CHAPTER 8

HYPOGLYCEMIC ACTIVITY

8.0 INTRODUCTION

8.1 Diabetes

Diabetes is a disorder of the carbohydrate, fat and protein metabolism. It is attributed to the diminished production of insulin or mounting resistance to its action. Chronic hyperglycemia during diabetes causes glycation of body proteins that in turn leads to secondary complications affecting the eyes, kidneys, nerves and arteries (Sharma *et al.*, 1993).

Diabetes can be classified into two types: (a) type 1 diabetes (insulin-dependent Diabetes mellitus or IDDM) and (b) type 2 diabetes (non-insulin dependent Diabetes mellitus or NIDDM). Exercise and having a controlled diet are recommended for the treatment of both types of diabetes. In addition, insulin is used to treat cases of type 1 diabetes. Oral hypoglycemic agents (such as sulfonylureas, biguanidines, thiazolidinediones and α-glucosidase inhibitors) are often used to treat cases of type 2 diabetes. When therapy with oral hypoglycemic agents is ineffective, insulin also can be used to treat type-2 diabetes (Liu *et al.*, 1996; Zhao, 1999).

In exception to western medicine, diabetes has been treated orally with various medicinal plants or their extracts based upon 'folklore' medicine. Diabetes comprised of a group of metabolic disorders characterized by an alteration in the metabolism of carbohydrates, proteins and fatty substances. This disorder causes a complete or relative insufficiency in insulin secretion and or its action. An investigation of hypoglycemic agents originating from plants used in traditional medicine would be of major public health importance (Almeida *et al.*, 2006).

8.1.1 Prevalence of Diabetes and role of plant derived drugs

Diabetes mellitus is the metabolic disorder with the highest rate of prevalence and mortality world-wide (Harris *et al.*, 1998). The incidence of type 2 diabetes is increasing worldwide. Although genetic factors may play a role, life-style changes such as the consumption of a Western diet which are high in fat, leads to obesity which can be a factor also contributing to the increase of this disease. Life-style factors, such as increased fat intake and reduced exercise, have been shown to be associated with obesity and insulin resistance (Lipman *et al.*, 1972; Lovejoy *et al.*, 1992). It is observed in rats that, high fat feeding induces a state of insulin resistance associated with diminished insulin-stimulated glycolysis and glycogen synthesis. This disease is a result of the peripheral insulin-responsive tissues, such as muscle and adipose tissue, displaying a significant decrease in response to insulin resulting in an increase in circulating glucose and fatty acids in the blood. The low response to insulin results in a decrease in glycolysis which in turn initiates gluconeogenesis and glycogenolysis in the liver, both of which are 'switched off' by insulin under normal conditions. Pancreatic β cells are able to cope with the initial insulin resistant phase by producing an excess of insulin and

increasing the amount of insulin secreted (Pirol *et al.*, 2004). The resulting hyperinsulinaemia to maintain normoglycaemia eventually brings about β cell dysfunction (Khan, 2003) leading to full blown diabetes. It is evident that type 2 diabetes is dependent on insults occurring both at peripheral as well as the β cell level (Khan *et al.*, 2000).

Biguanides, such as metformin, became available for treatment of type 2 diabetes in the late 1950s, and have been effective hypoglycaemic agents ever since (Vigneri *et al.*, 1987). Little is known about the exact molecular mechanism of these agents. As an insulin sensitizer, metformin acts predominantly on the liver, where it suppresses glucose release (Goldfine, 2001). Metformin has also been shown to inhibit the enzymatic activity of complex I of the respiratory chain and thereby impairs both mitochondrial function and cell respiration, and in so doing decreasing the ATP/ADP ratio which activates AMP-activated protein kinases causing catabolic responses on the short term and insulin sensitization on the long term (Brunmair *et al.*, 2004; Tiikkainen *et al.*, 2004). This drug has been proven effective in both monotherapy and in combination with sulfonylureas or insulin (Davidson *et al.*, 1997). However, their cost is very high and the development of more affordable alternative therapies would be an advantage. It is for this reason that scientists are investigating the efficacy of indigenous plant extracts in their own country (Chadwick *et al.*, 2007). Plants such as *Urtica parviflora, Callicarpa arborea* and *Morinda citrifolia* in my experimental work may be the answer to this problem.

Diabetes mellitus and its complications constitute a major health problem in modern societies (Li *et al.*, 2007). The prevalence of diabetes has increased over the past 20 years, adding urgency to the need of treatment with oral medications (Chehade *et al.*, 2000). Targeting postprandial hyperglycemia may be difficult with conventional diabetes therapy and in this regard, the availability of a-glucosidase inhibitors is helpful (Van de Laar *et al.*, 2005; Scheen, 2003). a-Glucosidase inhibitors are nontoxic and well tolerated, and with mild antihyperglycemic activity, either used as monotherapy or adjuncts to any other oral diabetic agents (Charpentier, 2002). It has long been recognized that many naturally occurring substances have inhibitory effect of aglucosidase, in plant materials such as fruits, leaves, seeds etc. (Shim *et al.*, 2003; Ye *et al.*, 2002). So the study of those bioactive constituents represents a promising approach to the discovery of new diabetes drugs. Teas, rooibos and honeybush are reported to have multiple biochemical and pharmacological activities, such as anticarcinogenic, antioxidant, antiangiogenic, antiviral and antidiabetic effects (Gomes *et al.*, 1995; Shukla *et al.*, 2002 and Skrzydlewska *et al.*, 2002).

Diabetes affects nearly 10% of population of the world (Yanardag *et al.*, 2003). Overall prevalence among Indians is about 1.73% (Park, 1997). Diabetes mellitus is an independent

risk factor for the development of coronary artery diseases, myocardial infarcation, hypertension and dyslipidemia. Clinically diabetic patients are characterized by marked increase in blood glucose level followed by normal or mild hyperlipidemia. It is a chronic disease characterized by high blood glucose levels due to absolute or relative deficiency of circulating insulin levels. Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes mellitus, there is an increase demand by patients to use the natural products with antidiabetic activity. Insulin cannot be used orally and continuous use of synthetic drugs causes side effects and toxicity. At present, second and third generation sulfonylurea are the oral pharmacological agents used to counteract insulin secretion deficiency in diabetes (Puri et al, 2002). Plants have long been a source of traditional antidiabetic medicines (Blumenthal, 2000 & Kirtikar, 1988). Herbal drugs are prescribed widely even when their biologically active compounds are unknown, because of their effectiveness, less side effects and relatively low cost (Venkatesh, 2003). Herbal preparations alone or in combination with oral hypoglycemic agents sometimes produce a good therapeutic response in some resistant cases where modern medicines alone fail (Anturlikar, 1995).

In recent years there has been an upsurge in the clinical use of indigenous drugs. Indian medicinal plants and their derivatives have been an invaluable source of therapeutic agents to treat various disorders including diabetes (Koehn and Carter, 2005). Polyherbal preparations, originally used in the traditional systems of medicine, are now being investigated and effectively tried in a variety of path physiological states (Shah *et al.*, 1997). Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes (Ajgaonkar, 1979; Alarcon-Aguilara *et al.*, 1998). The hypoglycemic activity of a large number of these plants been evaluated and confirmed in different animal models (Karaway *et al.*, 1984; Bopanna *et al.*, 1997; Bhandari *et al.*, 1998; Jouad *et al.*, 2000; Sugihara *et al.*, 2000; Takako *et al.*, 2005). Side effects and expenses associated with allopathic drugs have provoked the need for research into drugs, which are without the side effects, especially those belonging to the traditional systems of medicine like Ayurveda, Homoeopathy, and Unani etc (Sharma *et al.*, 2007).

Diabetes mellitus, both of the insulin-dependent diabetes mellitus (IDDM) and non-insulindependent diabetes mellitus (NIDDM) type, is a common and serious disorder throughout the world (Keen, 1986; Harris *et al.*, 1987). This metabolic disorder often leads to physical disability arising from the vascular complications of coronary artery disease and cerebrovascular disease, resulting in renal failure, blindness, and limb amputation in addition to neurological complications and premature death (Goldstein and Massry, 1978; Weidmann *et al.*, 1993; Strippoli *et al.*, 2003; He and King, 2004). Treatment of diabetes mellitus by insulin

and oral hypoglycemic drugs fails to prevent these complications in many patients, indicating that additional alternative treatment could be helpful (Cherng *et al.*, 2005).
Type 2 diabetes (DM2) is one of the primary threats to human health due to its increasing prevalence, chronic course and disabling complications. According to the World Health Organization (WHO, 2005) there were 150 million people over 20 years of age living with diabetes in 2000 and they project that by 2025 there will be 300 million people living with this condition. The increase is expected to be 42% in developed countries and 70% in developing countries (WHO, 2005; King, *et al.*, 1998).

One of the principal objectives when treating patients with DM2 is to control glucose levels. Presently, there is an arsenal of synthetic hypoglycemic drugs available; however, these drugs normally cause side effects prompting the patients to stop taking the medication and DM2 progresses with further acute and chronic complications and even death. For this reason, a phytomedicine capable of treating the disease at early stages, but with fewer side effects and less expensive, will be of great help to the diabetic patients specially due to the extended belief that natural treatments cause less harm to the organism (Revilla-Monsalve *et al.*, 2007).

Metabolic syndrome (MS) is also known as syndrome X or insulin resistance syndrome (Boehm and Claudi-Boehm, 2005). In accordance with World Health Organization (WHO), the MS components are: visceral fat obesity, hypertension, insulin resistance and dyslipidemia (Isomaa *et al.*, 2001). Therefore, International Diabetes Federation (IDF) mentions that MS is a cluster of the most dangerous heart attack risk factors such as diabetes and prediabetes, abdominal obesity, high cholesterol and high blood pressure (IDF on-line definition, 2005). Some authors included liver steatosis as an important factor to diagnosing this complex metabolic disease (Masuzaki *et al.*, 2004; Den Boer *et al.*, 2004). Although the pathogenesis of metabolic syndrome is still not fully clear, a large body of evidence indicates that insulin resistance could be the central abnormality (Wajchenberg *et al.*, 1994). These diseases occur due to several factors such as genetic background alterations, lifestyle and other related factors (Fowler *et al.*, 2005).

8.1.2 Hypoglycemic models

Hypoglycemic activity of drugs, including the products derived from medicinal plants, is conventionally assessed in diabetic animal models by observing drug-induced fall in fasting blood glucose (FBG) or suppression of glucose tolerance curve. Diabetes is induced experimentally by partial or total pancreatecctomy, exposure to antislet cell antibodies, but most commonly by injecting chemical agents, such as alloxan or streptozotocin, that cause widespread destruction of insulin-secreting pancreatic beta cells (Kaneto *et al.*, 1995).

However, beta cells being extensively eliminated/ or destroyed in the diabetic animals, several drugs that require functional pancreas cannot be satisfactorily tested in them (Puri, 2006).

Moreover, the drug-induced fall in FBG as a criterion for hypoglycemic effect has been reported to be a relatively intensive method of assessment and sometimes gives inconsistent results. Post-treatment suppression of glucose tolerance curve, though a sensitive method of assessment, suffers from a major drawback in the test is associated with high mortality in diabetic animals (Puri *et al.*, 2002).

Hyperglycemia is common and is due to multiple factors, including decreased insulin release, increased glucagons release and increased output of adrenal glucocorticoids and catecholamines and hypoglycemia is most commonly the result of taking drugs used to treat diabetes mellitus or other drugs, including alcohol. However a number of other disorders, including end-stage organ failure and sepsis, endocrine deficiencies, large mesenchymal tumors, insulinoma, and inherited metabolic disorders are also associated with hypoglycemia. Hypoglycemia defined as a plasma glucose level <2.5 to 2.8 mmol/L (45 to 50 mg/dL).

Experimental animal models in which diabetes is induced by administration of alloxan, streptozotocin or other agents have been used effectively to study etiologies, complications, treatment and prevention of disease (Park, 1997).

8.1.3 Ethnomedicinal antidiabetic of Sikkim and Darjeeling Himalayas

Sikkim and Darjeeling Himalayan region is characterized by a rich floral diversity and an equally rich ethnomedicinal tradition. Herbal medicine is the dominant system of medicine practiced by the local tribes of this region for the treatment of diabetes. It is reported that 37 species of plants belonging to 28 families are used as antidiabetic agents in the folk medicinal practices in the region and 81% of these plants are hitherto unreported as hypoglycemic agents. This finding may lead to serious research towards developing new and efficient drugs for diabetes (Chhetri *et al.*, 2005).

The efficacy of these ethnomedicinal plants needs to be subjected to pharmacological validation. Some antidiabetic plants may exert their action by stimulating the function or number of cells and thus increasing insulin release (Persaud *et al.*, 1999). In some other plants, the effect is due to decreased blood glucose synthesis due to the decrease of the activity of enzymes like glucose-6-phosphatase, fructose1,6-bisphosphatase, etc. In still other plants, the activity is due to slow absorption of carbohydrate and inhibition of glucose transport (Madar, 1984). However, these products may interact with the conventional diabetes medicines (Shane-McWhorter, 2001). Therefore, a cautious approach should be adopted before

administering these drugs. Of course, this primary information is important in view that it may lead to serious pharmacological research and can provide great value in selecting plant material for drug discovery (Lewis *et al.*, 2004).

Worldwide, over 1200 species of plants have been recorded as traditional medicine for diabetes (Marles and Farnsworth, 1995). Some of these plants have been evaluated in laboratories and in a number of cases their efficacy has been confirmed, for instance, *Panax ginseng (ginseng), Opuntia cactus (cactus), Tecoma stans* (trompeta), *Syzygium cumini* (jambol˜ao) (Li *et al.*, 2004). Specific chemical constituents of these plants, such as polysaccharides, alkaloids, triterpenoids and xanthones, are believed to be responsible for the hypoglycemic effects and they can be related to actions including increased insulin release and increased glucose metabolism in the body periphery, among others (Wang and Ng, 1999; Tatiana *et al.*, 2006). Many plants like *Allium cepa, Alium sativum, Ficus bengalensis, Gymnema sylvestre, Pterocarpus marsupium, Trignella foenumgraecum, Engenia jambolana* etc. have ben shown to possess potent antidiabetic activity (Melander, 1988).

The literature survey indicates plants such as *Cyamopsis tetragonoloba* Linn.(Mukhtar *et al.*, 2004), *Polygala arvensis, Polyalthia longifolia, Pterocarpus marsupium, Azadirachta indica, Abroma angusta, Ocimum sanctum, Murraya koenigii* (Vinuthan *et al.*, 2004), *Lantana camara* Linn. are tested for their antihyperglycemic potential against alloxan induced hyperglycemia in laboratory animals. Whereas plants like *Memordica charantia* Linn (Prasanna *et al.*, 2005), *Acacia catechu* Willd., *Ficus hispida* (Ghosh *et al.*, 2004) were tested against glibenclamide induced hypoglycemia and plants like *Cassia kleinii, Petroselinum crispum* (Yanardag *et al.*, 2003), *Brassica nigra* Linn. are tested against streptozotocin induced hyperglycemia in animals respectively. Many herbal agents have been described for the treatment of diabetis mellitus in ancient literature. They have been shown to have hypoglycemic action in both animals as well as humans.

The Ethnomedicinal plants which are practiced by the tribes of Sikkim and Darjeeling Himalayas are given in **Table 8.1.**

Table 8.1 Antidiabetic medicinal plants from Sikkim and Darjeeling Himalayas (Chhetri *et al.*, 2005).

Botanical name and family	Habit	Local name: N-Nepali; L-Lepcha; T-Tibetan	Method of use and administration
Abroma augusta (L.) L.f., Sterculiaceae	Shrub	Ulatkamal (N)	Stem bark and leaf decoction (10–20 ml) taken one time each alternate day in empty stomach for 4–6 week.
Abutilum indicum (L.) Sw., Malvaceae	Shrub	Ghantiphool (N)	Decoction of stem bark (25–50 ml) given two times daily (after principal meals) for 3–4 weeks.
Aconitum palmatum D. Don., Ranunculaceae	Herb	Seto bikhumma (N); Nyini (L); Bhongnanukpo (T)	Root decoction (10–15 ml) taken with a cup of milk one time daily (after lunch) for 7–10 days
Aloe barbadensis Mill, Liliaceae	Herb	Ghew kumari (N); Kumari (T)	Fresh leaf pulp (40–50 g) taken once a day in empty stomach for 10–12 weeks.
Asparagus racemosus Willd., Liliaceae	Climbing shrub	Kurilo (N); Neusiri (T)	Decoction of tender shoots (25 ml) taken once a day for 6–8 weeks.
Berberis aristata DC., Berberidaceae	Shrub	Sano Chutro (N); Sutangkung (L); Skyerba (T)	Root bark extract (5–10 ml) taken twice daily after breakfast and dinner) for 1–2 weeks
Boenninghausenia albiflora (Hook. f.) Reich ex Meissn., Rutaceae	Herb	Chirbirpatay (N)	The whole plant is crushed without water and the juice (5–10 ml) taken one to two times daily for 3–4 weeks.
Calamus rotanga (L.), Arecaceae	Climbing shrub	Bet (N)	Raw fruit (1–2) taken as masticatory two times daily (after breakfast and lunch) for 6–8 weeks.
Campylandra aurantiaca Baker, Liliaceae	Herb	Nakima (N)	Flowers are made into curry and taken with staple food two times per week for 4–6 weeks
Cannabis sativa (L.), Cannabaceae	Under shrub	Bhang (N)	Leaf extract (5–10 ml) taken two times daily for 3–4 weeks.
Catharanthus roseus (L.) G. Don., Apocynaceae,	Herb	Sada bahar (N)	Raw leaf (1–2) chewed daily for 2 weeks.
Cinnamomum tamala (Buch.-Ham.) Nees and Eberm., Lauraceae,	Tree	Sinkauli (N); Napsor (L); Mensing (T)	Decoction of stem bark taken three times daily for 3–4 weeks
Cissampelos pareira (L.), var.hirsuta (Buch.-Ham ex DC) Forman, Menisper-maceae	Climber	Batulpatay (N)	Root bark extract (5–10 ml) tken one to two times daily for 2–3 weeks
Coccinea grandis (L.) Voigt., Cucurbitaceae	Climber	Tilkor (N)	Fresh root extract (5–10 ml.) taken two times daily (before principal meals) for 3–4 weeks
Costus speciosus (Koening) Sm., Costaceae	Herb	Betlouri (N); Ruyang (L)	Decoction of rhizome (10–20 ml) taken two to three times daily for 2–4 weeks
Ficus racemosa (L.), Moraceae	Tree	Dumri (N)	Fruit juice (20–25 ml) taken two times daily (before meals) for 4–8 weeks
Girardiana heterophylla Decne., Urticaceae	Shrub	Bhangre sisnu (N)	Root decoction (25–50 ml) taken two times daily for 4–8 weeks
Gynocardia odorata R. Br., Flacourtiaceae	Tree	Gantay (N); Tukkung (L)	Fruit juice (10–15 ml) taken one time daily for 2 weeks

Botanical name and family	Habit	Local name: N-Nepali; L-Lepcha; T-Tibetan	Method of use and administration
Ipomoea batatus (L.) Lamk., Convolvulaceae	Herb	Sagarkhanda (N)	The juice of the aerial part of the plant (25–30 ml) taken two times daily for 3–4 weeks
Litsea cubeba Pers., Lauraceae	Tree	Siltimmur (N)	One raw fruit chewed as masticatory two times daily for 4–6 weeks
Momordica chrantia (L.), Cucurbitaceae	Climber	Karela (N)	Fruit extract (25 ml) taken two times daily for 12–14 weeks
Nardostachys jatamansi DC., Valerianaceae	Herb	Jatamansi (N), Spanpos (T)	Decoction of rootstock (30–50 ml) taken once daily for 2–3 weeks.
Oroxylum indicum (L.) Vent. Bignoniaceae	Tree	Totola (N), Phagorip (L), Sonaka (T)	Stem bark decoction (15–20 ml) or juice (5–10 ml) taken two to three times daily
Paederia foetida (L.), Rubiaceae	Climber	Birilahara (N), Takpoedrik (L)	Leaf infusion (50–60 ml) taken one time in the morning for 2–3 weeks
Panax pseudoginseng Wall., Araliaceae	Herb	Panch patay (N)	Dried rhizome powder (0.5–1 g) taken one time daily with warm milk
Picrorhiza kurrooa Royle ex Benth. Scrophulariaceae	Herb	Kutki (N), Putse sel (T)	Dry rhizome powder (0.5 g) taken with two tablespoon of curd and a pinch of pepper power one time daily for 1–2 weeks
Potentilla fulgens Wall., Rosaceae	Herb	Banmula (N)	Decoction of root (20–25 ml) taken two times daily for 4–8 weeks
Quercus lanata Sm., Fagaceae	Tree	Banj (N)	Decoction of stem bark (20–25 ml) taken one or two times daily for 2–3 weeks
Saraca asoca (Roxb.) De Wilde, Caesalpiniaceae	Tree	Asok (N)	Infusion of the dry flower (50–100 ml) taken two times daily (before principal meals) for 4–5 weeks
Stephania glabra (Roxb.) Miers, Menispermaceae	Climber	Tamarkay (N), Kanthey (L)	Root decoction (20–25 ml) taken with milk two to three times daily for 1–2 weeks
Swertia angustifolia Buch.-Ham.ex D. Don., Gentianaceae	Herb	Patlay Chireto (N)	Infusion of whole plant (40–50 ml) taken two times daily (before principal meals for 3–4 weeks
Swertia chirayita (Roxb. ex Flem.) Karst., Gentianaceae	Herb	Chireto (N), Rungkyon (L), Tagota (T)	Infusion of the whole plant (50–60 ml) taken one time daily in empty stomach for 2 weeks
Syzygium cuminii (L.) Skeels, Myrtaceae,	Tree	Kyamuna (N), Dzambu (T)	Decoction of stem bark (25–30 ml) taken three times daily for 2–3 weeks
Trigonella foenum-graecum (L.), Fabaceae,	Herb	Methi (N)	Sprouted seeds mixed with chilly, salt and garlic and ground into a paste. 5–10 g of the paste taken with two principal meals daily
Urtica dioica (L.), Urticaceae	Herb	Sisnu (N), Sarong (L)	Decoction of young leaves and shoots (50–100 ml) taken as curry one or two times daily with meals for 4–8 weeks
Zingiber officinale Rosc., Zingiberaceae	Herb	Adua (N), Heng (L), Beasga (T)	Decoction of rhizome (25–50 ml) taken as herbal tea with a pinch of salt two to three times daily for 8–12 weeks

8.2 MATERIAL AND METHODS

8.2.1 Plant materials

The fresh leaves of *Urtica parviflora (U. parviflora), Callicarpa arborea (C. arborea)* and root bark of *Morinda citrifolia (M. citrifolia)* were collected at Majhitar, East Sikkim and were authenticated by Botanical Survey of India (BSI), Gangtok, Sikkim and the herbaria were preserved in the institutional museum (HPI / PK/ No. 131, 132 and 133). The concentrated methanolic extract of the leaves of *Urtica parviflora, Callicarpa arborea* was used for the study whereas the concentrated chloroform extract of the roots of *Morinda citrifolia* was used. Further the compounds I,II, and III isolated from the leaves of *Urtica parviflora, Callicarpa arborea* and roots of *Morinda citrifolia* respectively were used in this study.

8.2.2 Animals

Male Wistar rats (6 weeks old) weighing 150-200g obtained from Ghosh Enterprises, Kolkata were used. The animals were kept in well ventilated and clean animal house of Himalayan Pharmacy Institute, Sikkim, at 23^0-27^0C, 55% humidity and allowed to acclimatized with free access to food (Local animal feed) and water *ad libitum* for one week under 12 hr each of light and dark cycles. The animal studies were approved by Institutional Animal Ethics Committee.

8.2.3 Induction of experimental diabetes

Diabetes was induced by a single intra peritoneal injection of freshly prepared Streptozotocin (STZ) in a dose of 50mg/kg body weight dissolved in citrate buffer pH 4.5 to overnight fasted rats. The hypoglycemic activity on these animals was carried out after one week of STZ injection when the stabilization of diabetes was ensured (Murali *et al.*, 2004). The animals with fasting serum glucose (FSG) of 240 mg/dl and above were used (Anand *et al.*, 2007).

8.2.4 Effect of different extracts and isolated compounds of the respective extracts on glucose tolerance test (GTT)

Different extracts and the isolated compounds were assessed by oral glucose tolerance test (GTT) based antidiabetic activity in STZ induced diabetic rats (Anand *et al.*, 2007). In this method, the advantage is that the same group of animals served as their self control. Six groups (Group I-VI) of six rats each (one group for each extract or compounds) were used in this experiment. The animals were fasted overnight and fasting blood samples were drawn from the tail vein. To get the GTT pattern in untreated animals, empty gelatin capsules were fed to the animals after withdrawal of fasting blood samples. Again after 90 minutes blood samples were withdrawn. This sample was taken as '0' hour value for GTT. The animals were given immediately aqueous glucose solution 2 g/kg of body weight orally and blood samples were withdrawn at 1, 2 and 3 hr after glucose administration to get the GTT pattern of the untreated diabetic animals (diabetic control). After a week, same animals were again fasted

overnight to carry out GTT with drug. Fasting blood samples were drawn. The extracts and isolated compounds were administered orally to rats in gelatin capsules. Animals of group I-III received methanolic extract of the leaves of *Urtica parviflora* (MEUP), methanolic extract of the leaves of *Callicarpa arborea* (MECA), and chloroform extract of the roots of *Morinda citrifolia* (CEMC). The animals of group IV to VI received the isolated compound I,II, and III at the dose of 50mg/kg body weight **(Table 8.2).** After 90 min (this time was allowed for their effect to take place in the body), blood samples were drawn again. This served as '0' hr blood sample of the treated diabetic rats in GTT. Then the GTT was carried on as described above to get the glucose tolerance pattern of the same diabetic animals but after treatment with the extracts. The GTT pattern of untreated diabetic animals and the GTT pattern of treated animals after one week showed no significant difference. So no separate group of untreated control animals for each drug was required.

8.2.5 Assessment of antidiabetic effect in rats (serum glucose level)

The effect of the extracts and their isolated compound on fasting serum glucose (FSG) in experimental diabetes induced by STZ (section 8.2.3) was assessed as follows. Nine groups (I-IX) each of six rats were used. Group I served as normal healthy controls and group II as untreated diabetic animals and was given water during the experiment period as the vehicle. Group III to VIII diabetic rats were given effective dose of MEUP, MECA, CEMC, compound I, II and III (dose in **Table 8.2**). Group IX received the standard drug glibenclamide (Sigma Chemicals, USA) in a dose of 0.20 g/kg body weight orally. The drugs were given once daily in the morning for seven days. The animals had free access to food and water. The fasting blood was collected in the beginning and on the 8^{th} day. Serum glucose (Fasting serum glucose, FSG) and urine glucose levels were also estimated at the same time. FSG was estimated using Kits of Ranbaxy Laboratories, New Delhi, India and the urine glucose level was estimated using Uristix of Bayer Diagnostics Pvt. Ltd, Mumbai, India.

8.2.6 Assessment of hypoglycemic activity of Plant drugs in normal healthy rat

It is essential to estimate the glucose tolerance of normal healthy animals. The animals were divided into six groups. Each group consists of six animals. The animals were caged for eight days. On the first day they were kept fasted for overnight and had free access to water. On the first day "fasting" blood glucose level was tested in the blood samples collected from the tip of the tails. The animals were allowed the normal feed and water.

On day eight, to the overnight fasted animals drugs were administered (as per the doses given in the **Table 8.2**). After 90 minutes of drug administration blood samples were withdrawn which served as '0' hr blood sample. After collection of blood samples, the animals were administered glucose solution 2g/kg of body weight orally. Again blood samples were collection at 1,2 and 3 hr after glucose administration to get the GTT pattern in normal healthy animals.

8.2.7 Statistical analysis

The result were expressed as mean ± S.D.. The statistical analysis involved in two groups was performed by means of paired *t* test, whereas analysis of variance (ANOVA) followed by Dunette's multiple comparison test were used in order to compare more than two groups. All the data were processed with Graph Pad Prism, version 4.01 software.

$P < 0.05$ was considered significant and $P < 0.01$ was considered more significant.

8.3 RESULTS OF HYPOGLYCEMIC ACTIVITY

8.3.1 Effect of extracts and isolated compounds on glucose tolerance test

The results of the antidiabetic activity on glucose tolerance in streptozotocin induced diabetic rat is presented in the **Table 8.3**. From the result it has been revealed that MEUP (methanolic extract of *Urtica parviflora*) succeeded to control the rise of serum glucose level (70.6%) within 1st hour of GTT in streptozotocin induced diabetic rats, followed by Compound I treated group i.e. Group IV (60.0%) and Compound II treated group i.e. Group V (55.2%). The serum glucose levels between '0' hour and 1 hour are generally compared in these type of studies because there is sudden rise of serum glucose level at the 1st hour after glucose loading in '0' hour. The immediate rise of serum glucose level challenges the efficacy of the test drugs to control it. The CEMC treated (24.24%) and compound III (isolated from *Morinda citrifolia*) treated group (17.8%) failed to normalize the rise of serum glucose level. Thus out of the three plants studied, the plant *Urtica parviflora*, was found to half remarkable hypoglycemic activity.

8.3.2 Assessment of antidiabetic effect (serum glucose level) in rats

The result of the antidiabetic effect of test drugs as well as of standard drug (Glibenclamide) is presented in the **Table 8.4**. The percentage reduction of serum glucose level by the MEUP treated group is maximum (27.2), which is much higher than the standard drug, Glibenclamide treated group (18.5). Even the percentage reduction of serum glucose level by MECA treated group is slightly higher (19.2) than the standard drug treated group. Compound II and Compound I treated groups showed this value at 16 and 15.2 respectively. The percentage reduction values are obtained when compared to diabetic control value. The result indicates high efficacy of MEUP and MECA in reducing serum glucose level in diabetic rats.

8.3.3 Assesment of hypoglycemic activity of the plants drug in normal healthy rats

The result of this activity is presented in the **Table 8.5**. The antidiabetic activity of the extracts and their respective isolated compounds was evaluated by considering the percentage reduction when compared to the value of the control group. Compoud I and Compound II exhibited same percentage reduction i.e. 14.1%, followed by MEUP and MECA respectively. Compound III and CEMC showed low values (3.5% and 2.3% respectively).

It is fairly evident from the studies performed in all the three models that maximum percentage reduction of serum glucose level was found with the MEUP and Compound I, isolated from the same plant *Urtica parviflora*. Hence the plant *Urtica parviflora* is having highest hypoglycemic activity, followed by *Calicarpa arborea* and *Morinda citrifolia*.

Table 8.2 The solvent extracts, their respective isolated compounds with doses and rout of administration used for this experiment.

Gr. No.	Plant Drug	Dose	Rout of administration
I	Methanolic Extract of *Urtica parviflora* (MEUP)	200 mg/kg body weight	Oral
II	Methanolic Extract of *Callicarpa arborea* (MECA)	200 mg/kg body weight	Oral
III	Chloroform Extract of *Morinda citrifolia* (CEMC)	200 mg/kg body weight	Oral
IV	Compound I	50 mg/kg body weight	Oral
V	Compound II	50 mg/kg body weight	Oral
VI	Compound III	50 mg/kg body weight	Oral
VII	Glibenclamide	0.20g/kg body weight	Oral
VIII	Streptozotocin	50 mg/kg body weight	Intra peritoneal
IX	Aqueous Glucose Solution	2g/kg body weight	Oral

Table 8.3 Antidiabetic activity on glucose tolerance in streptozotocin induced diabetic rats during GTT (Anand *et al.*, 2007).

(Group) Treatment	GROUP	Serum Glucose (mg/dl)					Increase in serum glucose (mg/dl) between 0 and 1 hr
		Fasting	0 hr	1 hr	2 hr	3 hr	
Group-I MEUP	C	278±7.0	287±6.4	362±6.7	351±5.8	332±6.0	75
	T	281±6.3	289±5.5	311 ±4.7**	300±4.8	287±5.1	22 (70.6%)#
Group-II MECA	C	287±6.1	280±5.8	358±4.4	330±4.7	309±4.8	78
	T	309±5.2	319±5.0	355 ±6.3**	333±6.2	296±6.7	36 (53.8%)#
Group-III CEMC	C	301±4.8	315±6.2	381±5.4	342±5.5	333±5.3	66
	T	313±5.7	318±4.8	368 ±6.1**	342±5.6	317±6.0	50 (24.24%)#
Group-IV Compound I	C	300±5.7	306±5.9	376±6.0	326±4.8	306±5.2	70
	T	289±5.1	290±5.3	318 ±5.6**	271±5.5	249±6.1	28 (60.0%)#
Group-V Compound II	C	311±4.4	317±4.6	384±5.2	361±5.2	336±5.4	67
	T	303±4.7	311±4.8	341 ±4.9**	312±4.7	292±6.1	30 (55.2%)#
Group-VI Compound III	C	307±5.2	316±5.3	389±6.2	364±4.8	343±4.5	73
	T	299±6.2	317±5.8	377 ±6.1**	350±6.8	326±6.7	60 (17.8%) #

[Values are mean ± SD from 6 animals in each group]

P values: *<0.05; **<0.01 as compared with control values at the same time
C = Control, T = Treated
# Percent of the untreated control of the same group.

Table 8.4 Effect of treatment of diabetic rats with glibenclamide, extracts and their respective isolated compouds for one week on glucose levels (Sharma *et al.*, 2006).

Groups of animals	Glucose levels (mg/dl) before and after one week of treatment			
	Before		After one week	
	Serum	Urine	Serum	Urine
(Gr I) Healthy Control	85±6.9	-	87±7.2	-
(Gr II) Diabetic Control	262±6.8	++	275±6.5	++
(Gr III) MEUP treated	255±5.5	++	200±7.0** 27.2#	+-
(Gr IV) MECA treated	260±5.8	++	222±6.1** 19.2#	+
(Gr V) CEMC treated	249±6.6	++	256±6.7** 6.9#	++
(Gr VI) Compound I treated	252±5.7	++	233±5.9** 15.2#	+
(Gr VII) Compound II treated	257±5.2	++	231±5.9** 16#	+
(Gr VIII) Compound III treated	251±5.6	++	263±6.2** 4.3#	++
(Gr IX) Glibenclamide treated	261±6.5	++	224±7.0** 18.5#	+

[Values are mean ± SD from 6 animals in each group]

P<0.01 when compared with the diabetic control values.

The percentage reduction is of one week value when compared with corresponding diabetic control value.

'+-' indicates presence of urine sugar in trace quantity, '++' indicates presence of urine sugar in more quantity, '-' indicates absence of urine sugar, '+' indicates presence of urine sugar in minimum quantity.

Table 8.5 Antidiabetic activity of the extract and isolated compounds on Serum glucose level during GTT in normal healthy rats (Anand *et al.*, 2007).

(Group) Treatment	Serum Glucose (mg/dl)					Increase in serum glucose (mg/dl) between 0 and 1 hr
	Fasting	0 hr	1 hr	2 hr	3 hr	
(Gr I) CONTROL	84 ±5.6	79 ±7.8	164 ±7.9	114 ±5.7	103 ±4.3	85
(Gr II) EEUP	81 ±6.1	75 ±6.6	150 ±5.1	97 ±4.5	76 ±3.3	75 (11.7%*)
(Gr III) EECA	82 ±6.3	76 ±6.2	154 ±5.3	104 ±5.0	86 ±4.1	78 (8.2%*)
(Gr IV) CEMC	80 ±5.9	76 ±6.5	159 ±6.0	116 ±5.7	104 ±5.1	83 (2.3%*)
(Gr V) Compound I	83 ±5.8	74 ±6.4	147 ±5.5	98 ±4.7	78 ±3.3	73 (14.1%*)
(Gr VI) Compound II	84 ±6.2	76 ±6.8	149 ±5.4	99 ±4.8	81 ±3.8	73 (14.1%*)
(Gr VII) Compound III	82 ±5.8	77 ±7.1	159 ±6.0	115 ±5.7	104 ±5.1	82 (3.5%*)

*Percent of the untreated control of the same group.

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