Chapter – II

LITERATURE REVIEW

2. LITERATURE REVIEW

Tea [*Camellia sinensis* (L.) O. Kuntze] is one of the world's oldest panaceas. The tea plant was first discovered by the ancient Chinese, who then spent many centuries perfecting the art of tea cultivation to manufacturing, which resulted in a variety of types available today. Tea-elixir is drunk in almost every country around the world and has reached a ceremonial status as a social and medicinal beverage (Helliwell *et al*., 1999).

2.1 EARLY HISTORY OF TEA CULTIVATION

The first attempt of tea cultivation in Darjeeling dates back to January 1834 when Lord Willam Bentinck proposed, to the Council of the East India Company, setting up of a tea committee to investigate and make recommendations on the suitability of tea cultivation in India. The tea committee decided to send their secretary G.J. Gordon to China in order to acquire tea seeds and some tea workmen familiar to tea cultivation and manufacture. From this original consignment of China seed around 42,000 young plants could be raised which were allocated to three main areas, 20,000 to the hill districts in the Kumaon in North India, 2,000 to the hills of South India and the remaining 20,000 to the then North-East (N.E.) frontier. Out of this initial trial, seed tried in Darjeeling grew well. As per the available records one Dr. Campbell, a civil surgeon, planted tea seeds in his garden at Beechwood, Darjeeling 2100 m above mean sea level (amsl) as an experiment with reasonable success. Subsequently the Government, in 1847, selected the area to raise tea nurseries. With the plants raised in the government nurseries, the first commercial tea gardens in Darjeeling hill area were Tukvar, Steinthal and Aloobari tea estates in 1852 (Pathak, 2004).

2.2 TAXONOMY OF TEA

The grouping of tea into an erect small-leaf China variety and a horizontal broad-leaf Assam variety was rather subjective. Plants with intermediate leaf characteristics could not always be assigned to either of these two varieties. Relying mostly on characteristics of styles, Wight (1962) divided tea plants into *C. sinensis* (L.), *C. assamica* (Masters) and a third southern form of tea or Cambod race, the sub-species *C. assamica* ssp. *Lasiocalyx*.

In the Indian sub-continent, the classification proposed by Wight (1962) is popular. Otherwise, the practice world over is to put tea plants under the name *C.*

sinensis (L.) O. Kuntze, irrespective of taxonomic variation. Reference to varieties is however still common; China, Assam and Cambod varieties are generally referred to as *C. sinensis, C. assamica* and *C. assamica* ssp. *lasiocalyx*, respectively (Banerjee, 1992). There are no absolute crossing barriers within the genus *Camellia*, creating the possible involvement of taxa other than *C. assamica, C. sinensis* and *C. assamica* ssp. *Lasiocalyx* in the tea genetic pool of particular interest is the suspected involvement of *Camellia irrawadiensis* Barua (Kondo, 1977). This sexual compatibility of *C. sinensis* with other *Camellia* species provides the potential for the introduction of other desirable genes into the *C. sinensis* gene pool. For example, using this breeding strategy, resistance to a wider range of pests can be obtained. Besides a few natural triploids, the chromosome number of all varieties of *C. sinensis* studied is 2n = 30 (Bezbaruah, 1971; Kondo, 1977).

2.3 PRESENT SCENARIO OF TEA CULTIVATION

Tea plantation of North Bengal is spread over three regions – the Darjeeling hills, its Terai region, and the plains of the Dooars. North Bengal produced some 10, 85,300 kgs of tea in March 2006. According to the statistics of Tea Board of India, there are 308 big and 1232 small tea gardens in North Bengal. Total area under tea is 5, 19,700 hectares and in 2005, total production of tea was 927.98 million kilograms. At present, there are 86 running gardens producing ‘Darjeeling Tea’ on a total land of 19,000 hectares. The cool and moist climate, the soil, the rainfall and the sloping terrain all combine to give Darjeeling tea its unique "Muscatel" flavour which is regarded as the "Champagne of Teas". The total annual production of such tea is in the range of about 10 to 12 million kilograms. Tea grown in the Darjeeling foothills, Terai and the Dooars plains are mostly high yielding clones.

Each tea growing region has its own distinctive features. During last three decades, several changes have taken place in the agronomic practices, which have also magnified our quality of tea.

2.4 CONCEPT OF FREE RADICALS

Free radicals are molecular fragments containing one or more unpaired electrons in the outer orbit. These fragments are unstable and usually give a significant degree of reactivity. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are expressed as free radicals and other non-radical reactive derivatives (Ghosal, 2014).

These radicals are less stable, and generally stronger than non-radical species (Pham-Huy *et al*., 2008; Ghosal, 2014). Free radicals are generated from molecules by the homolytic division of chemical bond and via redox reactions, and once formed these highly reactive radicals can start chain reaction (Bahorun *et al*., 2006; Valko *et al*., 2007). ROS includes superoxide ($O^{2\bullet\,-}$), hydroxyl ($^{\bullet}OH$), peroxyl ($ROO^{\bullet}$), lipid peroxyl ($LOO^{\bullet}$), alkoxyl ($RO^{\bullet}$) radicals. Nitrogen free radicals consist of nitric oxide ($NO^{\bullet}$) and nitrogen dioxide ($NO_2^{\bullet}$). Oxygen and nitrogen free radicals can be readily transformed to the other non-radical reactive species which are also harmful to health. Hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), ozone (O_3), hypochlorous acid (HOCl), nitrous acid (HNO_2), peroxynitrite ($ONOO^-$), dinitrogen trioxide (N_2O_3), lipid peroxide (LOOH) are not the free radicals and generally named oxidants and can easily lead to the free radical reactions in living organisms. The above reactive species are formed in animals under physiological and pathological conditions (Halliwell and Gutteridge, 1999; Fernandez *et al*., 2002; Valko *et al*., 2007; Pham-Huy *et al*., 2008).

Free radicals are formed from both endogenous and exogenous substances. They are continuously forming in the cells and environment. Free radical reactions take in three different individual steps: formation of radicals then propagation step where free-radicals are reproduced with repeated chain reaction and lastly termination step in which destruction of radicals occur (Ghosal, 2014).

ROS and RNS play a dual role in human beings, toxic and beneficial compounds. The slight balance between their two opposite effects is unquestionably a key aspect of the life (Sen *et al*., 2010). At low or moderate levels, these reactive species exert beneficial effects on cellular redox signaling and immune function; on the other hand at high concentration, they produce oxidative stress which is a harmful process that can damage cell function and structures (Pham-Huy *et al*., 2008; Sen *et al*., 2010).

2.5 ANTIOXIDANT MECHANISM OF ACTION

The efficacy and biological action of antioxidant molecules vary with homogeneous nature of the cellular system. So mechanism of action of antioxidants has changed with the chemical atmosphere. Natural antioxidants in biological mechanisms may serve as a physiological barrier to prevent generation of reactive oxygen species (ROS) access to important target sites (Ghosal, 2014). Occasionally antioxidants like carotenoids or anthocyanidins develop a chemical trap that absorbs energy and electron and quenching with ROS. Antioxidant enzymes like catalase; glutathione reductase and superoxide dismutases neutralize or divert ROS through catalytic mechanisms. Sometimes binding or chelation of metal ions by ferritene ceruloplasmin and catechin prevents generation of ROS. Besides this, there also chain breaking antioxidants such as ascorbic acids, tocopherol, uric acid, glutathione and flavonoids (Benzie and Strain, 1996) which scavenge and destroy ROS. It is also important to understand the mechanism and pharmaco-dynamics of antioxidant action for appropriate selection of antioxidants. The quantitative structure activity relationship is determined by chemical nature of antioxidants and governed by chemical reactivity towards free radicals and their stoichiometric ratio, fate of antioxidant derived radicals interaction with other antioxidants concentration and mobility at a particular microenvironment along with absorption, distribution, retention, metabolism and fate of antioxidant molecules in a particular biological system (Niki, 2010). Some antioxidants are present in free form while the others are existing as a metabolic intermediate in bound form. Hydrophilic antioxidants like ascorbic acid or uric acid scavenge the free radicals primarily in the aqueous phase. The biological action of lipophilic antioxidants is mainly observed within the membrane and lipoprotein. The efficacy of radical scavenging by antioxidants in the membrane and lipoprotein particles depend on physical nature like the fluidity of micro-environment and relative mobility of antioxidants. Such as peroxyl radical scavenging capacity by α-tocopherol in the membrane is irrelevant than that of homogeneous solution probably because of restricted mobility of α-tocopherol (Ghosal, 2014). It was also noticed that the side chain of lipophilic antioxidants reduces the mobility inside the membrane and lipoprotein, thus minimizing the apparent antioxidant capacity. It was previously reported that the radical scavenging capacity of ubiquinol didn't depend on the length of isoprenoid chain in homogeneous solution, but in heterogeneous membranes ubiquinols having short side chain significantly inhibit lipid peroxidation than their longer side chain homolog (Niki, 2010).

Some free radical scavenging antioxidants also inhibit oxidation of bio-molecules by synergistic co-operation with other antioxidants. The examples of the synergistic antioxidant interaction are efficient combination of vitamin C and vitamin E during oxidative stress. Vitamin E also induces another chain oxidation during scavenging of active free radicals by converting into vitamin E radical through which polyunsaturated lipids may be disrupted. Vitamin E also enhances the oxidation of isolated LDL and plasma lipids by phase transfer mechanism but combination of Vitamin E and vitamin C inhibit their oxidation completely. In contrast, another hydrophilic radical scavenging antioxidant uric acid present in plasma does not reduce vitamin E radical and inhibit pro-oxidants action of vitamin E. Phenoxy radical from polyphenolic antioxidants available in different medicinal plants having hydroquinone and catechol structure reacts rapidly with active oxygen and produces corresponding quinone and hydroperoxyl radical, which may elicit new chain reaction. It was also observed that ubiquinol and tocopherol hydroquinone undergo auto-oxidation *in vitro*. The reactivity of these hydroquinones towards free radical is larger than the α-tocopherol. So, the fate of antioxidant derived radical ultimately determines radical scavenging capacity in biological system (Ghosal, 2014).

2.6 SYNTHETIC ANTIOXIDANTS

Synthetic antioxidants are not available in nature and frequently mixed with food products as preservative to prevent the lipid peroxidation. Primary antioxidants stop the generation of free radicals and they are classified into free radical terminators, oxygen scavengers and chelating agents. The radical terminators include butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary butyl hydroquinone (TBHQ) and gallates (Ozkan and Erdogan, 2011). Oxygen scavengers is glucose oxidase, sulphites and ascorbyl palmitate, while chelating agents are known as transitional heavy metal like iron, copper with incompletely filled d-orbital. Secondary antioxidants like thiodipropionic acid and dilauryl theodipropionate act by breaking down hydroperoxides formed during lipid oxidation and produce stable end products (Sen *et al.*, 2010). Many antioxidants found in plants and are also prepared synthetically through different chemical processing *i.e.* gallic acid. These phenolic synthetic antioxidants have the similar common biological effect on molecular, cellular and organ levels (Kahl, 1984; Venkatesh and Sood, 2011). Commonly used synthetic antioxidants like BHT, BHA, ethoxyquinone and propyl gallate can produce beneficial interactions such as antimutagenic activity and antitumorigenic action, radioprotection, protection against acute toxicity of chemicals (Kahl, 1984). Besides the beneficial impacts of synthetic antioxidants, these chemicals also have some adverse side-effects, like they play a key role in radio sensitization, enhanced toxicity from other chemicals, increased tumor yield from chemical carcinogens and increased mutagenic activity (Venkatesh and Sood, 2011).

2.7 THERAPEUTIC & CHEMICAL CHARACTERISTICS OF TEA

Scientists are very concerned about escalating the occurrence of age related diseases like hyperlipidemia, hypertension, hyperglycemia and cancer. Tea extract directly control pro-inflammatory signaling. Daily consumption of green tea prevents the incidence of cancer (Tamara, *et al.*, 2004). Black tea polyphenols prevent pollutant induced hepatotoxicity (Chen *et al.*, 2004), delay skin ageing, act against oxidation in rheumatoid arthritis (Carol *et al.* 2004), attenuate lung injury in pleurisy (Paole *et al.*, 2005), act against *H. pylori* (Mabe *et al.*, 1999), cardiovascular diseases (Hitchen *et al.*, 2004), radiation induced skin toxicity (Pajohk *et al.*, 2006) and smoke induced damage in protein (Misra *et al.*, 2003). Mandal *et al*. (2010) revealed that there has been increasing interest in finding plants with high antioxidant capacities since they can escalate the progression of many chronic diseases. Serafini *et al*. (1996) analyzed young tea shoots which contain more than 35% of their dry weight in polyphenols. Also, tea polyphenols decrease capillary fragility, which is likely to accumulate the flavonoids like vitamin-P during apoptosis. Nonfermented green tea contains predominantly flavonols, flavan diols and phenolic acids like gallic acid, coumaric acid or caffeic acid, with those in green tea is higher than those in black tea. Misra *et al*. (2008) reported that phenolic compounds that are present in young tea shoots (also referred to as fresh green leaves, fresh tea shoots, or flushes) are known to be one of the main factors in determining the quality of the resulting tea drink.

Processed tea that is widely consumed now as popular health drinks or beverages commercially manufactured from the young tender leaves of the tea plant (Cabrera *el al*., 2003). Tea elixir has continued to be considered as medicine since the ancient time because of its richness in phytochemical constituents (Misra *et al*., 2016). Research on the effects of tea on human health has been fuelled by the growing need to provide natural healthy diets that include plant derived polyphenols. Research is going on to elucidate how functional component in tea cultivar could expand the role of diet in oxidative disease prevention and treatment (Misra *el al*., 2003. Hitchon *et al*., 2004, Marian *et al*., 2004, Pajohk *et al*., 2006). There is evidence that tea constituents play a therapeutic role in more than sixty different health conditions (Pandey *et al*., 2005, Vanessa *et al*., 2004, Yamamoto *et al.*, 2004, Paola *et al.*, 2005, Hang *et al*., 2003 and Hakim *et al*., 2004). Therefore tea appears to be an effective chemopreventive agent for toxic chemicals which are produced in the body during normal metabolic pathways or introduced from the environment. Many plant phenolics have been reported to have antioxidant properties that are even much stronger than vitamin-C and E (Karori *et al*., 2007). In addition, currently available synthetic antioxidant like BHA, BHT and gallic acid esters have been suspected to initiate negative side effects (Amie *et al*., 2003, Aquil *et al*., 2006) and hence the need to substitute them with natural antioxidants like that from tea with broad-spectrum action. Information on the tea antioxidant properties varies in tea cultivar and degree of leaf maturation, which are rare and grossly lacking (Misra *et al*., 2016). The antioxidants quality with some chemical constituents of tea cultivars is correlated with soil agronomic parameters for determining better cultivation practices to restore the antioxidant quality of tea (Misra *et al*., 2016).

The flavonoids act as anti-viral (influenza), anti-bacterial, anti-mutagenic, molluscicidal, deodorant and alter enzyme activity affecting cell division, proliferation, platelet aggregation and immune response. Such therapeutic properties of tea are most likely due to the presence of significant amount of polyphenols like catechins, gallocatechin, epicatechin, epigallocatechin. epigallocatechin-3-gallate, theaflavins, thearubigins, flavonols and their glycosides, gallic acid, chlorogenic acid, non flavonoid hydrolysable tannins etc which are known to protect against oxidants. There were studies related to the chemical composition of tea shoots and its constituent catechins are best known for their antioxidant properties, which has led to their evaluation in a number of diseases associated with reactive oxygen species (ROS), such as cancer, cardiovascular and neurodegenerative diseases. Several epidemiological studied by Yang *et al*. (2002) as well as studies in animal models have shown that green tea can afford protection against various cancers such as those of the skin, breast, prostate and lung. The free radicals *e.g.* reactive oxygen species (ROS), reactive nitrogen species (RNS) and other radical-centered species, e.g. S- and C-, abstract hydrogen (H) from hydrogen donors (polyphenols) and accept an electron from electron rich species. Hence, they act as oxidants and responsible for oxidative stress in aerobic organisms (Halliwell *et al.,* 1999). Natural antioxidants are commonly used to counter the deleterious action of free radicals (Ghosal, 1991). Radical scavenging can be considered as the principal mechanisms of the anti oxidative property of flavonoids. Flavonoids are highly effective scavengers of all types oxidizing radicals (Bors *et al.,* 1996). The radical scavenging activity is directly correlated with the number of phenolics OH groups in the flavonoid molecules. The flavonoids protect vitamin-C against oxidation (Bentsath *et al.*, 1936). The synergistic interaction of flavonoids with vitamin-C is documented (Sarata *et al.*, 1988) in Citrus fruit. Therefore the roles of flavonoids are very important to regenerate the antioxidant activity of vitamins in the tissues. According to Hudson and Levis (1983), the chelating ability of flavones and flavonone type molecules are OH group at C5 and C3 position, methoxy group at the C4 position and a double bond between C2-C3. The chelation of metal ions can be facilitated by the OH groups and therefore these molecules limit the metal catalyzed the degradation of hydroperoxides. The hydroxylation pattern of β- ring and different substitution of flavonoids (polyphenol), are principally important for executing their bioactivity. Tannin also has a potential role as an antioxidant. Electron Paramagnetic Resonance (EPR) experiments demonstrated the quenching activity of singlet oxygen, super oxide, hydroxyl radical by the tea extract. The f1avonoids are 20 times more powerful antioxidants than vitamin.-C in lipoprotein oxidation model. Their efficacy was evaluated by battery test which indicated that they can inhibit hydro peroxide formation and their scavenging property has been tested against a spectrum of super oxide radicals (offensive oxidant) and other free radicals. They exhibit modifying influence on the protein phosphorylation process and different catalytic activity of many enzymes especially the oxidative ones. They prevent the metal-catalyzed free radical formation caused by Cu and Fe chelate. Flavonoids do not quench with beta-carotene, Vitamin C and E mediated endogenous antioxidant protections system of the body. The increasing interest of alimentary application (i.e. dietary, nutraceuticals, flavouring agent etc) of tea matrices rich in antioxidants is due to the possible correlation between oxidant action of free radicals and the onset of some important pathologies (Misra *et al*., 2016). Scavenging of antioxidants from the plant matrices can be used in the nutritional industry for the formulation of preventive phytochemicals in future. Strict legislation on the use of synthetic food additives and consumer preferences has also shifted the attention from the toxic synthetic to less toxic natural antioxidant.

A few authors considered the accumulation of polyphenols in plant system and correlated their data with the agro-climatic conditions. It has been observed that nitrogen and phosphorus deficiency usually increase polyphenol level in plants (Horborne, 1980). High nutrients of soil enhance the level of bioactive phenolics as an antioxidant in cabbage (Kim *et a1.* 2004) and high level of potassium usually reduced the amount of theaflavins and thearubigins in leaves, which could lead to reduction in tea quality (Devchoudhury *et a1.,* 1988). In 1969, Willson claimed that the quality was reduced with increasing level of nitrogen, no change with potash and an improvement was observed with increasing phosphate status. The effect of different NPK status of soil, changes the amount of 32 volatile flavouring components, theaflavin and thearubigin and caffeine in black tea (Wuor *et a1.,* 1987). The effect of nitrogen increases the level of tannins in the four leaf flush (Lorntadze *et al.,* 1986), also increased the level of phosphate and nitrogen enhanced the amount of tannins and catechins in tea (Salukvadze, 1980). Though Zyrin *et al.* (1987) stated that the conjugate application of phosphate and potash applications unchanged the level of tannins. The quality of tea is reduced by high status of nitrogen and potash and improved by the high status of phosphate only and high status of all nutrients reduced the quality again (Sugianto, 1985).

Human civilization is going under serious crisis. The crisis is workload and social/professional pressure due to a better lifestyle and more comfortable life leading. The ultimate result of this is oxidative stress which is converted into metabolic disorders such as atherosclerosis, cataracts, cancer and osteoarthritis (Misra *et al.*, 2007). Excess oxidative stress might produce many free radicals which are the main cause of oxidation of lipids, DNA and protein. There are many evidences which can prove that antioxidants might prevent the primary ageing processes, as well as many of the age-associated secondary pathological complications. It has been recorded that the consumption of a higher amount of tea and fruits along with vegetables reduced the risk of degenerative diseases like cancer as well as atherosclerosis and the dietary flavonoids can restore a range of oxidative radical damage sustained by DNA (Anderson *et al.,* 2000). There is no confusion that the antioxidants present in tea are the best solution for oxidative stress-mediated disorders. Now-a-days, synthetic medicines were frequently consumed for rapid recovery from different disorders. But these synthetic medicines generate lots of trouble-shooting side effects and sometimes accelerate apoptosis in our body. So the question has now arisen regarding the therapeutic application of different synthetic medicines for all kinds of disorders. In this context, it should be noted that disease protection is better than cure. Several recent studies have already indicated that antioxidant molecules might play an important role in disease protection, particularly when it is associated with lifestyle mediated disorders. But sufficient care should be taken before consumption of antioxidants, particularly when they are synthetic or derived from semi-synthetic procedures; regarding their toxicity, bioavailability, metabolic fate, and accumulation inside body and side-effects both for shorter and longer term. One of the main side effects of these synthetic drugs for long term use is drug addiction and resistance. Synthetic antioxidants are now compiled with several drugs for minimizing oxidation process, as well as they are also used in food as preservatives through which adverse effects of synthetic antioxidants might be generated in the biological system. These adverse biological effects occur on modulation of growth and immune response and interfere with oxygen activation in the body. Due to their toxic effect, a few antioxidants like BHA, BHT, PG and TBHQ are currently permitted for their usage in food as preservatives. A well-known degradation products of BHT i.e. tert-butylhydroquinone (tBHQ) which is used for stabilization and preservation of freshness, nutritive value, flavour and colour of animal food products, is known to exert a carcinogenic effect by causing oxidative damage of DNA .

Antioxidants are almost universal in normally consumed herbal food products, they are pre-existing compounds in the form of natural secondary metabolites; and sometimes are also required during processing of synthetic antioxidants. As long as they are consumed in moderate concentration, natural antioxidants have been proven to have several positive health effects as compared to their synthetic counterparts (Daniel, 1986). The food which are used by common people, serve as a nutrient. Side by side if these foods would have been used for nutraceutical and antioxidant purpose then the problem of oxidative stress mediated disorders might be resolved. Recently it has been conceptualized that ideal food should have all the required nutrients as well as nutraceutical and antioxidant principles (Danesi, 2009). The bioactive food component refers to nonessential bio-molecules which are present in tea, exhibit the capacity to modulate several metabolic processes that result in the promotion of better health. The ethnic group of inhabitants has gathered this knowledge through trial and error, during their survival in such inhospitable environments for hundreds of years. Unfortunately, this traditional knowledge is masked under the light of modern medication and therapy. Although, recently the importance of plant based traditional medicine is being realized by the masses. People of different parts of the world have started recording and evaluating such herbal knowledge through assessing antioxidants as well as different pharmacological properties (Saha *et al.*, 2011). Those who are unable to collect their staple foods or not getting the chance to take lunch a day but they consumed a cup of tea per day.

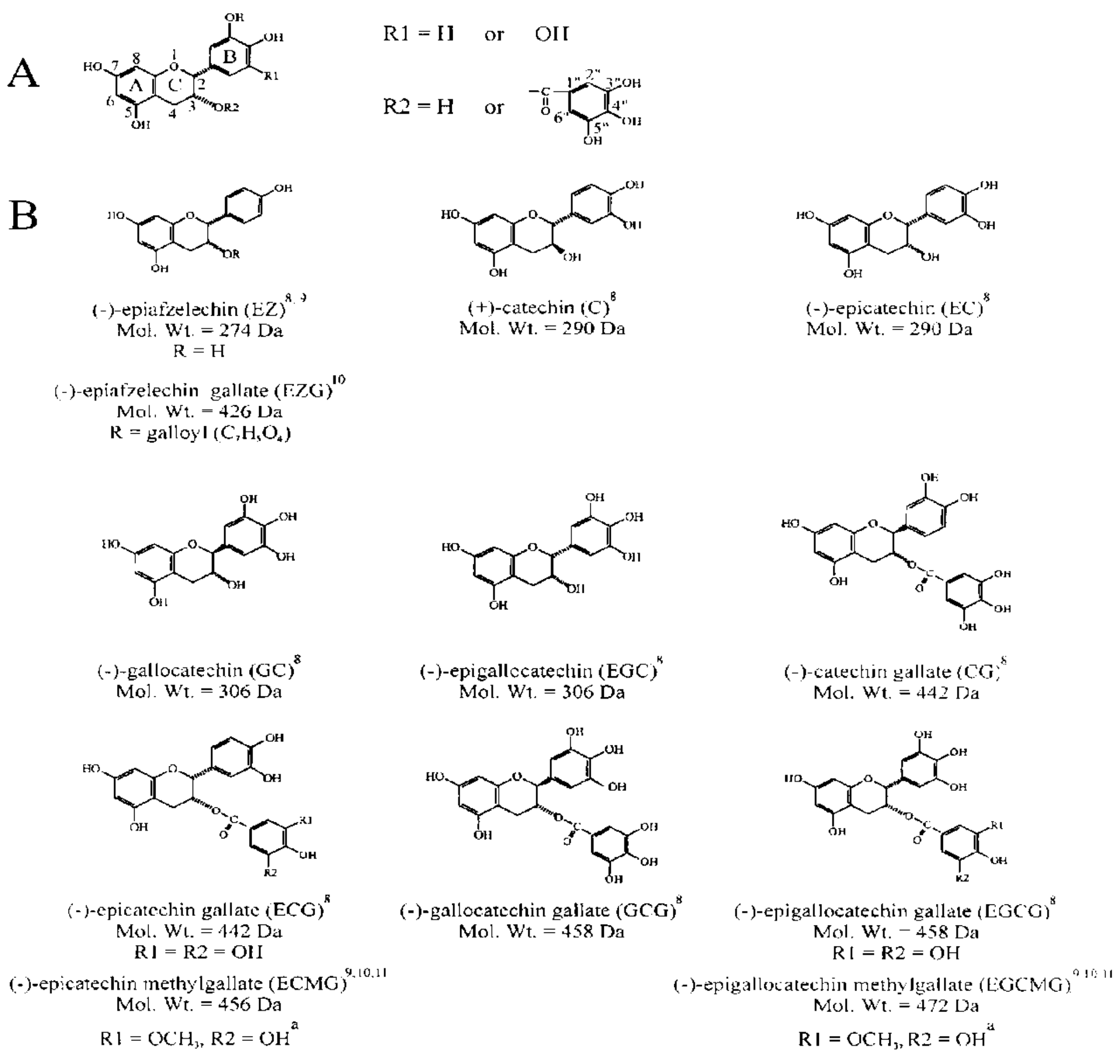

Figure 2.1 Catechin structures. (A) General structure of a Catechin. (B) Structures of the 12 tea catechins.

Regular consumption of tea may contribute to the daily dietary requirements of several elements and tea could be an important source of manganese and a large amount of potassium in comparison with sodium that could be beneficial for hypertensive patients (Misra *et al.*, 2016).

It has been reported that phenolic compounds that are present in young tea shoots (also referred to as fresh green leaves, fresh tea shoots, or flushes) are known to be one of the main factors in determining the quality of the resulting tea drink (Misra *et al*., 2008). There were studies related to the chemical composition of tea shoots and its constituent catechins are best known for their antioxidant properties, which has led to their evaluation in a number of diseases associated with reactive oxygen species (ROS), such as cancer, cardiovascular and neurodegenerative diseases. The natural polyphenols in orthodox tea include (-)-epigallocatechin-3 gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), and epicatechin (EC). Other minor catechins, (+)-gallocatechin (GC), (-)-gallocatechin gallate (GCG), (-)-catechin gallate (CG) and (+)-catechin (C) are also present in tea. The highest concentration is of EGCG followed by ECG, EGC and EC in decreasing order (Nakabayashi *et al*., 1991). The general structure of a catechin (A), structures of the 12 tea catechins (B) and Retro Diels-Alder fragmentation of a catechin (Daniel *et al*., 2000) is given in Figure 2.1 & 2.2.

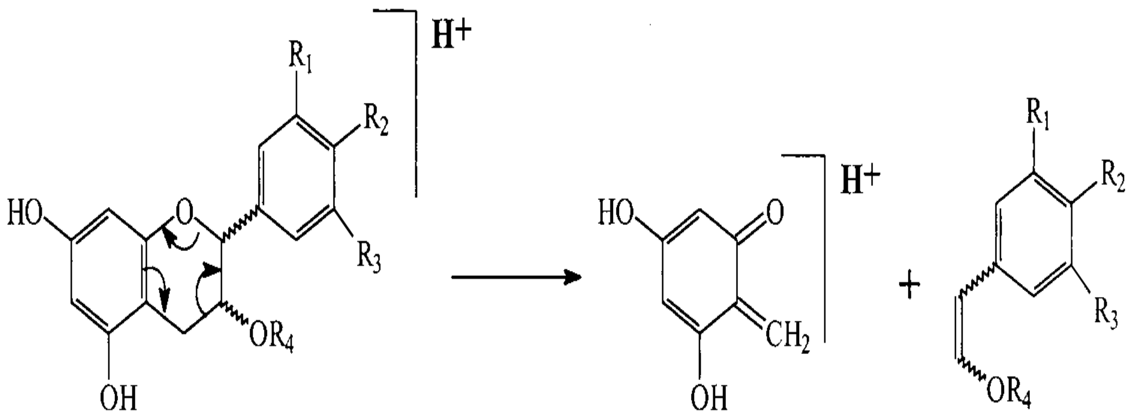

Figure 2.2 Retro Diels-Alder fragmentation of a catechin

2.8 ASSESSMENT OF FREE RADICALS SCAVENGING ACTIVITY

The functional properties of antioxidants are different in response to various oxidant sources. Free radicals involved in the oxidative stress have different physiochemical and biological properties within the cell. Therefore no single assay accurately reflects the antioxidant potential of a particular molecule against all radical sources (Huang *et al*., 2005).

The major free radicals that react predominantly with lipids, protein, carbohydrates and DNA are hydroxyl, alkoxyl, peroxyl, superoxide, nitric oxide along with sulfur and nitrogen cantered radicals. On the basis of inactivation mechanisms performed by major antioxidants, the reaction methods have been categorically differentiated into two groups: hydrogen atom transfer (HAT) reaction and electron transfer (ET) reaction (Karadag *et al.*, 2009). HAT based methods quantify antioxidant ability to scavenge free radicals by donating hydrogen and subsequent formation of the stable compound. They are considered as radical chain breaking antioxidant capacity (Prior *et al.*, 2005). ET based method detect the ability of antioxidant potential through the capacity to transfer one electron by reducing any compounds like metals, carbonyls and radicals (Ghosal, 2014).

$$M(III) + AH \rightarrow AH^{\cdot} + M(II)$$

Some methodical strategies are available for determining reaction which includes measurement at a fixed time, measurement of a reaction rate, lag phase measurement of end point change and integrated rate measurement with different orders of reaction kinetics (Antolovich *et al.*, 2002). In general, added antioxidants components with probes for the radicals are retardation of the oxidation of probe. Assays with these features include total radical antioxidant trapping assay (TRAP) and oxygen radical absorbance capacity (ORAC) assay (Huang *et al.*, 2005; Ghosal, 2014). These assays have the following attributes:

1. Thermo-labile azo-radical initiator which produces radical ($R^{\cdot}$) that react fast with oxygen give steady flusk of $ROO^{\cdot}$ radical.

2. Oxidizable molecular probe (UV/fluorescent for monitoring reaction process)

3. Antioxidants

4. Kinetic parameters of reaction (Magalhaes, 2008; Ghosal, 2014).

Other test includes carotenoids bleach via auto-oxidation induced by heat or peroxyl radicals (*e.g.* oxidized lipids). The assay measures decrease in the rate of β-carotene or decay of crocin probe by tea antioxidants. End point loss of colour was measured optically at 443 nm in phosphate buffer (pH 7.0) (Laguerre *et al.*, 2007). The major advantage is that the kinetic approach in the measurement allows the determination of total inhibitory effect and provides more precise evaluation and efficiency of antioxidant defense (Roginsky and Lissi, 2005). Although β-carotene is often used as a target in this assay, its discoloration at 470 nm can accomplish by multiple pathways. Consequently, interpretation of these results can be difficult. For overcoming this problem, carotenoid derivative protein which is natural compound with extremely strong absorbance in visible range has become the reagent choice recently (Prior *et al*., 2005).

The antioxidant capacity of a compound can be determined by two factors *i.e.* kinetics of scavenging radicals and number of radicals each antioxidant molecule can scavenge. These two attributes can be determined by the reaction with reference free radical like 2,2-diphenyl-1-pyicrichydrazyl (DPPH) and cationic radical ABTS. DPPH is long lived organic nitrogen radical with deep purple colour. Also DPPH is commercially available, stable, easy to handle and has a long visible spectrum with high molar extinction coefficient. The purple chromogenic DPPH radical is reduced by antioxidant compounds to the corresponding pale yellow hydrazine. The DPPH assay is technically simple and rapid and analyses of a large number of samples could be made by using microplate (Fukumoto and Mazza, 2000). ABTS, on the other hand, is a peroxidase substrate which is intensely coloured and spectrophotometrically monitored within the wavelength range of 600-750 nm. $ABTS^{\cdot +}$ can be solubilized in both organic and aqueous media and is not influenced by ionic strength; therefore the antioxidant capacity can be determined for both lipophilic and hydrophilic compounds (Arnao, 2000). In contrast DPPH can only be dissolved in organic media which is an important limitation for interpreting the role of hydrophilic antioxidants.

It is generally observed that a potent radical scavenging antioxidant can often behave like efficient reductant. The ferric reducing antioxidant power (FRAP) assay is based on the ability of an antioxidant to reduce ferric tripyridyl triazine complex (FeIII-TPTZ) to blue ferrous complex (FeII-TPTZ) by the action of electron donating capacity (Benzie, 2003). Here, ferric salt is used as an oxidant and redox potential is comparable to that of ABTS. FRAP assay is rapid, inexpensive, robust and does not require specialized equipment. The major limitation of this assay is that the ultimate product is a complex of $Fe(II)^+$ which is also known as pro-oxidant. The ferrous ion can react with H_2O_2 to produce hydroxyl radical ($OH^{\cdot}$) which is most harmful free radical found in biological system. Ultimately not all reductants that are able to reduce $Fe(III)^+$ are potent antioxidants (Prior and Cao, 1999). In addition FRAP cannot detect compounds that act by radical quenching (hydrogen transfer) particularly applicable for thiols and proteins (Ou *et al*., 2002). Sometimes polyphenols may bind to metal ions and form complex, which may perform another function of polyphenolic compounds as an antioxidants.

The assay methods so far discussed are particularly applicable on hydrophilic or hydrophobic targets. But lipids like free and esterified forms of polyunsaturated fatty acids and cholesterol are the sensitive target of free radicals. From different investigations, it was proved that peroxidation of membrane lipid induces disturbance and dynamic alteration of membrane functions. Obviously, it is also an essential task for scavenging antioxidants to suppress lipid peroxidation. One of the major advantages of lipid peroxidation of plasma is that both hydrophilic and lipophilic antioxidants and their interactions can be assayed through the biologically relevant system. The capacity of antioxidant for inhibition of lipid peroxidation can be assayed by determining the extent of suppression of peroxidation by the test antioxidants. In fact in this assay, the thiobarbituric acid reactive substances (TBARS) or malonaldehyde generation can be determined precisely through chromogenic reactions. TBARS in biological system can also be acceptable as biomarkers of oxidative stress. Overall it can be stated that elucidation, understanding and evaluation of antioxidant action can only be possible through determining several radical scavenging species by different *in vitro* and *in vivo* assays and understanding the correlation among them (Ghosal, 2014).

2.9 QUANTITATIVE VARIATION OF ANTIOXIDANT ACTIVITY WITH SOIL NUTRITIONAL PROPERTIES

Effects of various environmental factors on the accumulation of secondary metabolites were well known and documented elsewhere in this review, but only limited studies covering the response of secondary metabolites and antioxidant activities under different soil nutrient sources (Ibrahim *et al*., 2013). Recent research has uncovered the fact that the availability of plant nutrients can be important factors for elicitations of secondary metabolites and antioxidants within plants (Stewart *et al*., 2000). Mineral nutritional status and physical properties; generally pH, EC and micro-nutritional properties of soil greatly influence the phytochemical constituents present in different plant parts (Mandal *et al*., 2010). Macronutrients of soil like nitrogen, phosphorus, potassium and sulfur interfere with the biosynthesis of phenolic compounds produced through phenylpropanoid pathway (Coria-Cayupan *et al*., 2009). Interestingly while the enrichment of nitrogen, phosphorus, potassium and calcium in soil through fertilizer application have been shown to affect secondary metabolites production in some plants (Kraus *et al*., 2004), there are other plants also where polyphenol accumulation was not significantly influenced by mineral nutrition (Mogren *et al*., 2006). So, intensive research is required for determining the influential parameters of soil macronutrients on the biosynthesis and metabolic fate of antioxidant molecules. Among macronutrients of soil, nitrogen is one of the most important growth factors in regulating the quality and target plant parts. Nitrogen supply and accumulation in soil have both positive and negative effect on the biosynthesis of phenylpropanoids in plants. The enhancement of total plant phenolics and flavonoid compounds under limited nitrogen fertilization was previously reported (Koricheva *et al*., 1998; Felgines *et al*., 2000). Improvement of carbon based secondary metabolites under poor nitrogen soil condition was in agreement with Carbon Nutrient Balance theory proposed by Bryant *et al.* (1983). The increase in polyphenol based antioxidant molecules under low nitrogen condition might be attributed to the excess availability of unutilized phenyl alanine due to restricted protein synthesis under nitrogen deficiency (Awad and de Jager, 2002). Reduced nitrogen fertilizer also increased glutathione which has a strong correlation with total phenolics, flavonoids, ascorbic acid and saponin content (Ibrahim *et al.,* 2013). However, some studies also indicated that the available amendment of organic nitrogen fertilizer might improve leaf antioxidant status in association with soil biota (Montalba *et al*., 2010). In *Ziziphus jujuba* Mill., potassium enrichment in soil improved the accumulation of total phenolics, total flavonoids and total pro-anthocyanidin content (Wu *et al*., 2013). Potassium supplementation also helps in accumulation of phenylpropanoid components in apricot fruit (Radi *et al*., 2003). Interestingly, on onion, the addition of potassium in soil enhanced total polyphenols but bears negative correlation with free radical (DPPH) scavenging properties (Bystricka *et al*., 2013). In some cases, application of lime and phosphorus improved the plant nutrition, with enhanced dry matter content and plants antioxidative system (Mora *et al.,* 2008). But phosphorus also showed negative impacts on the accumulation of phenolics, expect for protocatechuic acid (Wu *et al*., 2013). Overall, it can be stated that natural growing practices and soil nutrient profile are important attributes for simultaneous improving yields, phenolics level and antioxidant activity (Ghosal, 2014).

2.10 AGRO-CLIMATIC ZONES OF NORTH BENGAL

Tea is most popular drink and that is why necessary to evaluate antioxidant activity of tea under various agro-climatic conditions of North Bengal. But unfortunately, through literature survey, it has to be known that the tea of Terai, Dooars and Hills are almost untouched (Misra *et al*., 2016).

Climatic variations are also important because of its fluctuating behaviour influence the polyphenol contents in the tea leaf. Warm days, long sunshine hours, high humidity and adequate rainfall, preferably over night showers, the optimum temperature range between 18°C to 30°C, minimum day length <11 hours, 150 ml average rain fall per month, are the primary criteria for quality tea. Mulching material or decomposed plant waste used in tea plantation may increase the antioxidant level and it is supported by the evidence from other plantation (Sivapalan *et al.*, 1993). Compost as soil supplements increases the level of antioxidant compounds and oxygen radical absorbance capacity in Straw berries (Wang *et al., 2003).* Some authors correlate quality parameters of tea with various agro-climatic conditions like off farm inputs, tea plant variety, processing technology, and industrial grades. But research on evaluation of antioxidant properties of tea grown in different agro-climatic conditions is still lacking. The correlations between the antioxidant level of tea with various production and processing parameters are also not found in the literature. Hence, the present work is being proposed to evaluate the antioxidant capacity of tea extracts from different grades of tea and also of different varieties of tea grown in different conditions.

Organic farming is not new to India, but the lack of practices of its culture creates problems, mainly in plant nutrition. Proper soil nutrition is the only way to restore productivity and quality of organic tea plantation (Singh, 2005). Tea should be free from any toxic chemical residues which have deleterious effects and should be rich in antioxidants to combat harmful oxidants in the bodies of consumers. Tea is rich in polyphenols, flavonoids etc. that have been shown to possess a wide range of biological and pharmaceutical benefits, including defense from any pathophysiological disorders. These beneficial effects may be attributed to tea's free-radical scavenging activity (Misra *et al.*, 2009, Ghosal *et al.*, 2014). Depending on the agronomic practices, tea is classified into two types: non-organically produced tea (NOT); when inorganic fertilizers and chemical pesticides are used in cultivation, and organically produced tea (OT) when environment-friendly organic techniques are employed in its agronomic practices. Indiscriminate use of chemicals in field, management techniques and profit making attitude has led to chemicalize the tea products, as a result of which antioxidant quality of tea is degrading day by day (Misra *et al*., 2009 & 2016).

2.11 IMPORTANCE OF TEA CULTIVATION

Willson *et al*. (1992) reported that the tea plant is an evergreen that is native to Northern India and China. There are two main varieties of the tea plant. The broad leaf variety, known as *Camellia sinensis* var *assamica*, grows best in the moist, tropical climates found Northeast India and the Szechuan and Yunnan provinces of China. The small leaf variety, known as *Camellia sinensis* var *sinensis*, thrives in the cool, high mountain regions of central China and Japan. India and China are the two major tea producers and exporters and followed by Sri Lanka, Kenya and Turkey, respectively.

The production of tea in India mainly started in the early 1758 AC of the British India along the Brahmaputra valley of Assam and Terai, Doors and Hills of Darjeeling. Many of the tea plantations are centered on the Darjeeling and Jalpaiguri District of West Bengal from the Nepal border to Jorhat and Kachar of Assam, reaching the altitude of around 1000 m to 3000m from mean sea level.

Halliwell *et al*. (1999) stated since 3000 B.C., traditional Chinese medicine has recommended green tea for headache, body aches and pains, digestion, enhancement of immune system, detoxification, as an energizer and to prolong life. The health benefits of tea are confirmed and therapeutic value of tea for the prevention and treatment of many diseases has become more and more commonly known (Misra et al., 2008). Tea also contains minerals and trace elements such as K, Mn, Cr, Ni and Zn which are essential to human health. Fernandez *et al*. (2002) showed that regular consumption of tea may contribute to the daily dietary requirements of several elements and tea could be an important source of manganese and a large amount of potassium in comparison with sodium that could be beneficial for hypertensive patients.

Mandal *et al*. (2010) revealed that there has been increasing interest in finding plants with high antioxidant capacities since they can help in the progression of many chronic diseases. Serafini *et al*. (1996) analyzed young tea shoots which contain more than 35% of their dry weight in polyphenols. Nonfermented green tea contains predominantly flavanols, flavan diols and phenolic acids like gallic acid, coumaric acid or caffeic acid, with those in green tea is higher than those in black tea. Misra *et al*. 2008 reported that phenolic compounds that are present in young tea shoots (also referred to as fresh green leaves, fresh tea shoots, or flushes) are known to be one of the main factors in determining the quality of the resulting tea drink. Several epidemiological studied by Yang *et al*. (2002) as well as studies in animal models have shown that green tea can afford protection against various cancers such as those of the skin, breast, prostate and lung. Henceforth, tea is an important panacea and popular health drink. Modern people interested drink tea elixir in the value added a form like cold tea, spices tea, tea ice-cream and much more. The demand for good quality Indian tea is worldwide.

The country is the second largest tea producer in the world with production of 1,197.18 million kg in 2014-15. Interestingly, India is also the world's largest consumer of black tea with the domestic market consuming 911 million kg of tea during 2013-14. India is ranked fourth in terms of tea exports, which reached 197.81 million kg during 2014-15 and were valued at US$ 619.96 million. The top export markets in volume terms for 2014-15 were Russian Federation (39.14 million kg), UK (18.58 million kg) and Iran (17.53 million kg). In terms of value, the top export markets were Russian Federation (US$ 94.43 million), Iran (US$ 75.73 million) and UK (US$ 57.74 million). All varieties of tea are produced by India. While CTC accounts for around 89 per cent of the production, orthodox/green and instant tea account for the remaining 11 per cent. Production of tea reached 1197.18 million kg in 2014-15. Around 955.82 million kg was produced in North India and 241.36 million kg was produced in South India.

India has around 563.98 thousand hectares of area under tea production, as per figures for December 2013. Tea production is led by Assam (304.40 thousand hectares), West Bengal (140.44 thousand hectares), Tamil Nadu (69.62 thousand hectares) and Kerala (35.01 thousand hectares). According to estimates, the tea industry is India's second largest employer. It employs over 3.5 million people across some 1,686 estates and 157,504 small holdings; most of them women.

2.12 PROBLEMS OF TEA PESTS

Every part of the tea plant is subject to the attack by pests. A steady loss of 10% due to overall pest attack is a generally accepted figure though it could be 40% in devastating attacks by defoliators (Banerjee, 1993). In addition to direct crop loss, pest damage can adversely affect the quality of processed tea. General observation and planters' experience indicate that looper (*Buzura suppressaria*), red slug (*Eterusia magnifica*), tea mosquito bug (*Helopeltis theivora*) and red spider mite (*Oligonychus coffeae*) are the most common tea pests of Darjeeling foothills, Terai, the Dooars areas with their incidence also in the plantations of North-East (N.E.) India. Planters of Terai and the Dooars are facing serious problems in combating the outbreaks of these folivores and sucking pests. *Oligonychus coffeae* breeds throughout the year and subsists on mature sustenance leaves. *Helopeltis theivora* causes extensive damage by attacking the tender leaves and the growing shoot. The defoliators like *Buzura suppressaria* and *Eterusia magnifica* have their share and defoliate tea in Terai, the Dooars and North-Eastern plantations. In the past, all tea phytophages were not active simultaneously and a well-marked seasonal appearance for each was evident with the seasonal cycle of the growth and productivity of the plants. Now a day, the planter's experiences indicate that most of the pest species remain active throughout the year with the overlapping seasonal cycle of the growth and productivity of the tea plants. The appearance of the pests needs timely management mostly by use of synthetic pesticides. Since 1962, increasing use of pesticides in the protection of tea plantations has been the common and popular practice.

2.13 SHADE TREES IN TEA CULTIVATION

The indigenous Assam tea was found in the under storey of the forest. From this basic knowledge, it was assumed that tea plants are grown ideally in shade environment. Most of the tea plantations, therefore, maintain a partial shade condition. Shade trees canopy protect tea bushes from excessive radiation or heat and efficiently conserves soil moisture (Ripley, 1967). In drought prone areas, deep rooted shade tree species are loped acropetally from lower branches which are very useful to protect the tea bushes below. Shallow rooted species seriously compete with tea for moisture in the dry season and are loped at the frequent interval to ensure a single leaf canopy. A happy balance between the temporary and permanent shade trees maintain proper light quality and ensure better photosynthesis. Sunlight is also associated with heat and these two factors affect plants in very different ways when considered in isolation. But under natural conditions, sunlight and heat always go together.

It appears that the primary function of a shade tree is merely to prevent excessive heating up of tea leaves by "trapping" some of the infra red rays from the sun before they reach the tea bushes. In this process, some of the useful visible spectrum of light might also be trapped by shade trees which the tea bush requires for photosynthesis. Practically, it is the balance between the amount of heat removed and the amount of light let through which determines the efficiency of a shade tree. In North-East India, only 20 to 30 % of the sunlight of clear sunny day of June was utilized by tea plants and is sufficient for maximum photosynthesis. Information regarding the suitability of shade trees in different soil status is scanty and yet to attract much attention of botanists or even tea scientists.

2.14 VARIATION OF ANTIOXIDANTS ATTRIBUTE WITH AGRO-CLIMATIC CONDITIONS AND SUITABILITY

Sivapalan *et al*. (1993) examined the comparative effects of phenol rich and phenol poor plant residues after prolonged decomposition on the urease activity of an acid soil. The addition of organic residues increased soil urease activity over that of control, but soil amended with phenol rich residues had urease activity which was about 50% lower than that of soil amended with phenol poor residues. This reduction in soil urease activity was attributed to the higher content of soil polyphenols present in the soils amended with phenol rich residues. Wang Sy *et al*. (2003) augmented compost as a soil supplements and increase the level of antioxidant compounds and oxygen radical absorbance capacity was observed in strawberries. He showed that compost as a soil supplement significantly enhance the levels of ascorbic acid (ASA) and glutathione (GSH) and the ratio of ASA/DHASA and GSH/GSSG in the fruit of two strawberry cultivars were considerably improved. The peroxyl radical $ROO^{\cdot}$ as well as superoxide radical ($O_2^{\cdot -}$), hydrogen peroxide, hydroxyl radical [$OH^{\cdot}$] and singlet oxygen [$^1O_2$] absorbance capacity in strawberries, increased significantly with increasing fertilizer strength and compost use. The planting medium × fertilizer interaction for phenolics and flavonoids was significant. Fruit from plants grown in full strength fertilizer with 50% soil + 50% composts and 100% compost yielded fruit with the highest levels of phenolics, flavonol and anthocyanin content. A positive relationship between antioxidant activities and contents of ASA and GHA and ratio of ASA/DHASA, GSH/GSSG existed in the fruit of both cultivars. The polyphenols of fresh green and dried leaves, litter of superficial humus from mull of moor site were examined by Coulson *et al*. (1960) and observed the greatest diversity and quantity of phenolic substances obtained in the extract of fresh beech leaves from moor site. There was a change in the quantity of simple polyphenols grading from a maximum in fresh growing green leaves, falling through senescent leaves to dead leaves to freshly fallen leaves, to minimum decayed leaves and humus or stored dry leaves. Tannin stripping and especially hydrolysis and reduction of the decayed leaves and superficial humus particularly release polyphenolic substances in the soil.

Total polyphenols, antioxidant capacity and flavonoids at juvenile cabbage genotypes (*Brasica Oleracea* var. capitata) were evaluated by Kim *et al*. (2004) and observed that nutritional soil supplements could provide enhanced levels of bioactive phenolic compounds as antioxidants in cabbage and therefore to consumers.

It has been discussed that nitrogen and phosphorus deficiency usually increase the flavonoid levels and boron deficiency might increase hydroxy-cinnamic acid levels at the expense of flavonoids or lignin. The application of compound fertilizers increased available P, K and Mg content in soil but decreased alkali-hydrolysable-N and NPK content, compared with the urea treatment. Application of compound fertilizers could improve the quality of tea and increased their yield by 0.551 to 1.3 tons/hector and enhanced their economic profit significantly by 10.0% to 15.7%. [Wang Rui *et al.,* 2006]. The total and the available sulfur varied with agro-climatic condition and region, with the gradual replacement of ‘S’ based fertilizers by urea and rock phosphate. The tea soils require Sulphur fertilizer for enhancing crop quality and enriched level of Theaflavin, Thearubigin and flavonol glycosides in leaf buds after processing (Chakravartee, 1996).

Effect of different doses of fertilizers on the concentration of catechins in tea shoots and theaflavin and thearubigin contents of CTC tea were examined in different clones (Dev Choudhury *et al.,* 1985). Increase in different doses of nitrogen has no significant effects on the synthesis of catechins. Gogoi *et al*. (1993) studied the effect of applied phosphate fertilizers on the quality of tea and assessed biochemically from bushes treated with different doses of P_2O_5 in different clones. They showed that the P_2O_5 concentration of 50 Kg/hac enhances the major catechins and caffeine content in tea shoots. No additional benefit was observed with higher doses of $P_2 O_5$.

The concentration of flavonoids in tea leaves changes during the development of tea shoots *i.e.* spring tea; summer tea and a 3rd crop tea differ to some extent. It is evident that flavonoid accumulation is higher in summer that in spring and also apical bud + 2 leaves are richer in polyphenols than old leaves. It is quite difficult to assign a physiological role of the majority of plant phenolics; there is increasing evidence that a considerable number of these substances play an ecological role in plants. The flavonoid pigments are contributed to flower and fruit colour for the purposes of attraction of bees and green fly in tea plant for pollination and seed dispersal. The relation between flavonoid structure and plant colour has recently been reviewed. The contribution of polyphenols in the cell vacuoles is self-evident flavones and flavonols present in the flower tissues are essential as co-pigments to the anthocyanin and also occasionally concerned as a hidden UV honey guides for attracting green fly to the tea flowers. Other ecological roles for phenolics are observed in nature. Some phenolic derivatives act as allelopathic agents. Certain chemicals are excreted by the plant which may be auto toxic or affect the growth of other plant in the environments. It has also been found that flavonoids, especially tannins, have a role as feeding deterrents, protecting plants from over-grazing by many animal species (Swain *et al.*, 1977). Other important role for certain classes of phenol is as antimicrobial agents in providing resistance to various fungal, bacterial and viral infections. Phenolics are significant not only as antifungal compounds but also for the production of phytoalexins formed post inflectionally.

Dev Choudhury, (1984) pointed out that the ecological effectiveness of many phenolics may lie in their ability to modify growth process in other organism through hormonal interactions. Thus progress in understanding the ecological function of phenolics undoubtedly illuminates and at the same times our comprehension of their physiological importance in the plant. N-ethyl glutamine (Threonine) content of tea shoots varies with clones and season. During tea processing, threonine contents of tea shoots get gradually decreased. High level of threonine in tea appears to be detrimental to tea quality of some clones.

Do Phenolics compounds play a physiological role in plant growth and metabolism? The answer is still uncertain but many phenols are clearly able to exert significant effects on growth process when applied to plant tissues at physiological concentration. This doesn't necessarily imply that they have an endogenous role. Another important role of phenolics is hormonal control, which might affect the biosynthesis of ethylene. Thus it is known that a Para-coumaric acid ester is a co-factor for ethylene biosynthesis from methionine in cauliflower floret (Mapson, 1970). It is a co-factor of a peroxidase-like enzyme on the pathway and it is interesting that caffeic acid at the site of the synthesis could theoretically provide a regulation of ethylene synthesis. Phenolics might react with other hormones by synergism or inhibition and both situations has been recorded in case of plant growth stimulated by gibberellic acid. There is evidence that dihydro-coniferyl alcohols in lettuce have a synergistic effect on GA_3 stimulated elongation of hypocotyls (Kamiska *et al*., 1977). By contrast, substitution of dihydro coniferyl alcohol by any one of several common hydroxy cinnamic acids reverses this effect. It may be noted that tannins in other plant system have an antagonist effect on GA_3 activity (Corcoran *et al*., 1972). It is clear that phenolics appear to interact specially with plant hormones to produce an effect on growth and may have an indirect effect on physiological processes. Many phenolics are capable of inhibiting ATP synthesis in mitochondria, uncoupling respiration and inhibiting ion absorption in roots (Stenlid, 1970). Flavonoids may also affect the polar transport of auxins (Stenlid, 1976) and protoplasmic streaming in root hairs (Popovici *et al*., 1976). There are also a variety of enzyme activities which may be inhibited in the presence of compounds such as quercetin (Van Sumere *et al*., 1975). How significant these effects are in the normal growth pattern of the plant has yet to be determined. The Recent discovery of certain phenolics, particularly caffeic acid ester and flavonoids occur in plant chloroplasts (Saunder *et al*., 1976) in small amounts raises the question of a further possible function in relationship to photosynthesis or to the effect of light on plant processes. It is conceivable that phenolic constituents, because of their intense UV absorption, can provide protection from damaging UV radiation in the atmosphere and might be physiologically useful in absorbing this radiation which otherwise interferes with the more vital processes in the chloroplast. Their occurrence in the chloroplast may equally be accidental and further studies are required to see whether they are in fact important in the overall metabolism of these organelles.

2.15 INDUSTRIAL PROCESSING OF TEA

Industrially processed tea is one of the most popular beverages all over the world and is manufactured from young tender shoots of tea plant (Cabrera *et al*., 2003). From tea leaves, two types of products are most commonly consumed: Black or CTC and Green Tea. The chemical composition of tea shoots and the biochemical reactions that continue during industrial processing will ultimately determine the quality of tea. Besides these two quality products, some special categories of tea like semi-fermented oolong tea, decaffeinated teas, herbal tea, and scented teas are also quite popular in North Bengal and Darjeeling Hills. The manufacturing techniques of three types of tea products: viz. Crush, Tear and Curl (CTC) tea, Green tea and Orthodox tea may vary widely during industrial processing and have a pronounced effect on the kinetics of degradative and formative patterns within cellular components.

Tea beverages have continuously being considered as therapeutics from ancient age because of the existence of polyphenols. The benefits of tea polyphenols are already well established. Tea polyphenols are a source of valuable functional components whose consumption might affect health in a positive mode and defend against various pathologies like cardiovascular disorders, cancer, diabetes, hypertension, renal disorder etc. From a therapeutic viewpoint, green tea has been most widely studied due to its richness in phenolic compounds; among them, most complexes are various categories of flavonols. The major flavic flavonols of green tea are different catechin derivatives: (-) catechin-3-gallate (ECG), (-) epicatechin (EC), (-) epigallocatechin (ECG) and (-) epigallocatechin-3-gallate (ECGC). Thearubigin and theaflavin are the most vital components of black tea. These polyphenols are highly bioactive molecules and most often acting as antioxidants, which can able to scavenge free radicals. The ability to scavenge free radicals by different kinds of polyphenols might be due to possession of phenolic hydroxyl groups attached to B-ring of Flavan structure. In human beings, free radicals are known to contribute numerous disorders like cancer, ischemia, central nervous system injury, arthritis, gastritis, dementia and renal disorders (Rao *et al*., 2006). The sources of free radicals are mainly environmental pollutants like radiation, synthetic drugs and chemicals, toxins, mental stress and anxiety, hormonal imbalance and apoptosis along with oxidation process of preserved foods (Pourmorad *et al.*, 2006; Karori *et al*., 2007). Currently available synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyl anisole (BHA), different gallic acid esters are suspected to prompt numerous health hazards (Amie *et al*., 2003; Aqil *et al*., 2006). Therapeutic and prophylactic properties of tea are due to the presence of significant amount of antioxidants which protect us against oxidative stress induced free radical mediated diseases (Cao *et al*., 2011). These properties are lost if not mentioned in proper conditions of processing and the tea manufacturer has no scientific idea for restoring this quality.

2.16 TEA AS POWERFUL ANTIOXIDANTS

Our body contains its own antioxidant system made up of enzymes like catalase, superoxide dismutase and metal binding proteins. The endogenous defense mechanism against oxidative damage is complemented by antioxidants like flavonoids mainly found in tea and other dietary sources. An imbalance between the free radical production of protection by antioxidants leads to damage in proteins, lipids and DNA. Among the exogenous antioxidants, polyphenolic compounds especially flavonoids present only in plant like tea is potent phytochemicals in the diet protecting the body from oxidative damage.

The most commonly consumed flavonoids, catechins are present in tea. These compounds have a wide range of antioxidant activities. Electron paramagnetic resonance experiments demonstrated the quenching of singlet oxygen, superoxide anion, and hydroxyl radicals by black and green tea extracts (Thiagaragan *et al*., 2001). H_2O_2 and primaquine induced lipid peroxidation is prevented by prior incubation with tea polyphenols. The tea polyphenols inhibit hydroxy radical fluxes generated by an iron-ascorbic acid system, suggesting that iron chelation might have a role of polyphenol action. Tea flavonoids inhibit macrophage of human umbilical vein endothelial cell induced LDL oxidation (Yoshida *et al*., 1999). Theaflavin-digallate was most effective. The mechanism of action may be the chelation of iron and decreased the formation of superoxide anion. Green tea catechins also were effective in the preventions of cell mediated LDL oxidation (Yang *et al*., 2006). Black and green tea polyphenols protect *in vitro* red blood cell against oxidative stress.

Flavonoids found in tea show 20 times more powerful antioxidant property than vitamin-C in the lipoprotein oxidation model. The antioxidant efficacy of polyphenols is evaluated by battery tests, indicated that they can inhibit lipid hydroperoxides formation. Scavenging property of polyphenols has been demonstrated against a spectrum of offensive oxidants like superoxide radicals and other free radicals. Polyphenols exhibit modifying influences on the protein phosphorylation process and the different catalytic activity of many enzymes especially the oxidative ones. They are able to prevent metal-catalysed free radical formation caused by copper and iron chelate. An important property of flavonoids is that they do not affect β-carotene, Vita-C and E, which attributes for the endogenous antioxidant protection system of the body. Production of offending N-nitroso compounds is a consequence of the interaction of nitrogen containing compounds with nitrososating agents which is prevented by polyphenols. Flavonoids are the presence of some metals or in high concentration can also act as pro-oxidants for inhibition of P-450 catalyzed activity. Tea contains wide spread of flavonoids which are observed in other costly food supplements, so same quantity of tea will supply the entire body requirement of antioxidants of all ages of people. It is also reported that black tea contains Vita.-B complex group, Vita-K of large number of minerals like K, Mn, Mg, Cu, Zn, Na and F.

2.16.1 Black tea may prevent cigarette smoke-induced oxidative damage of proteins

The degenerative diseases that have been linked to oxidative damage caused by cigarette smoking are emphysema, atherosclerosis, lung cancer and other malignancies. Epidemiological reports and other experimental studies suggest that tea has chemo-preventive effects against cigarette smoking and tobacco use. Almost one-third of the worlds are direct or indirect smokers and the hazardous effect of smoking is a global public health problem of great alarm. Disappointing smoking would unquestionably be the best measure to eradicate this deleterious practice. However, notwithstanding the warnings and anti-smoking campaign, smoking continues. Thus, a practicable approach is to find a way to prevent the deleterious possessions of smoking. Many scientific studies reveal that regular intake of tea may protect smokers from nicotine-induced oxidative damage and the consequent degenerative diseases.

2.16.2 Oxidation in rheumatoid arthritis

Oxygen metabolism has a crucial role in the pathogenesis of rheumatoid arthritis. Reactive oxygen species formed in the course of cellular oxidative phosphorylation, and by activated phagocytes cells during the oxidative burst, go above the physiological buffer capacity and resulting in oxidative stress. The unwarranted production of reactive oxygen species can damage protein, lipids, nucleic acids, and matrix components. They also serve as important intracellular signal molecules that amplify the synovial inflammatory – proliferative response. Repetitive cycles of hypoxia and deoxygenating conditions associated with changes in synovial perfusion are postulated to activate hypoxia-inducible factor-1α and nuclear factor-κB, two key transcription factors that are regulated by changes in cellular oxygenation and cytokine stimulation, and that in turn orchestrate the expression of a spectrum of genes critical to the doggedness of sinusitis. A thoughtful of the complex relations involved in genes is critical to the persistence of sinusitis. As understanding of the complex interactions involved in this pathway might be the improvement of novel therapeutic strategies for rheumatoid arthritis.

2.16.3 Tea catechins against *Helicobacter pylori*

Tea catechins have an antibacterial effect against *H. pylori* and may have a therapeutic effect against gastric mucosal injury induced by this organism. A new, safe, and effective therapeutic regimen against *H. pylori* infection may be manufactured by the use of catechins combined with a proton pump inhibitor, perhaps in a delivery system which prolongs the gastric-transit time of catechins.

2.16.4 Acute radiation induced skin toxicity

Tea extracts are competent for patients who are suffering from acute radiation-induced skin toxicity. The molecular mechanism underlying the advantageous effects are complex, and most likely not solely dependent on effects of tea polyphenols such as epigallocatechin-gallate.

Tea extracts supported the restitution of skin integrity. Tea extracts inhibited proteasome function and suppressed cytokine activity; it was altered by tea extracts in a complex, caspase-dependent manner, which is due to the effects of epigallocatechin-gallate. Moreover, both tea extract, as well as epigallocatechin-gallate, slightly protected macrophages from ionizing radiation.

2.16.5 Toxin-induced hepatotoxicity

Liver injury, on the other hand, there is also growing evidence that excessive NO production by iNOS plays an important role in the induction of toxin-induced liver injury. The reaction of NO with superoxide anions produces peroxynitrite, which is a highly oxidative species capable of nitrating tyrosine residues of numerous proteins, which leads to the formation of nitro tyrosine. Nitro tyrosine formation, detected by a specific antibody, was increased in carbon tetrachloride-treated mice and was significantly decreased by EGCG treatment. The fact that iNOS induction and nitro tyrosine formation occurred in the cells exhibiting necrotic changes suggests evidence for a role of NO in liver injury, at least in the model used in the current study. Other pathways through which NO and peroxynitrite mediated tissue injury include inhibitors and formation of free radicals. Of note, the decreased production of NO-derived free radicals in the mice treated with carbon tetrachloride and EGCG provides further evidence for a role of EGCG in down-regulating NO-mediated injury. The main findings of the current study were that elicited acute liver injury as indicated by a significant increase in hepatocellular damage increased expression of iNOS, and extensive nitro tyrosine formation. By comparison, the degree of liver injury and expression of iNOS and nitro tyrosine decreased significantly in the EGCG, it is important to point out that the overall protective effect of green tea might require the combined actions of several components of tea. Relevant to the findings of the current study, which shows that a concentration of green tea extract equivalent to the consumption of 10 cups tea per day, exerts inhibitory actions on cytokine-induced tyrosine phosphorylation and subsequently blocks the expressions of iNOS along with reducing NO production. Because green tea can be consumed over long periods of time without any obviously known side effects, its possible role as an adjunct therapeutic agent in human inflammatory liver disease deserves consideration.

2.16.6 Green tea polyphenol extract may attenuate lung injury in pleurisy

Green tea extracts exerts a potent anti-inflammatory effect on an acute inflammatory response characterized by fluid accumulation in the pleural cavity that contained many neutrophils (PMNs), an infiltration of PMNs in lung tissues and increased production of nitrite/nitrate, tumor necrosis factor alpha. All parameters of inflammation were attenuated by green tea extract treatment.

2.16.7 Anti-carcinogenic effects of polyphenols and cerebrovascular diseases

Carcinogenesis can be viewed as a multistage, micro-evolutionary process. The progression of tumor formation may be slow, often taking ten or more years. It is generally agreed that tumors can be derived from single abnormal cells, and work with experimental systems shows that carcinogenesis is divisible into three major stages: initiation, promotion, and progression. Initiation is a heritable aberration of a cell. Cells so initiated can undergo transformation to malignancy if promotion and progression follow. Initiation appears to be irreversible and can result from DNA damage. Promotion, on the other hand, is affected by factors that do not alter DNA sequences and involves the selection and Clonal expansin of initiated cells. This process is partly reversible and accounts for a major portion of the lengthy latent period of carcinogenesis. The final stage of tumor formation is the progression of a benign growth to a malignant neoplasm. There is a loss of growth control, an escape from the host defense mechanism and metastasis. Certain initiators, such as radiation or chemical carcinogens, can induce the production of various free radicals and subsequent DNA base sequence alternation. In addition, cells of the immune system, such as neutrophils and macrophages, produce $O_2^{\cdot\,-}$ and H_2O_2 that have been associated with the induction of experimental cancers. Oxygen free-radicals and methyl radicals are known to damage DNA. In some cases, such free radicals may arise in reactions catalyzed by ferric and cupric ions localized in the vicinity of cellular DNA. Free radical mediated DNA damage can have serious consequences on an organism unless the damages are repaired. Although oxygen free-radical effects can lead to DNA damage, they may also directly affect the protein components of the DNA repair apparatus. Unrepaired DNA alterations are inherited as mutations. Phase-I metabolizing enzymes (*e.g.* cytochrome P_{450}) play an important role in the initiation stage. Through the catalytic activity of these enzymes, a polar reactive group is added to lipophilic carcinogens/xenobiotics to form an electrophile, which can react with DNA. Later, the xenobiotics can be detoxified by phase-II metabolizing enzymes, with sugars, amino acids, and glutathione for example. Green and black tea extract strongly inhibit neoplastic transformation in mammary organ cultures or epithelial cells. In animals, green or black tea also induces the phase-I metabolizing enzyme CYP1A2.

It is generally believed that the generation of growth promotion oxidants is a major trigger of the tumor promotion and progression stages. Tumor-promoting phorbol esters not only can induce changes in cellular genes leading to some of the phenotypic characteristics of tumor cells, but they also can stimulate inflammatory leukocytes to release superoxide. The release of superoxide by phagocyte cells following stimulation with phorbol esters is proportional to their tumor promoting activity. Low levels of both $O_2\cdot^-$ and H_2O_2, products of the “respiratory burst”, can promote fibroblast growth, possibly fibroblasts that harbour an oncogenic or a mutated proto-oncogene. Also, low levels of superoxide can stimulate growth or growth responses in a variety of cell types when added exogenously to the culture medium. In particular, these species stimulate the activation and translocation of protein kinase C as well as the expression of early growth-regulated genes, such as the proto-oncogene’s *c-fos* and *c-myc.* Superoxide and/or hydrogen peroxides might function as mutagenic stimuli through biochemical processes common to natural growth factors. Thus, signaling of growth responses involving released superoxide or hydrogen peroxide may be mediated through the oxidative modification of components of the signal transduction pathway. It is also possible that oxidative inactivation of serum protein inhibitors allow proteases to remodel the cell surface, thereby facilitating or modulating, the action of normal growth factors (Burdon, 1993). Non-steroidal anti-inflammatory drugs or COX-2 inhibitors have shown potent chemopreventive activity in animal colorectal carcinogenesis models (Cuendet & Pezzuto, 2000; Krishnan *et al*., 2000). Dietary polyphenolics, such as curcumin, chlorogenic acid, caffeic acid, resveratrol, or the flavonoid silymarin have also been shown to prevent colon carcinogenesis. A final and decisive step in carcinogenesis is the invasion and metastatic spread of the tumor to various body spaces and cavities. This appears to be facilitated by the activation of genes for the release of proteolytic enzymes. Whereas high levels of immune cells appear to favour metastasis. Again, the release of superoxide may serve to promote metastatic growth. Alternatively, superoxide could inactivate serum antiproteases, some of which are extremely sensitive to oxidative inactivation. Chemoprevention by flavonoids or polyphenolics could also result from tumor cell death, apoptosis, caused by the cytotoxic effect of flavonoids/polyphenolics. An accumulation of evidence has shown some anti-cancer properties of resveratrol are related to down regulating of the activation of NF-κB, which contributes to the progression of organ independence of prostate cancer and increase invasive and metastatic properties (Tsai *et al*., 1999). Recently, another possible antitumor molecular mechanism of resveratrol was shown, which involves mutagen-activated protein kinase-mediated p53 activation and subsequent induction of apoptosis.

Lipid peroxidation is associated with some phases of carcinogenesis. There is increasing evidence that covalent binding of carcinogens or toxic substances to cellular macromolecules, particularly those carrying genetic information, is a primary event in the initiation of carcinogenesis. Thus, covalent binding to macromolecules could be the basis of many pathological changes induced by toxic substances. The ultimate forms of xenobiotics are believed to be reactive electrophile metabolites, which combine with nucleophilic groups of macromolecules. It is also possible that miscoding or mutagenesis may be of minor importance in the initial events of chemical carcinogenesis and that genetic transposition, including relatively large regions of the genome, may be more relevant (O'Brien, 1994). The DNA adducts, deoxyadenosine and deoxyguanosine, which are induced by malondialdehyde, the end product of lipid peroxidation, accumulate in human breast cancer cells compared to normal breast cells. Serum antioxidative vitamin levels and lipid peroxidation were compared in gastric cancer patients (Choi *et al.,* 1999). The level of serum ascorbic acid, α-tocopherol, β-carotene, and retinol were assessed. The levels of ascorbic acid in patients with gastric carcinoma were less than one-fifth of that in the control group, and the production of β-carotene and α-tocopherol was decreased, as well.

Current information supports the importance of polyphenols in cancer protection (Dragsted, 2003; Rasmussen & Breinholt, 2003). A number of studies suggest decreased the risk of colon, breasts and lung, stomach and oesophageal cancer by polyphenol intake. Nonetheless, the overall incidence of cancers such as lung, breast and prostate has increased. It is reasonable to expect that the incidence of cancer can be substantially reduced by diet modification, regular exercise, and avoiding tobacco smoke.

2.16.8 Cardiovascular and cerebrovascular disease

Polyphenolic compounds are generally known to possess antioxidant properties (Tapiero *et al*., 2002). Despite intake of a high-fat diet, the low incidence of coronary heart disease in France – the “French Paradox” – has been attributed partly to consumption of red wine (Sun *et al*., 2002). Compounds such as resveratrol, quercetin, catechin, and proanthocyanidins are enriched in grapes skins and seeds, and the ability of these compounds to inhibit platelet aggregation and protect low-density lipoproteins from oxidation has been demonstrated (Xia *et al*., 1998). Also, the intake of flavonoids in relationship to cardiovascular disease has been explored by several groups of investigators. Hertog *et al*. (1997) reported that a high intake of flavonols was associated with a decreased risk of coronary heart disease mortality. They also reported a general decrease in mortality with increasing flavonol intake. Knekt *et al*. (1996) reported similar results, with a decreased risk of coronary mortality associated with flavonoids. In contrast, a U.S. study of heart disease in males 40-75 years of age found no significant association with flavonoid intake (Rimm *et al*., 1996). Finally, in a 10-years follow-up study of more than 34,000 postmenopausal women from Lowa, flavonoids intake was associated with a decreased risk of heart disease. However, no association was found between flavonoid intake and stroke mortality after 10 years of follow-up (Yochum *et al*., 1999).

The syndrome of ischemia/reperfusion (I/R) injury has been characterized for the heart, brain intestine, kidney, and other organs. This phenomenon consists of a paradoxical increase in tissue injury during the reperfusion period in an organ that has sustained relatively minor damage during a period of ischemia. It is now evident that reperfusion tissue injury is mediated through oxidant mechanisms associated with the generation of oxygen-based radicals. ROS have been implicated in both the myocardial dysfunctions that are observed during reperfusion following short periods of ischemia (the stunned myocardium) and the irreversible injury to cardiomyocytes that occurs during reperfusion after longer periods of ischemia. Infusions of high concentrations of the catecholamine’s epinephrine or nor-epinephrine into experimental animals are known to produce myocellular mitochondrial swelling, myofibrillar disruption, plasma membrane blabbing, and myocardial necrosis. It has been suggested that these cardio toxic effects result not from the catecholamine themselves but from the production of $O\cdot_2{}^-$ and H_2O_2 formed by a complicated series of reactions during the antioxidation of catecholamine. It was observed that vitamin E-deficient rats were more sensitive to the cardio toxic effects of isoproterenol; whereas myocardial damage induced by this synthetic catecholamine was reduced when the diet was supplemented with vitamin E. However, the results of studies demonstrating protection by antioxidants against catecholamine-induced myocardial necrosis must be interpreted with caution. As the accumulation of neutrophils, a major source of oxygen radicals has been observed in such models (Singla *et al*., 1982).

Carvedilol, a potent antioxidant, prevents the lipoperoxidation of mitochondrial membranes, which suggests a strong contribution to the known cardio protective activity of this compound through protection of mitochondrial function (Moreno *et al*., 1998). A similar cardio-preventive benefit is achieved by agents and antioxidant enzymes that scavenge hydroxyl radicals (or reduce their formation), but not agents that reduce superoxide anion production. Some examples of compounds of plant origin that have shown protective effects against ischemic injury are procyanidine from *Vitis vinifera* L. (Facino *et al*., 1996), resveratrol from red wine (Ray, 1999) and ginseng extracts (Facino *et al*., 1990).

The ability of polyphenolic compounds to offer cardiovascular system protection has also stimulated efforts to investigate whether this compound may offer neuroprotective effects. Few studies have explored flavonoid intake and risk of stroke. An inverse association has been shown with increasing dietary quercetin consumption. Tea consumption, which comprised the major source of flavonoids intake, was associated with decreased risk of stroke (Keli *et al*., 1996). Knekt *et al*. (2000) showed that quercetin intake is not associated with cerebrovascular disease.

Lipid-peroxidation of biological membranes gives rise to degeneration of synapses and neurons and may be observed in stroke of neuronal disorders such as Alzheimer, Parkinson, and Huntington diseases. Oxidative stress and damages are accepted features of neutral degeneration. The pathological presentation of Alzheimer disease, the leading cause of senile dementia, involves regionalized neuronal death and accumulation of intraneuronal and extracellular lesions (Smith *et al*., 1997), 4-Hydrosynonenal mediates oxidation-induced impairment of glutamate transport and mitochondrial function in synapses (Keller *et al*., 1997). Amyloid β-protein may be related to modulation of membrane lipid peroxidation. Amyloid β-protein fragment inhibits lipid peroxidation at low concentrations as a result of physicochemical interactions with the membrane lipid layer (Walter *et al*., 1997). Further, there is close association between increased levels of the antioxidant enzymes superoxide dismutase and heme oxiygenase-1 and cytoskeleton abnormalities found in Alzheimer disease.

2.16.9 Delay skin ageing

Cells that migrate toward the surface of the skin normally live about 28 days and by day 20, they basically on the upper layer of the skin getting ready to die. But the effects of the host abundant green tea polyphenol, EGCG on human skin and found that EGCG reactivates with dying skin that if dying skin cells could be energized, skin condition could be improved to benefit psoriasis, roses lea wrinkles of wounds. In these regional CCA industries already marketed mega tea supplements which are claimed to induce weight loss and skin ageing (Smith *et al*., 1998).

2.17 METABOLOMICS OF TEA POLYPHENOLS

2.17.1 Absorption

The absorption of the flavonoids in tea will depend on its physicochemical properties like molecular size, configuration lipophilicity, solubility and pKa. Till date, only a little information is available regarding on metabolomics of plant food matrix on absorption and its effect.

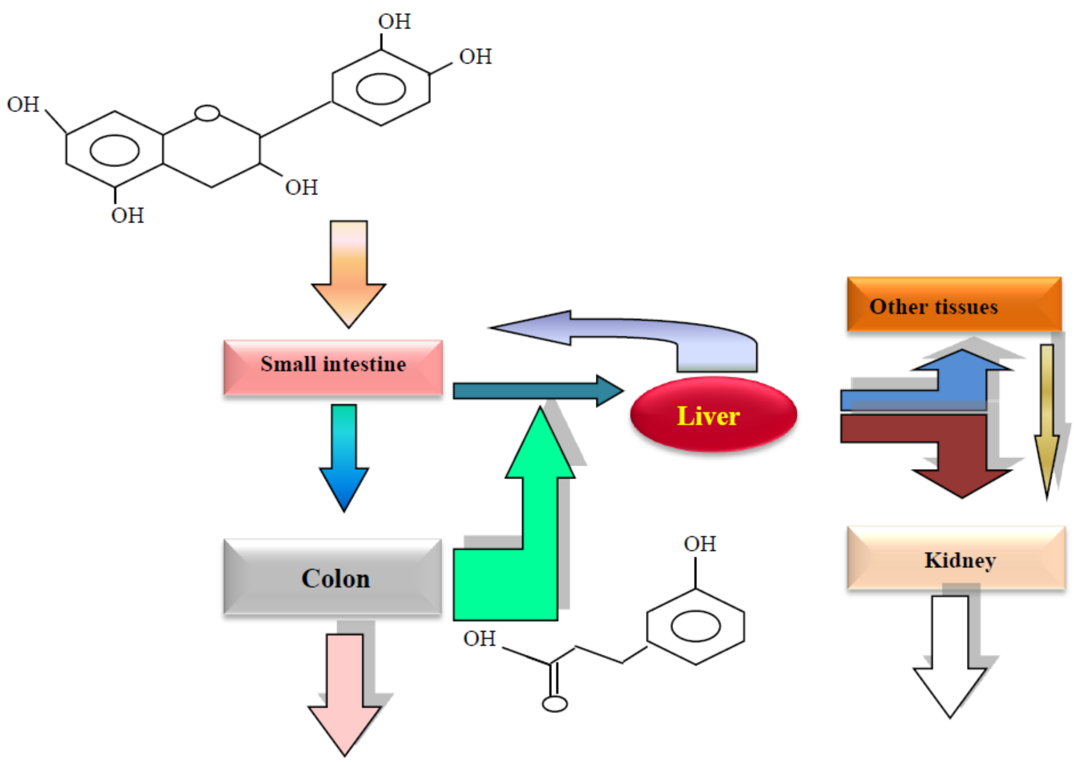

Figure 2.3 Absorption, bio-availability and metabolism fate of tea polyphenols.

Most of the flavonoids except catechins are usually present in the diet as β-glycosides. (Figure 2.3). Hydrophilic glycosides are absorbed by passive diffusion in the small intestine, therefore aglycones part are only to be absorbed. Hollman *et al*. (1997) studied that β-glycosides are unexpectedly highly absorbed in onion formed, in this context quercetin glycosides are also absorbed in the small intestine and confirmed absorption of aglycones by the pharmacokinetics studies in a human model. This is explained by the hydrophilic quercetin glycosides, which are transported across the small intestine; it is proposed that the Na^+ dependent glucose cotransporter is involved (Hollman *et al.,* 1999). It will imply that intact glycoside can be transported across the enterocyte and might appear in the blood plasma although intact quercetin-3-glucoside was absent in plasma after intake with quercetin-3-glucoside, which does not strengthen the SGLT1 hypothesis (Senink *et al.,* 2001). An alternative mechanism was that quercetin glycosides hydrolyzed by lactase phloridzin hydrolase (LPH), a β-glucosidase on the outside of the brush border membrane of the small intestine. The substrate specificity of LPH enzyme varied considerably in a broad range of glycosides, it is evident that glycosides are competently hydrolyzed by LPH, more or less independent of the aglycone part of the glycoside.

Colon bacteria are able to hydrolyze flavonoid glycosides but simultaneously it will be degraded & liberated flavonoid aglycones. This is also explained by the fact that absorption capacity of the colon is less than that of the small intestine. The data supplied by Hollman *et al*. (1999) strongly imply that the sugar moiety of quercetin glycosides is a major determinant of their absorption and bioavailability. Oligomeric flavonoids, like 17 catechins occur in plant foods which have two forms, aglycones & galloylated form in the tea extracts. These two forms are absorbed in the small intestine. Oligomers can be hydrolyzed to monomers & dimers due to an acidic condition in the stomach. Larger molecules reach the colon & degraded by bacteria. Therefore it is concluded that only anthocyanidins up to 3- catechins are absorbed from the colon.

2.17.2 Bioavailability

Tea flavonol glycosides are absorbed very rapid to very slow. Comparative studies (Hallmann *et al.,* 1999) show that the bioavailability of different food supplements is dissimilar rates, it is implied that important determinant of their bioavailability is the presence of sugar moiety in quercetin glycosides. It is also evident that catechins are quite absorbed from the small intestine; galloylated EGCG form is not different in this respect. Bioavailabilities of various catechins monomers are quite similar although dimerization decreases the bioavailability.

2.17.3 Metabolism

The two compartments; small intestine, liver kidney and other compartments of colon are main sites of the metabolism of tea flavonoids. An absorbable form of flavonoids is secreted with bile and reaches the colon.

Mainly in the first compartment, enzymes act upon the substrate and their colonic metabolites; kidney also contains enzymes which are capable of biotransformation. Conjugation of the polar hydroxyl group of flavonoids with glucuronic acid sulfate or glycine occurs. Inactivation of catechol moiety takes place by the enzymes, catechol-o-methyltransferase *i.e. O*-methylation plays an important role. Side groups of flavonoids are attached or detached in the tissues.Flavonoids molecules are split in the colon and the microorganism degraded products are ultimately absorbed. Because the degradation of flavonoids ring by the colonic bacteria secreted enzymes such as glucosidase, glucuronidases and sulfates can trip flavonoid conjugates of their sugar moieties. Figure 2.3 shows the different compartment involved in the metabolism of polyphenol and their impact on human health.

2.17.4 Extent of metabolism

Metabolism of the total quercetin occurs in the human body because ingested tea extract containing quercetin is extracted from urine as conjugated forms. It is correlated with quercetin in plasma flavonoids and also with low urinary excretion of the same.

Polyphenols are widely distributed in the plant kingdom; among them, tea plant represents an abundant antioxidant component of the human diet. This section offers a brief description of the use of polyphenol which may reduce or prevent much health risk in human. Considerable evidence is now available, showing anti-carcinogenic effects of polyphenolic compounds, as well as potential to prevent cardiovascular diseases, toxin-induced hepatotoxicity, acute radiation-induced toxin toxicity, offers a novel therapeutic approach for the risk of smoking, oxidation in rheumatoid arthritis and delay skin ageing.

2.18 FUTURE PROSPECTS

After a long period, it is now time to illuminate the outputs of antioxidant research and their further development for mankind. Most of the degenerative diseases are related to radical damage, which requires more secure data. It can be expected that antioxidants can check and cure a number of pathophysiological problems also which require intensive attention. Many novel approaches are made in antioxidant research and significant findings have come out in last few years with improved phytotherapeutic activities. The traditional Indian diet, spices as well as medicinal plants are the rich sources of natural antioxidants. Higher intake of these foods & drink with functional attributes including a high level of antioxidants is one strategy that is gaining importance in the advanced countries and also it is making a formal shape in India. Coordinated investigation involving biomedical scientists, botanists, nutritionists and physicians will definitely create a significant difference in phytotherapy research for overcoming critical human disorders in the coming decades. Research on the free radicals and antioxidants is one attempt for inventing the nutraceutical and pharmaceutical products.