significant reduction in the fasting blood glucose levels within 50 days compared with the control groups (Ruvin Kumara *et al.*, 2005). Pari and Latha (2002) demonstrated the levels of blood glucose in normal and experimental animals while they have worked on the antidiabetic activity of *Cassia auriculata* flowers. There was a significant increase in blood glucose. The effect of administration of *Cassia auriculata* flower extract at 0.15g was not significant, whereas 0.30 and 0.45g/kg body weight of *Cassia auriculata* flower extract and glibenclamide tended to bring the blood glucose toward a normal value. The leaf juice of *Catharanthus roseus* and the seed powder of fenugreek were tested for their hypoglycemic action individually and in combination in normal and alloxan-induced diabetic rabbits. *Catharanthus roseus* at dose levels of 0.5, 0.75 and 1.0ml/kg body weight produced maximum blood glucose reduction of 16.66% ($p<0.05$), 28.65% ($p<0.05$) and 38.49% ($p<0.001$) respectively. Duration and intensity of action increased in a dose-dependent manner. Fenugreek seed powder produced signicant reduction in blood glucose of 26.98% ($p<0.01$) and 39.21% ($p<0.001$) at a doses of 100 and 150mg/kg body weight, respectively. The combination of *Catharanthus roseus* (0.5ml/kg) and fenugreek (50ml/kg) produced significant ($p<0.001$) reduction in blood glucose 35.41% at 10h and increased the duration of action compared to either one (Satyanarayana *et al.*, 2003). The butanolic fraction of *Helicteres isora* root at a dose of 250mg/kg produced maximum fall (48.86%) in the blood glucose of diabetic rats (Venkatesh *et al.*, 2003). The whole seed powder of *Trigonella foenum-graecum*, its methanol extract and the residue which remained after methanol

extraction were reported to lower blood glucose levels in normal and sreptozotocin-induced diabetic rats (Liaquot *et al.*, 1995). The effect of treatment with *Anacardium occidentale* on fasting blood glucose in normal and fructose-fed rats was investigated by Olatunji *et al.*, (2005). Fasting plasma glucose levels of fructose-fed rats were significantly higher than those in normal rats. Treatment with extract of *A. occidentale* significantly abolished the increase in fasting plasma glucose levels induced by high-fructose diet. Some genera of cucurbitaceae have been mentioned anti-diabetic by Marles and Farnsworth (1995) such as *Benincasa, Bryonia, Citrullus, Coccinia, Cucumis, Cucurbita, Lagenaria, Luffa, Melothria, Mamordica* and *trichosanthes*.

In the present investigation it has been observed that the aqueous extract of *C. tamala, M. oleifera* and *S. dulcis* showed better hypoglycemic response on tested mice, at a dose of 250 mg/kg body weight than the dose of 125 mg/kg body weight.

Diabetes mellitus is associated with a marked decrease in the level of liver glycogen (Pugazhenthi *et al.*, 1991). The regulation of glycogen synthase and glycogen phosphorylase is of a recipropal nature. Roessler and Khandelwal (1986) have observed the increased glycogen phosphorylase and decreased glycogen synthase activity in the liver of diabetic mice. The reduced glycogen store has been attributed to reduced activity of glycogen synthase and increased activity of glycogen phosphorylase. All these activities result in an increased blood glucose level typical for diabetes. Pari and Latha (2004) have shown that the hepatic and skeletal muscle glycogen content was reduced significantly in diabetic control. Glycogen synthesis in the rat liver and skeletal muscles is impaired during diabetes (Huang *et al.*, 2000). In diabetic animals treated with the extract, the significant increase in the liver glycogen may be due to the activation of the glycogen synthesis system by the aqueous extract. Glycosylated hemoglobin is known to increase in patients with diabetes mellitus (Koeing *et al.*, 1976), and the increase has been found to be directly proportional to the fasting blood glucose level (Jackson *et al.*, 1979).

Similar result were also found in the present investigation, where the level of glycogen content decreased significantly in STZ- diabetic rats in compare to normal (both distilled water and citrate buffer treated). But after 20th day's treatment with *C. tamala,* and *S. dulcis* at a dose of 250 mg/kg body weight, the level of glycogen content increased better than the dose of 125 mg/kg body weight. Since Streptozotocin (STZ) - induced hyperglycaemia has been described as a useful experimental model to study the activity of

hypoglycaemic agents (Junod *et al.*, 1969). Streptozotocin (STZ) destroys β-cells of the pancreas and induces hyperglycemia (Palmer *et al*, 1998). Due to this observed effect, enhancement of peripheral utilization of glucose may be associated in the possible mechanisms involved in the hypoglycemic action of *C. tamala, M. oleifera* and *S. dulcis* leaves extracts.

An elevated level of lipid peroxides in the plasma of streptozotocin diabetic rats and lipid peroxidation is one of the characteristic features of chronic diabetes (Maxwell *et al.*, 1997). The increased levels of thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD), malonaldehyde and hydroperoxides are indices of lipid peroxidation (Krishnakumar *et al.*, 1999). Lipid peroxide mediated tissue damage has been observed in the development of both type I and type II diabetes mellitus. Increased con00000.centration of TBARS and hydroperoxide were observed in liver during diabetes. Previous studies have reported that there was an increased Lipid peroxidation in the liver of diabetic rats (Prince and Menon, 2000). Kamalakkannan and Stanely (2004) investigated on the antidiabetic and antioxidant activity of *Aegel marmelos* in streptozotocin-induced diabetic rats. The diabetic rats showed a significant increase in TBARS and hydroperoxides in liver and kidney. Oral administration of *Aegel marmelos* fruit extract maintained the tissue TBARS and hydroperoxides to near normal status. Pari and Latha (2002) demonstrate the levels of TBARS in normal and experimental animals while they have worked on the antidiabetic activity of *Cassia auriculata* flowers. There was a significant elevation of lipid peroxides in liver and kidney during diabetes when compared to the normal group. Administration of *Cassia auriculata* flowers extract at 0.45g/kg body weight was more effective than glibenclamide was found in their study. The effect of treatment with *Anacardium occidentale* on lipid peroxidation index (MDA) in normal and fructose-fed rats was investigated by Olatunji *et al.*, (2005). The MDA levels were significantly higher in fructose-fed rats compared with those in normal rats. Treatment with *Anacardium occidentale* produced significant reduction in MDA.

Our study showed that after administration of leaves of *C. tamala, M. oleifera* and *S. dulcis* aqueous extracts at a dose of 250 mg/ kg body weight tended to be near normal. This indicates that the study plants may inhibit oxidative damage of hepatic tissues.

Antioxidant effect of methanol extract of *Enicostemma littorle* was evaluated by measuring blood GSH levels erythrocyte CAT activity and LPO. Significant changes were observed in the above parameters in the extract treated diabetic rats. That extract treatment

caused a 23% increase in GSH levels in diabetic rats. There was a 29% decrease in erythrocyte CAT activity and a 21% decrease in erythrocyte LPO levels in extract treated diabetic rats. In their case of extract treated control rats, no significant changes were observed in the above antioxidant parameters (Maroo *et al.*, 2003).

Kamalakkannan and Stanely (2004) reported that there was a significant reduction in glutathione and glutathione peroxidase in liver and kidney in diabetic rats. Oral administration of *Aegel marmelos* fruit extract to diabetic rats significantly increased the liver and kidney glutathione and glutathione peroxidase to near normal. It was also observed in the present study that there was an increase in hydroperoxides in the liver and kidney of diabetic rats. Our results are also consistent with other reports on an increase of hydroperoxides in liver of animals with experimental diabetes (Kowluru *et al.*, 1996; Halliwell and Gutteridge, 1984; Yagi, 1987). This phenomenon is possibly caused by the decreased activity of antioxidant enzymes, which is a favorable factor for uncontrolled generation of free radicals and subsequent generation of lipid hydroperoxides (Halliwell and Gutteridge, 1984; Yagi, 1987). Pari and Latha (2002) demonstrate the levels of GSH in normal and experimental animals while they have worked on the antidiabetic activity of *Cassia auriculata* flowers. Their study showed that GSH level was significantly lower in diabetic rats than in normal rats. Administration of *Cassia auriculata* flower extracts at a0.45g/kg body weight and glibenclamide increased significantly the GSH levels as compared with the levels in diabetic rats.

Under *in vivo* condition glutathione (GSH) acts as an antioxidant and its decrease is reported in diabetes mellitus (Baynes and Thrope, 1999). Hydroperoxides are molecules with high toxicity and a high potential for destroying enzymatic proteins and cell membranes (Wang *et al.*, 1996). A significant decrease in GSH levels in liver during diabetes was also observed in the present study. The decrease in GSH levels represents increased utilization due to oxidative stress (Anuradha and Selvam, 1993). The increased GSH content in the *C. tamala, M. oleifera* and *S. dulcis* leaf extracts treated rats may be a factor responsible for inhibition of lipid peroxidation. In a study by Bharali *et al.*, (2003) it was revealed that administration of hydroalcoholic extract of *Moringa oleifera* at both dose levels (125 and 250 mg/kg body weight) for 7 and 14 days daily enhanced the levels of hepatic cytochromes b_5, cytochrome P_{450} and glutathione- S- transferase elucidating that *M. oleifera* acts as a biofunctional indeces as it induces both Phase-I and Phase-II system enzymes that finish the balance of xcuobiotic metabolism towards detoxification.

Treatment with *C. tamala, M. oleifera* and *S. dulcis* leaf extracts lowered oxidative stress in STZ diabetic rats by lowering glucose level in blood and by increasing the antioxidant enzyme activities. It has been previously reported that the supplementation with Vitamin C and Vitamin E were necessary to protect STZ diabetic rats against oxidative stress (Ihara *et al.*, 2000; Garg and Bansal, 2000; Head, 2000). In the present study it was found that high amount of ascorbic acid (Vitamin C) was present in *C. tamala* and *M. oleifera* which also could suppress diabetes significantly.

The present study was undertaken to verify the claim and evaluate the medicinal properties specially antioxidant and antidiabetic potential of *C. tamala, M. oleifera* and *S. dulcis*. Most of the available antidiabetic drugs are targeted towards hyperglycemia. Oxidative stress is believed to be a pathogenic factor in the development of diabetic complications. These plants extracts can act as free- radical scavengers *in vitro*. Therefore, antioxidant therapy is essential for diabetes along with hypoglycemic drugs. Our observations show that the leaf extracts of study plants viz, *C. tamala, M. oleifera, S. dulcis* have a glucose lowering effect as well as an antioxidant effect. Such plants having both hypoglycemic as well as antioxidant properties are very useful for the treatment of diabetes and have great potential for development as antidiabetic agents. All the parameters studied with *C. tamala, M. oleifera* and *S. dulcis* leaves extracts at 250mg/kg body weight showed higher antioxidant action. Thus, the claim made by the traditional Indian system of medicine regarding the use of leaf extracts of these plants in the treatment of diabetes stands confirmed.

Among the tested three plants, i.e, *C. tamala, M. oleifera* and *S. dulcis* showed good antioxidant activity, but *C. tamala* showed the maximum, followed by *M. oleifera*. Similarly, when tested for hypoglycemic activity, *C. tamala* was the most effective, followed by *M. oleifera*. *C. tamala*, or bay leaves, is one of the common ingredients of Indian cooking.The high medicinal potential of the leaves point to the fact that this is also a case where age old practice is based on preliminary knowledge. Similarly, *M. oleifera*, also consumed as vegetable has high potential. On the other hand, *Clerodendrum viscosum*, a weed showed minimum beneficial activity.

It is known that oral antidiabetic agents produce hypoglycemic effect through various mechanisms of action. This ranges from suppressing hepatic gluconeogenesis, stimulating glycolysis and inhibition of glucose absorbtion from the intestine (biguanides), stimulation of insulin release (sulphonylureas); inhibition of conversion of dietary disaccharides to

monosacchrides (alpha-glucosidase inhibitors); and exerting transcription of fatty acids by activating a specific sub-class of perioxisome proliferators-activated receptor (phiazolidine-diones) (Hardy and McNutty, 1997).

It is therefore probable that these modes of action may also be present in the aqueous leaves extracts of *C. tamala, M. oleifera, S. dulcis* in which the presence of several bioactive constituents have been demonstrated. It is not known which of the recorded groups of biologically active compounds are responsible for this observed hypoglycemic effect. Neither is the mechanism of action clearly understood. However, this work has clearly brought out the importance of common plants in medicine, which if used properly, can be as good as any other forms of medicine. The involvement of several biochemical components in their action has also been established. Several lines of action are still open for further research which can pinpoint the actual component(s) involved and their specific modes of action.

Chapter - 6

SUMMARY

1. A review of literature pertaining to this investigation has been presented which deals mainly with ethnobotanical study, biochemical, antimicrobial and antioxidant properties of the plant and hypoglycemic suppression.

2. Materials used in this investigation and experimental procedures followed have been discussed in details.

3. The entire work was divided into two phase. In the first phase the traditional knowledge about medicinal plants of the villagers was collected from the selected areas of the district and in the second phase detailed investigation of a few selected plants was carried out.

4. An ethnobotanical study has been carried out during 2005-2006 in the selected areas of Dakshin Dinajpur district (latitude $25^0 10' 5''$N and longitude $89^0 0' 30''$E) of West Bengal.

5. The data were collected through repeated interactions particularly by the participatory rural appraisal method with the help of elder peoples especially tribal, using a structured questionnaire.

6. In the ethnobotanical study, the uses of 107 medicinal plants of 96 genera of 47 families have been found.

7. The information on scientific name along with family name, local name, part(s) used, uses and method of doses have been provided.

8. Plant specimens were collected from the study area and the herbarium specimens were prepared using conventional method. Identification of plant specimen was done with the help of literature and comparison with authentic specimens at Central National Herbarium (Cal) and North Bengal University Herbarium and the specimens were deposited to the North Bengal University herbarium.

9. The maximum numbers of plants used by the villagers for the diseases were dysentery, diabetes, stomach/ liver diseases, cold-cough, rheumatism, gynecological problems, male sexual problems, urinary tract infection etc.

10. For the treatment of diabetes plants used by the villagers were *Abroma augusta, Aegel marmelos, Cajanus indicus, Catheranthus roseus, Cinnamomum tamala, Coccinia cordifolia, Enhydra fluctuans, Ficus carica, Melia azadirecta, Mimosa pudica, Momordica charanta, Moringa oleifera, Murraya koenigi, Musa paradisiacal, Piper longum, Punica granatum, Scoparia dulcis, Syzigium cumini, Tamarindus indica.*

11. After analysis of investigated plant specimens, four plants viz., *Clerodendrum viscosum, Cinnamomum tamala, Moringa oleifera* and *Scoparia dulcis* have been selected for the detailed study.

12. The effectiveness of biochemical, anti-microbiological, antioxidant properties of the leaves of *C. tamala, C. viscosum, M. oleifera* and *S. dulcis* has been studied.

13. Preliminary phytochemical screening of *C. viscosum, C. tamala, M. oleifera* and *S. dulcis* revealed that the presence of active principles including alkaloids, flavonoids, tannins, saponins, cardic glycosides, terpenoids.

14. Results revealed that steroid is present in *C. viscosum,C. tamala, M. oleifere* but absent in *S. dulcis.* Results also revealed that *C. tamala* contained the highest percentage crude yield of alkaloid while *S. dulcis* contained the lowest yield of alkaloid.

15. *M. oleifera* contained the highest amount of protein among the four studied leaves extracts and *C. viscosum* contained the least amount.

16. *M. oleifera* also contained the highest amount of total sugar while *C. viscosum* contained the least amount of total sugar. Again results revealed that leaves of *M. oleifera* contained higher quantity of reducing sugar; *C. tamala, S. dulcis* also contained moderate amount but *C. viscosum* contained the least amount.

17. *C. tamala* contained the highest amount of carotenoids followed by *M. oleifera* and *S. dulcis.*

18. Among the antioxidant capacities of *C. tamala, M. oleifera, C. viscosum, S. dulcis* studied highest quantity was detected in *C. tamala.*

19. *C. tamala* contained highest amount of ascorbic acid and phenol followed by *M. oleifera*, *S. dulcis* and the least amount was in *C. viscosum.*

20. Solvent extracts analyzed by UV-spectrophotometry showed maximum absorbance in the range 199-298 nm. None of the extracts showed antimicrobial activities except small inhibitory zone was observed against *Poria hybrunnea.*

21. For detecting the hyperglycemia in rats the parameters tested were fasting blood glucose, urine sugar, and glycogen content in liver tissue, TBARS and GSH of liver tissue.

22. The body weight of the diabetic rats decreased significantly after the treatment with STZ. The body weights of *C. tamala, M. oleifera* and *S. dulcis* treated groups increased significantly after the 20^{th} day compared with control and diabetic control.

23. The results of urine test were positive (+++) in diabetic rats and amount of sugar was nil in *C. tamala, M. oleifera* and *S. dulcis* (250mg/kg) treated groups.

24. Diabetic rats treated with *C. tamala, M. oleifera, S. dulcis* showed a significant reduction in the fasting blood glucose levels within 20 days compared with the control groups.

25. Diabetic rats treated with leaves of study plants showed a significant increase in glycogen level of liver tissue. *C. tamala* treated group showed maximum increase and brought the level of glycogen near normal.

26. *S. dulcis* treated group exhibited the most significant TBARS lowering effect in liver tissue followed by *M. oleifera* and *C. tamala.*

27. There was a significant reduction in glutathione in liver in diabetic rats. Oral administration of *C. tamala, M. oleifera* and *S. dulcis* leaves extracts to diabetic rats significantly increased the liver glutathione to near normal.

28. Significant correlation was obtained between body weight increase in diabetic treated rats and other parameters like blood sugar, TBARS, glycogen and GSH.

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