CHAPTER IV

4. DISCUSSION

Now-a-days there is a renewed interest in drugs of natural origin simply because they are considered as green medicine is always supposed to be safe. Another factor which emphasizes this attention is the incidences of harmful nature of synthetic drugs which are regarded as harmful to human beings and environment. The advantage of natural drugs is their easy availability, economic and less or no side effects but the disadvantage is that they are the victims of adulteration (Dineshkumar, 2007).

Recently naturally occurring phytochemicals are receiving increased attention because of their promising efficacy in several cancer models. Phytochemicals, including those obtained from fruits, vegetables, nuts and spices, have drawn a considerable amount of attention due to their ability to selectively kill tumor cells and suppress carcinogenesis in preclinical animal models.

4.1. Phytochemicals analysis of Aplotaxis auriculata root

Plants have basic nutritional importance by their content of protein, carbohydrate, fats and oils minerals, vitamins and water responsible for growth and development in man and animals. Phytochemical simply means plant chemicals. -Phyt- is the Greek word for plant. Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary metabolism is important for growth and development of plants include the common sugars, aminoacids, proteins, purines and pyrimidines of nucleic acids etc. Secondary metabolism in a plant plays a major role in the survival of the plant in its environment. Attractions of pollinators, natural defense
system against predators and diseases, etc., are examples of the roles of secondary metabolites (Sofowara, 1993). The phytochemical, inorganic elements and vitamins were analysed in *Aplotaxis auriculata* root. The result of the present study shows that the extract of *Aplotaxis auriculata*, which contains Phlobatannin, Saponin, Flavanoids, Steroids, Terpenoids, Triterpenoids, Carbohydrate, Protein and Polyphenol while alkaloids, tannin and anthroquinone absent.

Leo Stanley *et al.*, (2011) reported that leaves of *C. pedata* showed the presence of alkaloids, carbohydrates, steroids, tannin, phenolic compounds, flavonoids and terpenoids. Dineshkumar *et al.*, (2011) has been reported to terpenoids, flavonoids and tannin are present in *C. trifolia*.

Rajmohanan *et al.*, (2014) investigated the preliminary phytochemical analysis of various extracts of leaves of *C. pedata* and showed the presence of carbohydrates, flavonoids, tannins and phenolic compounds and terpenes.

Abuzar *et al.*, (2013) reported the phytochemical analyses of *Heliotropium dasycarpum* L were evaluating the presence of secondary metabolites in drug sample. The results showed the presence of alkaloids and cardiac glycosides while the saponins, anthraquinone glycoside and tannins were absent in the plant extract.

Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action. Flavonoids are 15 carbon compounds generally distributed throughout the plant kingdom. Some isoflavones widely used in insecticides. They might
also play a role in disease resistance. Some flavonoids such as quercetin and rutin, are known to support human health by serving antiinflamatory, antihistaminic and antiviral agents (Okwu, 2004). Flavonoid compounds exhibit inhibitory effects against multiple viruses. Numerous studies have documented the effectiveness of flavonoids, such as glycyrrhizin and chrysin (Duraipandiyan, 2006) against HIV. Flavonoids are potent water soluble antioxidants and free radical scavengers which prevent oxidative cell damage and have strong anticancer activity (Del-Rio et al., 1997). Flavonoids have been referred to as nature’s biological response modifiers, because of inherent ability to modify the body’s reaction to allergies and virus and the showed their anti-allergic, anti-inflammatory, antimicrobial and anti-cancer activities (Duraipandiyan, 2006).

It has been recognized that alkaloids and flavonoids shows antioxidant property and their effects on human nutrition and healthcare are considerable. Flavonoids also known as nature’s tender drugs possess numerous biological and pharmacological activities. The anti-inflammatory capacity of flavanoids has been long utilized in Chinese medicine and the cosmetic industry as a form of crude plant extracts (Duraipandiyan, 2006).

Anthroquinones possess antiparastic, bacteriostatic, antidepressant and antimicrobial and antioxidant activities. Their potential effects against cancer through different mechanisms have been studied. Many human physiological activities such as stimulation of phagocytic cell host mediated tumour activity and a wide range of anti-infective actions have been assigned to tannins. Tannins have stringent properties,
hastening of wounds and inflamed mucous membrane. Tannins are responsible for colour changes in food (Agoha., 1974).

Alkaloids have established broad spectrum antibacterial activity and are also used as analgesics and narcotics for pain relief. Alkaloids are very important in medicine and constitute most of the valuable drug. They have marked physiological effect in animals (Edeoga and Eriata, 2006). Alkaloids such as solasodium have been indicated as a starting material in the manufacture of steroidal drug. Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antispasmodic and bactericidal effects. They exhibited marked physiological activity when administered to animals (Okwu, 2004).

Tannins are phenolic phytochemicals, which are natural constituents of green tea, are considered to have cancer- preventive properties (Lambert and Yang, 2003; Niraimathi et al, 2012). Condensed tannins, isolated from black beans, did not affect the growth of normal cells, but induced cell death in cancer cells in a dose-dependent manner (Awika et al, 2004). Studies in animal models and with cultured human malignant cell lines have demonstrated both the antitumor and cancer preventive activities of methanolic extract of Psidium guajava leaves and its main ingredients. It was suggested that these effects of methanolic extract might be due to their content of flavonoids, tannins, alkaloids and saponins reported earlier (Vikrant Arya et al, 2012).
4.2. Qualitative Analysis of Inorganic Elements in *Aplotaxis auriculata*

Qualitative or quantitative determination of mineral elements present in plants is important because the concentration and type of minerals present must often be stipulated on the label of a food. The quality of many foods depends on the concentration and type of minerals what they contains, also play a very significant role against a variety of degenerative diseases and processes, they may also prevent and reduce injury from environmental pollutants and enhance the ability to work and learn, some minerals are essential to a healthy diet (e.g. Calcium, Phosphorus, Potassium and Sodium) where as some can be toxic (e.g. Lead, Mercury, Cadmium and Aluminium). It is clear that mineral nutrition is important to maintain good health and because of that determination of As, Ca, Fe, Mg, Na, K, Zn, Ni, Co etc. have been added to Ayurvedic Pharmacopoeia of India (The Ayurvedic Pharmacopoeia of India, 1999). From ancient times, Swarnabhasma (gold ash) has been used in several clinical manifestations including loss of memory, defective eyesight, infertility, overall body weakness and incidence of early aging. Hence, their presence is vital for the health and to cure diseases. Mineral content indicates the nutritive value and potentially act as a cofactor for the biological activity exhibited by the plant extracts studied.

The inorganic elemental characters of the *Aplotaxis auriculata* root were investigated and roots were found to be Calcium, Sodium, Potassium, Sulphate, Phosphorus, Chloride and Nitrate, while Magnesium, Iron and Carbonate were absent. Mineral content indicates the nutritive value and potentially act as a cofactor for the biological activity exhibited by the plant extracts studied.
Ciura et al., (2007) reported to contain Cadmium, lead, zinc and copper in selected vegetables and fruit from garden.

Kumudhaveni Babu et al (2013) examined the heavy metals and inorganic element content in Stereospermum colais leaves. Heavy metals (Lead, Copper, Cadmium, Arsenic and Mercury) and inorganic elements (Sulphate, Fluoride, Chloride) were qualitatively and quantitatively estimated from the leaves of Stereospermum colais. Magnesium, Sulphate, iron, Fluroide, Phosphate and Chloride were qualitatively found to be in Stereospermum colais leaves. He obtained results revealed that the content of heavy metals was within the permissible levels and hence the plant was safe to be utilized in herbal drug formulation.

Ramy a et al. (2015) investigated the micronutrients and vitamin analysis of Bryonopsis Laciniosa fruits. Study revealed that the fruit contains Vitamins like C,D and E. Iron was found to be very much abundant and Calcium, Magnesium, Potassium, Chloride are in high amount. Sulphate and sodium are in a moderate level. Carbon, phosphorous, sodium, sulphur, zinc and manganese are substantially present while copper, Boron, selenium and molybdenum are present in trace amounts.

4.3. Qualitative Analysis of Vitaminsin Aplotaxis auriculata

Vitamins are organic substances that are essential in tiny amounts for growth and activity of the body. They are obtained naturally from plant and animal foods. Organic in this definition refers to the chemistry and molecules of vitamins. The word organic means that the molecules of the substance contain the element carbon. The term also means that vitamins can be destroyed and become unable to perform their functions in our bodies.
Too much heat, certain kinds of light and even oxygen can destroy some vitamins. The amounts of vitamins ingested from food are measured in micrograms or milligrams (Okwu, 2004).

Vitamins work with other substances in the body like enzymes and minerals. Together they perform such functions as strengthening bones, healing wounds, keeping the skin healthy, building cells, and helping to resist infections. Vitamins are separated into two groups, fat soluble and water soluble. The fat soluble vitamins are A, D, E, and K, and can dissolve in dietary fats and are stored in the liver and body fat. The body stores them for a longer amount of time, so they are not needed every day. Too much of these vitamins can become toxic and cause health problems. The water soluble vitamins are made up of eight B vitamins and vitamin C. Water soluble vitamins dissolve in water, and are not stored in the body. Rather they travel through the bloodstream and need to be replenished every day. These vitamins are easily destroyed during food preparation and storage. In the present study, vitamins A, D and E were present in Aplotaxis auriculata root.

Vitamin D is important in bone formation. Most vitamin D is made when sunshine hits the skin. Too much sun can contribute to skin cancer, and using a sunscreen of SPF 15 or more will block vitamin D formation. Milk and margarine are both fortified with vitamin D. Those over the age of 65 only make about half as much vitamin D as children from the same amount of light exposure, so it is recommended to take a supplement for these people to get enough vitamin D. A vitamin D deficiency can cause an older disease called rickets, and it is cured by cod-liver-oil, which has a high
concentration of vitamin D. Vitamin D is stored in the liver and as little as 5 times the Daily Value can produce unhealthy weight loss, vomiting, and calcium deposits in the lungs and kidneys (Clark, 2008).

Vitamin E remains the most mysterious of vitamins. The body needs it but its lack does not lead to any known disease. Vitamin E is the most exploited vitamin in that it is sold as a cure-all and even as an anti-aging potion. Vitamin E, vitamin C and beta carotene are antioxidants. Some studies suggest that the trio might help to strengthen the body's immune system and play a role in cancer prevention (Okwu, 2004).

Vitamin C or ascorbic acid, is one vitamin humans cannot make; they have to get it from food. Vitamin C helps hold the cells together, heal wounds, and build bones and teeth. The best sources for vitamin C are citrus fruits, strawberries, melons and leafy green vegetables. Vitamin C also helps to absorb and use Iron. It is important to protect the vitamins in fruits and vegetables from being destroyed; simple ways of doing this include refrigeration, washing them before cutting them, storing them in airtight containers, and avoiding high temperatures and long cooking times (Okwu, 2004).

Akubugwo et al (2007) studied the nutritional potential of the leaves and seeds of Solanum nigrum L. Mineral analysis revealed the order Mg>K>Ca>Fe>Na>Mn>Zn in the leaves and Mg>K>Fe>Ca>Na>Mn>Zn in the seeds. Phosphorus and sulphur levels were 75.22 and 8.55 mg/100g in the leaves and 62.50 and 14.48, g/100g in the seeds. Vitamin content indicate the order vit C>vit B,>Folic acid>Vit E>Vit A in both the leaves and seeds.
Pachkore and Dhale (2012) investigated the Phytochemicals, vitamins and minerals content of three *Ocimum, basilicum Linn.*, *Ocimum gratissimum Linn.* and *Ocimum sanctum* species. The medicinal plants contained Vitamins like thiamine (0.20 to 0.36 mg/100g) and riboflavin (0.22 to 0.37 mg/100g). The plants are good source of minerals such as major mineral elements comprising calcium, Phosphorus, Sodium, Potassium, Magnesium and trace elements (Iron, Zinc, Copper). These substances may be responsible for the health related properties of the plants.

### 4.4. Identification of bioactive compounds in *Aplotaxis auriculata* root extract by GC MS analysis

Twenty six compounds were identified in *Aplotaxis auriculata* root by Gas Chromatogram- Mass spectrometry (GC-MS) analysis. GC MS Studies of AARE indicates that the prevailing compounds were Ascorbic acid 2,6-dihexadecanoate, Hexadecanoic acid, methyl ester, Andrographolide, Octadecanoic acid - Stearic acid, Tetradecanoic acid - Myristic acid. The presence of various bioactive compounds justifies the use of the whole plant for various ailments by traditional practitioners.

Sarumathy *et al.*, (2011) screened seventeen compounds from *Caesalpinia italica* leaves by GC-MS analysis. The identified compounds possess many biological properties. For instance, 9,12,15-Octadecatrienoic acid, *(Z,Z,Z)*- Linolenic acid possesses anti-inflammatory, insectifuge, hypocholesterolemic, cancer preventive, nematicide, hepatoprotective, antihistaminic, antiezemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic, antiarthritic and anticoronary properties. n-Hexadecanoic acid - palmitic
acid can be an antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant activities and hemolytic 5-alpha is a reductase inhibitors. Phytol- Diterpene is an antimicrobial, anticancer, antiinflammatory and diuretic agent (Praveen kumar et al., 2010). 9, 12, 15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-, n-Hexadecanoic acid, 1,2-Benzenedicarboxylic acid and di-isoctyl ester were present in Caesalpinia sappan ethanol extract. Similar types of compounds were identified among the twenty six compounds of this present study.

Karpagasundari and Kulothungan (2014) screened the bioactive components of Physalis minima leaves have been evaluated using GCMS. GC/MS analysis of extract of Physalis minima leaves revealed the existence of Heneicosanoic acid (25.22), Bicyclo [4.1.0] Hepta-2, 4-dien (27.41) Octadecanoic acid (CAS), Stearic acid (31.19) and Octadeca-9, 12-dienoic acid (32.02).

Karthikeyan et al (2015) Analysis of Bioactive Components Of KarisalaiKarpa Chooranam- A Siddha Poly Herbal Formulation by GC-MS. They found 2,2'- Bioxirane (0.20%), Butanoic acid (0.55%), Methyl 2- oxopropanoate (0.23%), Phenol (3.03%), 2,4 dihydroxyacetophenone – (2.44%),D-Erythro-Pentose, 2- Deoxy- (0.88%), Cyclopentasiloxane, decamethyl(0.26%), 3-Isopropoxy-1,1,7,7,7- hexamethyl-3,5,5-tris(trimethyl siloxy) tetrasiloxane (0.25%), Dodecanoic acid (1.87%),2 (3H)-Naphthaleneone,4,4a,5,6,7,8- hexahydro-4a-methyl-(0.29%), Myristic acid (7.20%),9-Octadecanoic acid (Z) (0.22%),2,6,10-Trimethyl,14- Ethylene-14-Pentadecen- (0.55%),2- Pentadecanone,6,10,14-Trimethyl- (0.47%), Penta decanoic acid(0.65%), 3,7,11,15-Tetramethyl-2-Hexa decen -1-ol (0.26%),Hexadecanoic acid, methyl ester
(0.67%), cis-10-Nonadecenoic acid (1.29%), 1-(+)- Ascorbic acid 2,6-dihexadecanoate (31.30%), Andro grapholide (1.23%), Methyl 9-cis,11-trans-octadecadienoate (0.54%), 11-Octadecanoic acid, methyl ester (1.53%), Methyl stearate (0.81%), Cyclopentadecanone, 2-hydroxy- (29.89%), Octadecanoic acid (13.36%). Similar types of compounds were identified among the twenty six compounds of this present study.

4.5. IN VITRO ANTIOXIDANT ACTIVITY OF APLOTAISIS AURICULATA 4.5.1. ROOT

The phytochemical characters of the Aplotaxis auriculata roots were investigated. The qualitative phytochemical analysis of ethanolic extract of Aplotaxis auriculata roots extract contains flavonoids, saponin, terpenoids, steroids, polyphenols, saponin and triterpenoids which are important in disease prevention and health preservation.

4.5.2. DPPH Assay

Recently, the use of the DPPH’ reaction has been widely diffused among food technologists and researchers, for the evaluation of free radical scavenging activity on extracts from plant, food material or on single compounds. In the DPPH assay, the antioxidant was able to reduce the stable radical DPPH to the yellow colored 1, 1-diphenyl-1, 2-picryl hydrazine. The molecule of 2, 2-diphenyl-1-picryl hydrazine is characterised as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole. The proton transfer reaction of the DPPH’ free radical by a scavenger causes a decrease in absorbance at 517 nm, which can be followed by a
common spectrophotometer set in the visible region. The effect of antioxidants on DPPH’ is thought to be due to their hydrogen donating ability (Sindhu and Abraham, 2006). DPPH radical scavenging activity of plant extract of AARE and standard as ascorbic acid. The DPPH radical was widely used to evaluate the free-radical scavenging capacity of antioxidants (Nuutila et al., 2003). The half inhibition concentration (IC₅₀) of ascorbic acid and plant extract were 47.55μg ml⁻¹ and 59.62 μg ml⁻¹ respectively. The plant extract exhibited a significant dose dependent inhibition of DPPH activity. The potential of L-ascorbic acid to scavenge DPPH radical is directly proportional to the concentration. The DPPH assay activity is near to standard as ascorbic acid.

4.5.3. Total antioxidant activity

The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/ Mo (V) complex with a maximal absorption at 695 nm. The assay is successfully used to quantify vitamin E in seeds and, being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant extract (Prieto et al., 1999). Moreover, it is a quantitative one, since the antioxidant activity is expressed as the number of equivalents of ascorbic acid. The yield of the ethanol extract of the plant extract and its total antioxidant capacity. Total antioxidant capacity of AARE is expressed as the number of equivalents of ascorbic acid. The study reveals that the antioxidant activity of the extract is in the increasing trend with the increasing
concentration of the plant extract. The half inhibition concentration (IC$_{50}$) of ascorbic acid and plant extract were 48.08μg ml$^{-1}$ and 51.81 μg ml$^{-1}$ respectively.

4.5.4. Superoxide anion radical scavenging activity

Superoxide is biologically important since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals, is very harmful to the cellular components in a biological system (Korycka-Dahl & Richardson, 1978). The superoxide anion radical scavenging activities of the extract from Aplotaxis auriculata assayed by the PMS-NADH system. The superoxide scavenging activity of Aplotaxis auriculata was increased markedly with the increase of concentrations. The half inhibition concentration (IC$_{50}$) of Aplotaxis auriculata was 50 μg ml$^{-1}$ and ascorbic acid were 31.62μg ml$^{-1}$ respectively. These results suggested that Aplotaxis auriculata had notably superior superoxide radical scavenging effects.

4.5.5. The ferrous ion chelating activity

Ferrozine can make complexes with ferrous ions. In the presence of chelating agents, complex (red colored) formation is interrupted and as a result, the red color of the complex is decreased. Thus, the chelating effect of the coexisting chelator can be determined by measuring the rate of color reduction. The formation of the ferrozine– Fe$^{2+}$ complex is interrupted in the presence of aqueous extract of Aplotaxis auriculata, indicating that have chelating activity with an IC$_{50}$ of 54.87 μg ml$^{-1}$ and ascorbic acid was 30.96μg ml$^{-1}$ respectively. Ferrous iron can initiate lipid peroxidation by the Fenton reaction as well as accelerating peroxidation by decomposing lipid hydroperoxides into
peroxyl and alkoxy radicals (Halliwell, 1991; Fridovich, 1995). Metal chelating activity can contribute in reducing the concentration of the catalyzing transition metal in lipid peroxidation. Furthermore, chelating agents that form s bonds with a metal are effective as secondary antioxidants because they reduce the redox potential, and thereby stabilize the oxidized form of the metal ion (Gordon, 1990). Thus, *Aplotaxis auriculata* demonstrate a marked capacity for iron binding, suggesting their ability as a peroxidation protector that relates to the iron binding capacity.

**4.5.6. Reducing power activity**

The measurements of the reducing ability, the $\text{Fe}^{3+} \rightarrow \text{Fe}^{2+}$ transformation was investigated in the presence of *Aplotaxis auriculata*. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition-metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Diplock, 1997; Yildirim *et al*, 2000). The reductive effect of *Aplotaxis auriculata*. Similar to the antioxidant activity, the reducing power of *Aplotaxis auriculata* increased with increasing dosage. All the doses showed significantly higher activities than the control exhibited greater reducing power, indicating that *Aplotaxis auriculata* consist of hydrophilic polyphenolic compounds that cause the greater reducing power.
4.5.7. Nitric oxide radical scavenging activity

Nitric oxide (NO) is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical which plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilation and antimicrobial and antitumor activities (Miller et al, 1993). *Aplotaxis auriculata* extract also moderately inhibited nitric oxide in dose dependent manner with the IC\textsubscript{50} being 48.33 \( \mu \text{g ml}^{-1} \) and ascorbic acid is 46 \( \mu \text{g ml}^{-1} \).

On the basis of the results of this study, it clearly indicates that *Aplotaxis auriculata* root had powerful *in vitro* antioxidant capacity against various antioxidant systems as DPPH, nitric oxide, superoxide anion scavenging and metal chelator. From our results, the antioxidant activity of *Aplotaxis auriculata* root was concentration dependent. The extracts could exhibit antioxidant properties approximately comparable to commercial synthetic antioxidants as ascorbic acid. From the above assays, the possible mechanism of antioxidant activity of *Aplotaxis auriculata* root includes reductive ability, metal chelator, hydrogen donating ability and scavengers of superoxide and free radicals.
4.6. Protective effect of *Aplotaxis auriculata* root on DEN induced hepatocellular carcinoma in rat liver.

Hepatocellular carcinoma (HCC) is one of the most frequent cancers among humans, with 0.50–1 million newly diagnosed cases each year (Feo *et al.*, 2006). The highest frequencies are found in sub-Saharan Africa and far eastern Asia, where hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are endemic and in regions where food contaminated with Aflatoxin B1 is consumed (Feitelson *et al.*, 2002). HCC incidence appears to be rising, even in countries with relatively low incidence, especially in Southern Western Europe and Asia (Llovet *et al.*, 2003).

Carcinogenesis may arise as a result of chemical or biological damage to normal cells in a multistep process that involves changes at the initiation level followed by promotion and progression, which lead to malignancy. The promotional stage of cancer is a reversible stage and appears to be most appropriate target stage for chemopreventive intervention (Hala *et al.*, 2011). Chemoprevention is one of the strategies by which we can revert or delay the response of carcinogen. Cancer chemopreventive agents are able to reduce the incidence of tumorigenesis by intervening in one or more stages of carcinogenesis initiation, promotion or prolongation (Digiovanni, 1994).

In developing countries about 35% of prescribed drugs are derived from natural products. Many investigations are being carried out worldwide to discover naturally occurring compounds, which can suppress or prevent the progress of carcinogenesis (Cheng *et al.*, 2004). It is well known that many anticancer compounds derived from plants include Taxol from Pacific Yew tree, Vinblastine and Vincristine from
Catharanthus roseus, Rohitukine from Dysoxylum binectariferum, Broccoli and Red Cabbage (Aml et al., 2010), diterpene from Tinospora cordifolia (Dhanasekaran et al., 2009), derivatives of Podophyllin from Podophyllum peltatum and Camptothecin from Camptotheca acuminata. It is important to continue efforts aimed at discovering anticancer agents based on natural products (Pezzuto, 1997).

In recent years, there has been considerable emphasis on the identification of plant products with antioxidant property, as free radicals are considered to play a major role in most of the diseases, including cancer. The medicinal value of the chosen plant Aplotaxis auriculata root has been extensively worked out. However, its therapeutic efficacy in anticancer activity has not been evaluated.

Tumorigenesis is a multistep process that begins with cellular transformation and progresses to hyper proliferation leading to metastatic lesions. This progress can be activated by the carcinogenotoxic substances which are widely employed to develop cancers in specific organs of experimental animals. DEN is a potent carcinogen for HCC. DEN is well known to cause perturbations in the nuclear enzymes involved in DNA repair and is normally used as a carcinogen to induce liver cancer in animal models and these compounds are considered to be effective health hazards in causing HCC (Hahn and Weinberg, 2002).

Liver plays a pivotal role in regulation of physiological processes such as metabolism, secretion and storage. Unfortunately, it is a common target for a number of toxicants. The multitude of pathological changes caused by the progression of tumor as well as its inhibition through chemotherapy is expected to be reflected in the biochemical
and histological parameters of the host system, particularly pertaining to the liver which is known to be the major organ affected in carcinogenesis (Mayer and Kulkarni, 2001).

Oxidative stress is associated with damage to a wide range of macromolecular species, including lipids, proteins and nucleic acids thereby producing major interrelated derangements of cellular metabolism, including peroxidation of lipids. Free radicals and non-radicals oxidizing species were produced in animals treated with carcinogens and also in human tissues (Sun, 1990). Reactive oxygen species (ROS) is formed from endogenous or exogenous sources are highly reactive, toxic and mutagenic (Halliwell, 1994). DEN has been shown to generate free radicals an uncompromising free radical generation in the liver overwhelms the antioxidant status and ultimately proceeds to oxidative stress paving way to carcinogenesis (Gey, 1993). Lipid peroxidation plays an important role in carcinogenesis (Banakar et al., 2004), is the most studied biologically relevant free radical chain reaction and measured as malonaldehyde (MDA). Induction of DEN has been reported to generate lipid peroxidation products like malondialdehyde and 4-hydroxy nonenal that may interact with various molecules leading to cause oxidative stress and carcinogenicity (Hietanen et al., 1987).

Increased level of LPO was reported during DEN-induced hepatocarcinogenesis. This dynamic action may further lead to uncompromised production of free radicals overwhelming the cellular antioxidant defense (Klaunig and Kamendulis, 2007). Our result agrees with the earlier reports (Aml et al., 2010; Dhanasekaran et al., 2009). It has been extensively reported that free radicals participated in DEN-induced hepatocarcinogenesis. MDA generation at the initiation stage can be prevented by free
radicals scavengers and antioxidant action of AARE. Animals treated with AARE exhibited significantly lowered the levels of LPO in liver when compared with animals induced with DEN. This shows the anti-lipid peroxidative and anti-protein oxidation role of AARE that is probably mediated by its ability to scavenge free radical generation.

Antioxidants possess a variety of biological activities, including the induction of drug-metabolizing enzymes, inhibition of prostaglandin synthesis, inhibition of carcinogen-induced mutagenesis and scavenging of free radicals (Hirose et al., 1994). Antioxidants may protect membrane from ROS toxicity by prevention of ROS formation by the interruption of ROS attack, by facilitating the repair caused by ROS and by providing cofactors for the effective functioning of other antioxidants (Sen, 1995). Development of life threatening diseases like cancer is linked to the availability of these antioxidants (Gutteridge, 1994). Natural antioxidants are capable of inhibiting the ROS production and thereby reducing the associated intracellular oxidative stress.

SOD is the first line of defense in the antioxidant system against the oxidative damage mediated by superoxide radicals (Oberley and Oberley, 1986). Superoxide dismutases catalyze the dismutation of superoxide radical to hydrogen peroxide and water. Furthermore, CAT or GPx catalyze the transformation of H₂O₂ to harmless byproducts. Glutathione is a cysteine containing tripeptide, it required to maintain the normal reduced state of cells and to counteract all the deleterious effects of oxidative stress. GSH is said to be involved in many cellular processes including the detoxification of endogenous and exogenous compounds. DEN, an electrophilic carcinogen may interact with the large nucleophilic pool of GSH thereby reducing the macromolecule and
carcinogen interaction (Chasseaud, 1979). In AARE treated animals, there was a significantly higher level of GSH in liver when compared to DEN-induced animals consistent with the idea of attenuation of DNA carcinogen interaction and thereby averting a favorable environment for carcinogenesis.

Decreases in the activities of SOD, CAT, GPx and GSH are seen in tumor cells. The compounds that can scavenge excessive free radicals in the body are suggested to hinder the process of carcinogenesis (Sumathi et al., 1996). Such studies support our findings as we had seen a significant decrease in the activities of antioxidant enzyme in liver of animals treated with carcinogen in comparison with normal animals. Reduction in antioxidant enzyme (SOD, CAT and GPx) activities in liver by DEN is consistent with the earlier reports (Aml et al., 2010; Dhanasekaran et al., 2009). On the other hand, there is a significant increase in the activities of antioxidant enzymes in liver of the animals administered both AARE and carcinogen when compared with animals administered carcinogen alone.

Excessive liver damage and oxidative stress caused by diethylnitrosamine depleted the levels of non-enzymic antioxidants like GSH, vitamin-C and vitamin-E in our study. Non-enzymic antioxidants like vitamin-C and E act synergistically to scavenge the free radicals formed in the biological system. GSH acts synergistically with vitamin-E in inhibiting oxidative stress and acts against lipid peroxidation (Chaudiere, 1994). Vitamin-C also scavenges and detoxifies free radicals in combination with vitamin-E and glutathione (George, 2003). It plays a vital role by regenerating the reduced form of vitamin-E and preventing the formation of excessive free radicals (Das, 1994). The
decreased levels of these antioxidant vitamins and GSH observed during diethylnitrosamine administration might be due to the excessive utilization of these vitamins in scavenging the free radicals formed during the metabolism of diethylnitrosamine. AARE treatment effectively restored the depleted levels of these nonenzymic antioxidants caused by diethylnitrosamine. Increase in GSH levels in the present study observed that turn contributes to the recycling of other antioxidants such as vitamin-E and vitamin-C (Exner et al., 2000). This shows that AARE maintains the levels of antioxidant vitamins by maintaining GSH homeostasis, thereby protecting the cells from further oxidative stress.

The positive modulation of cellular damage in liver induced by the chronic feeding of carcinogens was also evident through the electron microscopic studies, like SEM of liver, the target organ. There were clear evidences of the AARE providing protective action to liver. The histopathological studies also supported the protective action of AARE.

In conclusion, the present study demonstrates that the AARE possesses potent free radical scavenging and antioxidant activities. From the results, it is evident that AARE is capable of modulating the levels of MDA, PCO, CD and significantly increases the enzymatic and non-enzymatic antioxidant defense mechanisms in DEN-induced hepatocellular carcinogenesis. The histological and electron microscopic studies also supported the chemopreventive properties of AARE. Our study confirms that AARE plays duel role by blocking carcinogen metabolic activation and enhancing carcinogen detoxification.
4.7. Effect of *Aplotaxis auriculata* root on tumor markers of diethyl nitrosamine (DEN) induced hepatocellular carcinoma in rats

Tumor Markers comprise a wide spectrum of biomacromolecules synthesized in excess concentration by a wide variety of neoplastic cells. The markers could be endogenous products of highly active metabolic malignant cells or the products of newly switched on genes, which remained unexpressed in early life or newly acquired antigens at cellular and sub-cellular levels. The appearance of tumor marker and their concentration are related to the genesis and growth of malignant tumors in patients. An ideal tumor marker should be highly sensitive, specific, reliable with high prognostic value, organ specificity and it should correlate with tumor stages (Malati, 2007).

Tumor Markers are biochemical substances elaborated by tumor cells either due to the cause or effect of malignant process. These markers can be normal endogenous products that are produced at a greater rate in cancer cells or the products of newly switched on genes that remained quiescent in the normal cells. A tumor marker produced by the tumor and when present in significant amounts, indicates the presence of a cancer. They may be present as intracellular substances in tissues or may be released into the circulation and appear in serum (Chu, 1987). Continuing search for suitable tumor markers in serum, tissue and body fluids during neoplastic process is of clinical value in the management of patients with various malignancies. The spectrum of biochemical tumor markers reported to date is very wide (Harnden, 1985). Tumor markers can be broadly classified as 1. Oncofetal antigens e.g., alpha-fetoprotein (AFP), Carcinoembryonic antigen (CA), Pancreatic oncofetal antigen, fetal sulfoglycoprotein. 2.
Tumor associated antigens /Cancer Antigens. Hormones e.g., Beta human chorionic gonadotropin, calcitonin, placental lactogen etc. 4. Hormone receptors e.g., estrogen and progesterone receptors 5. Enzymes and Isoenzymes e.g., prostate specific antigen (PSA), prostatic acid phosphatase (PAP), neuron specific enolase (NSE), glycosyl transferases, placental alkaline phosphatase (PALP), terminal deoxy nucleotidyl transferase (TDT), lysozyme, alpha amylase 6. Serum and tissue proteins (beta-2 microglobulin, monoclonal immunoglobulin/para proteins, glial fibrillary acidic protein (GFAP), protein S-100, ferritin, fibrinogen degradation products) other biomolecules e.g., polyamines (Virji et al., 1988; Bates and Longo, 1987).

Every tumor marker is specific to a group of malignancies or a single organ. Malignant process is known to elaborate a group of markers (William et al., 1986). Depending on the malignant cell type, a single organ can elaborate many cancer markers However, evaluation of tumor markers can be of valuable aid in diagnosis, prognosis, staging and in monitoring the growth of the tumor. Once the patient is positive for a particular marker before instituting therapy, the effective clinical use becomes evident only after its continued measurement throughout the patient’s clinical course. The rising or declining value of marker concentration in majority of malignancies predicts progression or remission (Esteva and Hortobagyi, 2004).

Several biochemical markers have been suggested for biomonitoring the actions of anticancer agents. Serum α- fetoprotein (AFP) is a useful tumour marker for the detection and monitoring of liver cancer development (Pepe et al., 2001). Recently, α2-macro-globulin (α2M), a homotetrameric major acute-phase glycolprotein has been
suggested as a novel cytochemical marker characterizing preneoplastic and neoplastic rat liver lesions. It is only recently that homocysteine (Hcy) has been implicated in increased cancer susceptibility and development (Wright et al., 2007).

The present study aims to carry out a systematic investigation of the tumour markers of AARE against DEN-induced hepatocarcinogenesis by analyzing serum α-fetoprotein, α2-macroglobulin (α2M), homocysteine (Hcy), DNA, RNA, Xanthine oxidase (XO) and Carcinoembryonic antigen (CA).

It is well known that liver cancer is one of the most important cancers in the world, resulting in more than 1 million patients and over 260,000 deaths per year (Liu et al., 2006). Therefore, the chemoprevention and treatment of liver cancer is very important. AFP, α2M, Hcy, DNA, RNA CA and liver weight are valuable references, widely used in animal studies to diagnose and observe the development of hepatocarcinogenesis (Thirunavukkarasu et al., 2005). In the present study, the values of previously mentioned parameters showed sharp alterations in DEN-group as compared with that of the normal control group.

Terpenoids exert antiproliferative and antitumour effects that are particularly pronounced in tumour cells (Lage et al., 2010). Terpenoids are believed to be active against cancer by enzymatically promoting glutathione transferase (Fahey and Sundquist, 1991). Our study showed significant decrease in body weight and normalization of liver weight caused in AARE -group. The decrease in body weight in AARE -supplemented group may be attributed at least partly to the slowing down of digestion by inhibiting enzymes such as amylase, protease and lipase activities at levels, which could affect
carbohydrates, protein and fat digestion and absorption. Our findings are in concordance with McDougall and Stewart (2005) study.

AFP, a tumour associated fetal protein, has long been employed as a serum fetal tumour marker to monitor disease progression (Abelev, 1971; Liu et al., 2006). The observed significant increase of serum α2-macroglobulin in DEN-induced rats is in harmony with Sukata et al., (2004) who stated that α2M might be tightly linked to the rat hepatocarcinogenesis from the initial stage to tumour progression even in conditions, which are undetectable, by established cytochemical markers such as placental glutathione-S-transferase (GST-P) and γ-GT-positive lesions. Sukata et al., (2004) also confirmed that the observed increases in serum α2M concentrations during hepatocarcinogenesis and in animals, bearing hepatic tumours was not a result of secretion by the host liver of α2M as an acute-phase reactant in response to inflammatory injury. α2M functions as a carrier protein and re regulator for various growth factors and cytokines such as transforming growth factor-β (known to be involved in the onset of hepatocyte apoptosis) (James, 1990). Furthermore, α2M partially counteracts the inhibitory effects of transforming growth factor-β on proliferation of neoplastic hepatocytes, suggesting that under some conditions, α2M can promote hepatocarcinogenesis by perturbing transforming growth factor-β-induced apoptosis (Wollenberg et al., 1991). In the present study observed that the increased content of AFP, CA and α2M concentrations in cancer bearing animals. Supplementation of AARE to cancer bearing animals restored the content of AFP, CA and α2M concentrations. Our findings are in concordance with Nermin et al., (2008) study, which have been reported
that supplementations of AARE decrease the content of AFP and α2M concentrations on DEN induced liver cancer.

Nucleic acid content of tumor is found to be an important indicator of prognosis, because it is well correlated with the size of the tumor in the cancerous condition (Gallagher, 1986). In diseased state, the degree of malignancy increases with the defective abnormalities in DNA. Reports reveal that abnormal amount of DNA was observed in various cancers including breast carcinoma, endometrial carcinoma and lung carcinoma (Ellis et al., 1991). In the present study, an increased activity was observed in DEN induced liver cancer animals and this may be due to the over expression of many enzymes, which are necessary for DNA synthesis in tumor cells.

RNA levels were found to be increased in the cancerous condition as DNA and RNA are directly related to each other, an abnormally increased content of DNA may lead to an increased transcription, which in turn increased RNA content in tumor cells. The mechanisms by which tea polyphenols may act includes the inhibition of promutagen activation, the inactivation of mutagens and carcinogens, blocking and scavenging of reactive molecules, modulation of DNA replication or repair, inhibition of promotion and inhibition of invasion and metastasis of tumor cells. These mechanisms are currently being progressively clarified. Most of the reports on mechanisms, however, still remain as suggestive or speculative (Kurodo and Hara, 1999). Present findings are similar to the Pakkir et al., (2011) study. In ethanolic extract of Aplotaxis auriculata root (500 mg/kg) treated animals, the nucleic acid levels were decreased due to its inhibition of mutagenesis process.
Increased serum Hcy was observed in the present study. Animal and human studies have increasingly demonstrated associations between folate deficiency, serum Hcy elevations and a variety of cancers. The observed increase in serum Hcy in our study is suggested to reflect inhibition of homocysteine metabolism due to folate deficiency reported in other studies (Eichholzer et al., 2001; Davis and Uthus, 2004). Folate is important for normal DNA synthesis, repair and converting homocysteine to methionine (Davis and Uthus, 2004). Therefore, increased demand of folate is postulated to be a result of increased hepatic levels of DNA and RNA and might indicate increased DNA and RNA synthesis and proliferation of cancer cells in response to growth stimulation. Supplementation of AARE to cancer bearing animals restored the content of serum Hcy. Our results concord with the earlier work done by Nermin et al., (2008) study, which have been reported that supplementations of AARE restored the serum Hcy on DEN induced liver cancer.

Feeding of the drug resulted in less number of rats showing liver tumors or slower tumor growth in rats that showed up tumors. Morphological and tissue-weight/ body-weight correlation studies supplemented with the electron microscopic studies were supportive of this statement. There was less damage in the liver tissue of the drug fed rats or better recovery.

In conclusion, restored the altered levels of serum α-fetoprotein, α2-macroglobulin (α2M), homocysteine (Hcy), DNA, RNA and Carcinoembryonic antigen (CA) on treatment with DEN and AARE. The protective properties of the ethanolic extract *Aplotaxis auriculata* root may be due to the presence of phytochemicals such as
flavonoids, terpenoids alkaloids etc. and all these observations clearly indicate a significant antitumor activity of ethanol extract of *Aplotaxis auriculata* root. The tissue-weight/ body-weight and morphological studies also supported the chemopreventive properties of AARE.


Cancer cells display a broad spectrum of alterations that include gene rearrangements, point mutations and gene amplifications, leading to disturbances in molecular pathways regulating cell growth, survival and metastasis. When such changes manifest in majority of patients with a specific type of tumour, these can be used as biomarkers for detection and developing targeted therapies, besides predicting responses to various treatments (Ludwig and John, 2005).

Every cell type has a unique molecular signature, referred to as biomarkers, which are identifiable characteristics such as levels or activities (the abilities of genes or proteins to perform their functions) of a myriad of genes, proteins or other molecular features. Biomarkers are therefore, an objective measure or evaluation of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention. This includes all diagnostic tests, imaging technologies and any other objective measures of a person's health status. Biomarkers are subject to dynamic modulation and are expected to enhance our understanding of drug metabolism, drug
action, efficacy and safety. These can also facilitate molecular definition of diseases, provide information (Anant Narayan Bhatt et al., 2010)

Technologies to recognize and understand the signatures of normal cells and how these become cancerous, promises to provide important insights into the aetiology of cancer that can be useful for early detection, diagnosis and treatment. Biomarkers are therefore, invaluable tools for cancer detection, diagnosis, patient prognosis and treatment selection. These can also be used to localize the tumour and determine its stage, subtype and response to therapy. Identification of such signature in surrounding cells or at more distal and easily sampled sites of the body viz., cells in the mouth (instead of lung) or urine (instead of urinary tract) can also influence the management of cancer (Sawyers, 2008).

A major challenge in cancer diagnosis is to establish the exact relationship between cancer biomarkers and the clinical pathology, as well as, to be able to non invasively detect tumours at an early stage. Similarly, identification of subtle changes in the genomics and proteomic status specific to malignant transformation will allow molecular targets to be used for developing therapeutics. Biomarkers employed currently in clinical oncology for diagnosis and therapy as well as potential ones that particularly hold promise as targets for therapy (Srinivas et al., 2001). In the present study to evaluate the Aplotaxis auriculata root extract (AARE ) on Hepatospecific enzymes such as transaminases, ALP, LDH, GGT and protein in DEN induced hepatocellular carcinoma rats.
During carcinogenesis, some enzymes can be used as biochemical indicators of tumor response to therapy (Thirunavukkarasu and Sakthisekaran, 2003). Hepatospecific enzymes were activated when hepatocellular damage gave rise to abnormalities of liver function and these enzymes are remarkably increased in HCC. AST and ALT activities in blood serum are generally accepted as an index of liver damage and this tendency is also known to be distinct in rodents. There was a good correlation between the activities of ALT and AST with tumor volume during therapy. Rocchi et al. (1997) reported that there was an increase in the levels of these transaminases activity in serum of HCC patients. In concurrent with the above findings an elevated serum aminotransferase activities were observed in animals bearing HCC with simultaneous decrease in the liver tissue; AARE treatment significantly attenuated this alteration thereby showing its anticarcinogenic activity.

Elevation of alkaline phosphatase is one of the signs, suggesting space-occupying lesions in the liver. An increased activity of ALP was seen in blood serum of animals with HCC, this may be due to the disturbance in secretory activity or due to altered gene expression in these conditions. Development of tumor results in tissue damage that lead to the release of ALP into circulation (Iqbal et al., 2004) and this enzyme level have been elevated in blood serum of the tumor-bearing animals and this elevation is significantly suppressed by the supplementation of AARE in diet. GGT has been shown to play an important role in the metabolism of foreign substances and also during cell growth and differentiation (Thusu et al., 1991) and is over expressed in tumor cells resistant to therapeutic drugs (Bailey et al., 2001). Experimental studies have shown that GGT was
strikingly activated during the course of hepatocarcinogenesis induced by several hepatocarcinogens in animals (Fiala and Fiala, 1973); chemical carcinogens may initiate some systematic effects that induce GGT synthesis (Vanisree and Shyamaladevi, 1998). This elevation reflects the progress of carcinogenesis, since its activity correlates with tumor growth rate, differentiation and survival of the host (Koss and Greengurd, 1982); in concurrent with above findings, there was an increase in the levels of GGT in the serum of animals bearing HCC. This elevation indicates the basic tumor burden and AARE treatment significantly decreased the elevation of the level of this enzyme.

LDH is a fairly sensitive marker of solid neoplasm (Lipport et al., 1981) and very high LDH levels correlate with treatment failure (Pui et al., 1985); numerous reports revealed increased LDH activity in various types of tumors (Thangaraju et al., 1998). The elevated levels of LDH may be due to its overproduction by tumor cells. Proliferating malignant cells exhibit very high rates of glycolysis, which subsequently lead to elevated LDH activity. The results of the present study are in agreement with literature data and show elevated levels of LDH in blood serum of the DEN administered rats and this elevation was attenuated in AARE-treated rats.

In the present results demonstrated that AARE treatment significantly attenuated the increased activities of these enzymes. AARE helps in parenchymal cell regeneration in liver, thus protecting membrane integrity and thereby minimizing enzyme leakage. This result suggested that AARE possess potential hepat regenerating activity.

Glycoproteins are carbohydrate linked protein macromolecules found in the cell surface, which is the principle component of animal cell. It contains oligosaccharide chains (glycans) covalently attached to their polypeptide chain. The oligosaccharide moieties of glycoproteins, hexose, hexosamine, fucose and sialic acid have an important role in protein stability, function and turnover. Compositional analysis following acid hydrolysis is one method of identifying sugars, qualitatively and quantitatively. The level of different types of glycoproteins are maintained within a narrow range in health, but is elevated in many pathological conditions viz. tuberculosis, autoimmune disease, cardiovascular disease, diabetes mellitus, cancer of cervix, uterus and breasts, trauma, prolonged bed rest and arthritis, including psychiatric disorders (Nandave et al., 2005). Glycoproteins play a significant role in contributing to the surface properties of the cells and also important role in tumorigenesis and as mediators of immunological specificity. They also have a central role of functioning in biological systems such as stabilizing the conformation of glycoproteins on cellular membranes, assisting in cell-cell recognition and interaction and serving as chemical messengers in body fluids & tissues (Kurtul et al., 2004).

Sialic acid, one of the glycoprotein components is used as a tumor marker. It is acetylated derivative of neuraminic acid and exists as terminal component of nonreducing end of carbohydrate chains of glycoprotein. Levels of sialic acid can be useful in early detection of cancer indicating progress of the disease, degree of metastasis and possible
recurrence (Shanmugam and Nagarajan, 1985). Increased activity of sialyltransferase leads to an increased expression of sialic acid in cancer conditions. The influence of sialic acid on the oncogenicity of tumor cells has been studied by many investigators as the main determinant of the cell surface negative charge electromobility and the loss of contact inhibition. It also acts as an antigen-masking agent and as component of cell surface involved in the adherence of tumor cells to mesothelial membrane to form metastasis (Prasad, 1986; Sivagnanam et al., 2012).

Carbohydrates moieties of glycoproteins such as hexose, hexosamine, fucose and sialic acid have also been implicated in the transport of metabolites across cell membranes and also observed a direct relationship between glycolproteins and tumorigenesis (Thirunavukkarasu and Sakthisekaran, 2003). In the present study to analyse the glycoproteins as hexose, hexosamine and sialic acid in plasma and liver tissues of control and experimental rats.

The crucial role of cell surface and cell membrane constituents in neoplastic behaviours and the changes in serum and tissue glycoconjugates have long been associated with malignancies (Patel et al., 1990). The presence of cancer-specific sialic acid-rich glycopeptides was first demonstrated in proteolytic digests derived from the surface of malignant cell (Van Beek, 1973). Thus, the combined evaluation of hexose, hexosamine and sialic acid residues of glycoproteins might help to establish a useful aid in strengthening the diagnosis and treatment monitoring of cancer patients (Patel et al., 1990; Dube and Bertozzi, 2005).
Over expression of glycoconjugates in the cell surface of carcinogen treated experimental animals has been reported (Senthil et al., 2007). A large number of experimental studies pointed out that glycoproteins were synthesized enormously in the tumor and liver tissues during cancerous conditions and subsequently entered into circulation (Thirunavukkarasu and Sakthisekaran, 2003). Over expression of glycoconjugates in the tumor cells with subsequent shedding into plasma could account for increased levels of plasma protein bound hexose, hexosamine and sialic acid were reported (Shimizu and Funakoshi, 1970). The increased levels of plasma glycoprotein components in cancer condition may be due to the leakage of the disturbed membrane components from either disintegrating or dying neoplastic cells or as a consequent shedding of plasma membrane and due to increased synthesis by sequential addition of monosaccharide units to parent protein molecule catalysed by multiple glycosyltransferases such as sialyltransferase (NeuAc -T), galactosyltransferase (Gal-T), fucosyltransferases (Fuc-T A and Fuc-T B) (Manju et al., 2002).

On drug treatment, glycoprotein components levels were reverted back to near normal levels. An increased expression of glycoprotein components in malignant liver tissue was decreased when compared to normal rats observed in our investigation is in line with previous reports (Thirunavukkarasu and Sakthisekaran, 2003; Sivagananam et al., 2012). This could be due to the cytostabilising property of the drug. Limtrakul et al (2005) showed that the flavonoids possess inhibitory action against carcinogenesis. Thus the flavonoids, alkaloids and other bioactive components of the drug may significantly alter the expression of glycosyltransferases thereby modulate glycoprotein synthesis and
protected the structural integrity of cell surface and membrane, indicating its potent anticancer property.

This study shows that AARE administration decrease the glycoprotein synthesis in tumor cells. This may be due to the inhibitory action of AARE on the initiation of N-nitrosodiethylamine activation/detoxification process or alter cell membrane glycoprotein synthesis and structure. Thus the ethanolic extract of Aplotaxis auriculata shows protective effect on carbohydrates moieties as glycoproteins against DEN treated rats.

4.10. Modulatory role of Aplotaxis auriculata root on glucose metabolizing enzymes in diethyl nitrosamine (DEN) induced hepatocellular carcinoma in rats.

In developing countries about 35% of prescribed drugs are derived from natural products. Many investigations are being carried out worldwide to discover naturally occurring compounds, which can suppress or prevent the progress of carcinogenesis (Cheng et al., 2004). In recent years, there has been considerable emphasis on the identification of plant products with antioxidant property, as free radicals are considered to play a major role in most of the diseases, including cancer.

The development of tumors is accompanied by characteristic alterations in the activities of enzymes, particularly those involved in carbohydrate metabolism (Herling et al., 2011). The growth rate of hepatomas and their glycolytic enzymes activities are significantly correlated. Many tumors accelerated the rate of glucose transport, alteration in the cellular levels and regulatory properties of key glycolytic enzymes. Previous studies show that alteration in the patterns of glucose metabolism and relevant genes is
coordinated with activities of glycolytic and gluconeogenic enzymes during the development of tumor (Weber and Cantero, 1960). As a definite correlation exits between tumor progression and the activities of glycolytic and gluconeogenic enzymes (Warburg, 1930), alterations in their activities can be used as a marker of diagnosis and prognosis. In the present study the effect of Aplotaxis auriculata root extract has been studied on glucose-metabolizing enzymes in DEN induced hepato cellular carcinoma (HCC) in rats.

The cancer cells possess an abnormal pattern of energy metabolism, when compared with the normal cells. Studies on experimental hepatomas have shown that metabolic alterations in the tumors are often accompanied by changes in the activities of various enzymes, including key enzymes of carbohydrate metabolism. (Annibaldi and Widmann, 2011). Many cancer cell lines have shown a marked preferential utilization of glycolytic metabolism to meet their increased energy demands. Rapidly growing, highly malignant tumour cells can obtain up to 60% of their total ATP production from glycolysis (Herling et al., 2011). An elevated rate of glycolysis in tumour cells results in an increase in the intracellular concentration of glucose-6-phosphate, a key precursor in the de novo synthesis of nucleic acids, phospholipids and other macromolecules. An enhanced rate of synthesis of the above mentioned compounds are essential to keep pace with rapid cell division and membrane biosynthesis during tumor growth (Shonk et al., 1965).

A direct correlation has been observed between glycolytic activity and hexokinase in a variety of tumor cell lines. Hexokinase levels are important in determining the glycolytic capacity of cancer cells (Dang et al., 2009). Increased activities of hexokinase
and phosphoglucoisomerase during development of tumor cells observed in the present study are in agreement with the finding of earlier study (Sharma et al., 2011), wherein the increased activities of glycolytic enzymes have been found to correlate with the degree of malignance in tumor tissues. High levels of hexokinase reported in Novikoff and Zajdela cell line hepatomas and Aflatoxin-B1 Induced Liver Carcinoma (Sharma et al., 2011) signify the functional importance of hexokinase in tumor cells to utilize excess glucose for the production of ATP. Elevated level of phosphoglucoisomerase reported in sarcoma and in cancers of lung, rectum and breast is an indicator of metastatic growth and increases specifically after metastasis. Its increased activity in liver of DEN induced HCC rate may be due to its level in malignant tissues (Langeswaran et al., 2012).

Gluconeogenesis is a biochemical process almost completely restricted to the liver (Quistorff, 1985). Gluconeogenic enzymes, glucose-6-phosphatase and fructose 1,6-bisphosphatase have shown a preferential localization in different zones of hepatic lobules, thus diseases affecting this organ can be diagnosed by the measurement of activity of certain enzymes of this pathway (Weber and Cantero et al., 1960). The progressive failure of gluconeogenesis, manifested most extensively in rapidly growing tumors such as hepatomas is explained partly by marked decrease or complete absence of glucose-6-phosphatase and fructose 1,6-bisphosphatase activities.

The inhibition of activities of gluconeogenic enzymes glucose -6-phosphatase and fructose-1,6-bisphosphatase in group II DEN-induced rats was in accordance with the earlier report (Balasubramanian and Premkumari, 2012). Glucose-6-phosphatase is reduced in residual liver tissue of group III DEN-induced rats was in accordance with the
earlier report (Balasubramanian and Premkumari, 2012). Glucose-6-phosphatase is also reduced in liver tissue of Aflatoxin-B1 Induced Liver Carcinoma (Sharma et al., 2011). Decreased rate of glucose-6-phosphatase mediated dephosphorylation is also reported in malignant cells (Graham et al., 1989). Decreased activity of fructose-1,6-bisphosphatase, the key regulatory enzyme for the synthesis of glucose-6-phosphate from pyruvic acid observed in liver of group III rats are supported by the earlier report (Sharma et al., 2011), which reported that in Aflatoxin-B1 Induced Liver Carcinoma, there appears to be a decreased fructose-1,6-bisphosphatase in the tumor and consequently, a block in the pathway, leading to the synthesis of glucose-6-phosphate from pyruvate.

A sharp drop in the activities of hexokinase and phosphoglucoisomerase and a significant increase in the activities of liver glucose-6-phosphatase and fructose-1, 6-bisphosphatase observed on oral administration of the extract of *Aplotaxis auriculata* to DEN induced group III rats correspond to the return of the tumor towards its normal states and are consistent with earlier reports (Langeswaran et al., 2012) on the herbal extracts, which have shown effect on glucose-metabolizing enzymes.

Comparison of groups I and II animals are shown that no significant variation in the key regulatory enzyme activities of both glycolytic and gluconeogenic pathways. It could be presumed that the *Aplotaxis auriculata* extract has modulatory activity on the carbohydrate metabolism in DEN induced HCC bearing rats through a mechanism that which does not provoke any acute biochemical disturbances in the metabolic pathways of glycolysis and gluconeogenesis. The modulatory effect of *Aplotaxis auriculata* extract may be attributed to the presence of active compounds such as polyphenols and
flavonoids. Earlier studies have also shown that *Semecarpus anacardium, Hygrophila Auriculata* and *Terminalia arjuna*, which are rich in flavonoids and polyphenols modulate the glucose-metabolizing enzymes in HCC rats (Premalatha *et al*., 1997; Balasubramanian and Premkumari, 2012). The extract treatment might lead to depletion of energy metabolism in cancer tissues by inhibiting the glycolytic enzymes and regulating the gluconeogenic enzymes.

The elevated hepatic activity of G-6-PD and LDH in this study may be related to enhanced glucose metabolism. It was discovered in the 1920s that cancer cells constitutively up regulate glucose metabolism (Warburg, 1930). Thus, cancer cells tend to synthesize ATP mainly through „glycolysis“, a metabolic state that is linked to high glucose uptake and local acidification owing to lactate production. Gatenby and Gillies (2004) and Zu and Guppy (2004) have reported that when glycolysis prevails, pyruvate is reduced to lactate in order to reoxidize NADH to NAD that is required for sustained glycolysis. Increased glucose breakdown provides building blocks for the synthesis of nucleotides via the pentose phosphate pathway. In addition, local acidification of the tumour microenvironment may facilitate tumour invasion (Kroemer, 2006). Glycolytic enzymes are induced by oncogenes (Plas and Thompson, 2005) or by the hypoxia-inducible transcription factor (King *et al*., 2006) or a dysfunctional tricarboxylic acid cycle owing to loss of function of mitochondrial tumour suppressor genes (Gottlieb and Tomlinson, 2005).

In this study, an alteration in the levels of carbohydrate metabolizing key enzymes were observed on DEN treated rats. It can be concluded from the present data that the
altered levels of hexokinase (HEX), phosphoglucoisomerase (PGI), fructose-1,6-bisphosphatase, Glucose-6-phosphatase, G-6-PD and LDH in HCC bearing rats were reverted significantly to near normal with the ethanolic extract of Aplotaxis auriculata root treated rats. The plant extract might interrupt the energy requirement of tumor tissue and lead to the suppression of tumor growth due to the presence of phenols and flavonoids.

4.11. Effect of AARE on Homolysate lipid peroxidation and antioxidant defence in control and experimental rats.

The simplicity, availability and ease of isolation make erythrocyte membrane as an excellent model for membrane studies (Nalecz, 1989). As cell membrane is an important target for radical damage and blood can reflect the liability of the whole animal to oxidative condition, erythrocytes have been used extensively for determining the effect of toxicological studies concerning the possible involvement of free radicals. Erythrocytes, the unique carriers of oxygen are highly susceptible to oxidative stress conditions. The rich polyunsaturated membrane lipids and iron, a potent catalyst for free radical reactions makes erythrocyte a good substrate for oxidative damages (Vani et al., 2010).

Erythrocyte membrane proteins are susceptible to covalent damage, including cross-linking and aggregation by free radical-induced peroxidation products. Extensive peroxidation of lipids causes changes in fluidity, e.g., a fall in the membrane potential and an increase in the permeability to different ions that finally lead to hemolysis.
Therefore erythrocytes are very sensitive to oxidative injury (Bernabucci et al., 2002). To defend themselves against oxidative stress (OS), erythrocytes are equipped with an effective and complex antioxidant system, including protective enzymes and biological antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione (GSH), vitamin C and vitamin E. Despite the efficacy of this antioxidant system, the capacity to repair oxidative damage to RBCs is limited (Nagababu et al., 2003).

It is well known that OS results in damage to cells, which may lead to the loss of cell function. The biochemical changes taking place during DEN intoxication is high altitude may make cells, particularly susceptible to oxidative injury (Gey, 1993). Thus there could be a role for antioxidant supplements in protecting the cells from oxidative injury.

Therefore, our study was concentrated on the role of AARE in augmenting the functions of antioxidants and determines the level of lipid peroxidation in Homolysate of DEN treated rats.

The mammalian erythrocyte red blood cell (RBC) is an ideal cell model in which to study free radical-mediated injury, since it is enucleated and has a short life span. Changes in membrane lipids can affect the RBC shape by disrupting the balance in area between the two lipid leaflets (Vani et al., 2010). Autoxidation of hemoglobin (Hb) produces superoxide as well as methemoglobin. It is known that Hb reacts with hydrogen peroxide to produce ferrylhemoglobin, which is a strong oxidant. Hb binds to membrane proteins of the RBC, particularly under hypoxic conditions. The reactive oxygen species (ROS) generated by bound Hb may not be accessible to cellular antioxidants, facilitating
the production of heme degradation products in close proximity to the membrane (Bakonyi and Radak, 2004; Nagababu et al., 2003).

Inactivation and removal of ROS depends on reactions involving the antioxidant system, the capacity of which is determined by a dynamic interaction between individual components comprising vitamin E, vitamin C and reduced glutathione (GSH) as well as SOD, GSH-Px and CAT. The latter two form a substantial defense network against oxidative stress imposed by several other factors such as physical exercise (Asha et al., 2005). The erythrocyte membrane encounters OS from both the interior and exterior of the cell and regardless of the site of origin of the OS, the primary protection against peroxidative damage is through α-tocopherol, which is recycled by ascorbate. Vitamin E a constituent of the plasma membrane, is an effective antioxidant since it is present at the site of free radical generation and can therefore neutralize the toxic effects of ROS. Vitamin E scavenges peroxide free radicals and converts them to less toxic lipid hydroperoxides, thereby protecting the cell membrane and decreasing hemolysis (Niki and Noguchi, 2004).

Oxygen radicals formed above the detoxifying capacity of erythrocytes can cause peroxidative breakdown of phospholipid fatty acids in the erythrocyte membrane, resulting in an accumulation of MDA (Ramazan et al., 2000). LPO is regarded as one of the basic mechanisms of tissue damage caused by free radicals and it acts as an important causative factor in carcinogenesis (Banakar et al., 2004). MDA is a major end product of lipid peroxidation, which can crosslink with DNA and other protein molecules, thereby it promotes tumoriogenesis (Niedernhofer et al., 2003). Recently, it has been reported that
administration of DEN causes increased level of LPO by an uncompromised generation of free radicals which overwhelms the antioxidant defense system leading to oxidative stress and carcinogenesis (Gey, 1993). These findings correlate with our results showing, the significant increase in the levels of LPO in erythrocyte of animals administered with DEN, when compared with control animals. LPO can be prevented at the initiation stage by free radical scavengers and antioxidants (Torel et al., 1986). An in vitro study is to be reported in our study that Aplotaxis auriculata root was able to efficiently can scavenge superoxide, hydroxyl and nitric oxide radicals. With this finding, there was a significant decrease in the level of LPO in erythrocytes of animals simultaneously treated with Aplotaxis auriculata when compared with cancer bearing rats, indicating the anti-lipid peroxidative property of Aplotaxis auriculata. This reveals that Aplotaxis auriculata is vulnerable to quench LPO chain and capable of shielding the membrane from free radicals caused injuries. Present finding is similar to the Janani et al., (2010) study.

Antioxidants are substances that either directly or indirectly protect cells against adverse effects of xenobiotics, drugs, carcinogens and toxic radical reactions (Sen, 1995). SOD is the primary step of defense mechanism in the antioxidant system against the oxidative stress; it demolish the superoxide radical by converting it to peroxide and molecular oxygen in turn that can be counteract by catalase or GPx reactions thereby reducing the level of cellular damage. During the reaction of H₂O₂ scavenging, GSH is oxidized to GSSG by the enzyme GPx and GR reduces GSSG to GSH. Deleterious effects of oxidants progression to neoplastic condition are affronted by primary
antioxidants such as SOD, CAT and GPx. In a variety of malignancies, the elevated LPO is associated with reduced activity of antioxidants. Deficiency of SOD and CAT results in decreased detoxification of oxygen radicals, which leads to attack of ROS on protein and nucleic acids. Hence, the activities of these enzymes are decreased in cancerous condition (Ray et al., 2000). Such studies substantiate with our findings as there was a significant depletion in the activities of enzymic antioxidants in hemolysate of animals treated with DEN when compared to normal animals. This indicates the severity of the oxidative stress during DEN metabolism, which could have inhibited the activities of these enzymes. Supplementation of AARE to DEN treated rats restored the enzymatic antioxidants. Our result agrees with the earlier report (Janani et al., 2010).

Vitamin C, Vitamin E and reduced glutathione are well known non-enzymic antioxidant defense system of cells. These are interrelated with each other by recycling process. GSH is the major cytosolic thiol compound and is required to maintain the normal reduced state of the cells and to counteract ROS thereby reducing the oxidative stress. GSH also preserves the cellular levels of active forms of Vitamin C and Vitamin E. Vitamin E is chain breaking antioxidant present in the cell membrane (Horwitt, 1976). It provides protection against superoxides as well as H₂O₂. Vitamin C is water soluble antioxidant and can react with Vitamin E radicals to regenerate Vitamin E. The levels of these non-enzymic antioxidants were decreased in hepatoma bearing animals (Thirunavukkarasu et al., 2002). The results of present study also correlate with such findings (Janani et al., 2010). It might be due to over utilization of these antioxidants to
scavenge free radicals. In other hands, the simultaneous administration of *Aplotaxis auriculata* reversed the changes induced by DEN exposure to near normal, supporting the hypothesis that *Aplotaxis auriculata* is an effective chemopreventive agent.

Supplementation of AARE to DEN treated rats may probably related to a counteraction of free radicals by its antioxidant nature of AARE. AARE strengthening of endogenous antioxidant defense by its ability to restored the levels of SOD, CAT, GPx, GR, vitamin C, vitamin E and increased GSH content and also its ability to decreased the levels of lipid peroxidation. The present investigation highlights the chemopreventive potential of *Aplotaxis auriculata* against DEN-induced HCC by quenching lipid peroxidation and enhancing antioxidant status in the erythrocyte through free radical scavenging mechanism and having potential of protecting endogenous enzymatic and non-enzymatic antioxidant activity.