CHAPTER V

5. DISCUSSION

In olden days itself the importance of medicinal plants have been discovered. At that ancient time there was no synthetic medicines, they have been using only the herbal medicines to treat all diseases. From this we can understand that plants are rich in medicinal properties and they are very useful in human health and wellbeing. Biological studies are essential to find more medicinal properties of the plants (Rukshana et al., 2017). But still the many medicinal plants and their medicinal properties are unexplored. Treating of diseases through natural medicine is the most ancient treatment known to mankind (Cragg and Snadder, 2003). In India the medical systems using medicinal plants are Ayurveda, Siddha, Homeopathy, etc., to treat diseases. Natural compounds are now gaining more pharmacological attention as many unexplored plant products are showing a wide range of activities like antioxidant and anti-cancer (Raghav et al., 2007).

Recently many of the research were being carried out in medicinal plants. The main reason was that the synthetic drugs which was now taking up by the human have many side effects that often lead to serious complications. The development of herbal medicine was done by the primary screening of the compounds in the plant extracts and evaluated its pharmacological activities. The present study to be made investigate the phytochemical screening, in-vitro antioxidant and anticancer activity in Corallocarpus epigaeus rhizome extract (CERE). Results of the present study presented as follows
5.1 Phytochemical analysis of Corallocarpus epigaeus rhizome

In recent years, medicinal plants receive considerable interest depending on type, number, and mode of action of the different components, so called as “phytochemicals”, for their presumed role in the prevention of various chronic diseases including cancers and cardiovascular diseases. Plants are rich sources of functional dietary micronutrients, fibers and phytochemicals, such as ascorbic acid, carotenoids, and phenolic compounds that individually or in combination may be beneficial for health since they demonstrate antioxidative activity in vitro (Liu, 2004; Yahia, 2009).

The secondary metabolites formed also are an important trait for our food plants (taste, colour, scent, etc.) and ornamental plants. Moreover, numerous plant secondary metabolites such as flavonoids, alkaloids, tannins, saponins, steroids, anthocyanins, terpenoids, rotenoids etc. have found commercial application as drug, dye, flavour, fragrance, insecticide, etc. Such fine chemicals are extracted and purified from plant materials (Das et al., 2010).

The qualitative phytochemicals characters of the Corallocarpus epigaeus rhizomes showed the presence of flavonoids, terpenoids, steroids, tannin, saponins, glycosides, phlobatannins, carbohydrate, triterpenoids, alkaloids, anthroquinones, polyphenol were present while phlobatannins was absent in ethanol extract, aqueous extract and hydro-alcohol extracts. Significant amount of total phenol (156.52±10.95mg/gm), Tannin (67.85±4.74mg/gm) steroids (35.45±2.48mg/gm) and
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flavonoids (95.32±6.67mg/gm) was present. This results agreement with earlier report (Bhavani et al., 2013). Over all, Corallocarpus epigaeus rhizome contain rich source of phytochemicals which are important in diseases prevention.

Waseem Ahmad et al. (2017) were designed to investigate the phytochemical screening and antimicrobial activities of Euphorbia hirta extracts. Phytochemical screening revealed the presence of alkaloid, flavonoid, saponin, terpenoid, steroid and sterols in the extracts of aerial part of Euphorbia hirta.

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

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 ethanolic extract might be due to their content of flavonoids, tannins, alkaloids and saponins reported earlier (Vikrant Arya et al., 2012).

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, anthroquinone, glycoside and tannins were absent in the plant extract.

5.1.1 Qualitative analysis of inorganic elements in *Corallocarpus epigaeus* rhizome

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) (Indrayan *et al.*, 2005).

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 (New Wall *et al.*, 1996).
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The inorganic elements were investigated in *Corallocarpus epigaeus* rhizome. Calcium, magnesium, sodium, potassium, sulphate, phosphate, chloride and nitrate were present while Iron was absent in *Corallocarpus epigaeus* rhizome extract. Mineral content indicates the nutritive value and potentially act as a cofactor for the biological activity exhibited by the plant extracts studied.

The vitamins of the *Corallocarpus epigaeus* rhizome were studied and showed that the presence of vitamin C, A and E while D was absent. Shalini and Velavan, (2017) investigate phytochemicals and inorganic elements in *Aplotaxis auriculata*. The results of this study clearly indicate that the preliminary phytochemical analysis of *Aplotaxis auriculata* (rhizome) revealed presence of flavonoids, polyphenol, steroids, tannin, saponins, glycosides, anthraquinones, steroids, alkaloids, carbohydrate, pholphenol while and protein were absent. Quantitative analysis revealed that *Aplotaxis auriculata* rhizome plant rich amount of total phenol (255.84±17.85mg/gm), alkaloids (40±2.80mg/gm), saponin (20±1.40mg/gm) and flavonoids (225.02±15.75mg/gm) were presented. The vitamin analysis of *Aplotaxis auriculata* rhizome plant showed that the presence of Vitamin C, D and E. Vitamin A were absent. The inorganic elements of *Aplotaxis auriculata* rhizome plant showed that the presence of calcium, sodium, potassium, sulphate, phosphorus, chloride, Nitrate magnesium while and iron was absent. The results of the present study concluded that CERE auriculata may be a good source of phytochemicals, vitamins and minerals.
Ramya 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 and 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.

Kumudhaveni Babu et al. (2013) examined the heavy metals and inorganic element content in *Stereospermum colais* leaves. Ciura et al. (2007) reported to contain cadmium, lead, zinc and copper in selected vegetables and fruit from garden.

### 5.1.2 UV-VIS spectral analysis of *Corallocarpus epigaeus* rhizome extract

UV-visible spectrophotometry is one of the most frequently employed techniques in pharmaceutical analysis. It involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in solution. Instrument which measure the ratio or function of ratio, of the intensity of two beams of light in the UV-visible region are called Ultraviolet-visible spectrophotometers (Davidson, 2002). Spectroscopy is a technique that measures the interaction of molecules with electromagnetic radiation. Light in the near ultraviolet (UV) and visible (vis) range of the electromagnetic spectrum has an energy of about 150–400 kJ mol\(^{-1}\). The energy of the light is used to promote electrons from the ground state to an excited state. A spectrum is obtained when the absorption of light is measured as a function of its frequency or wavelength. Molecules with electrons in delocalized aromatic
systems often absorb light in the near UV (150–400 nm) or the visible (400–800 nm) region (Brown, 1980).

Spectroscopic methods have become a powerful tool for secondary metabolite profiling as well as for qualitative and quantitative analysis of the pharmaceutical and biological materials. The application of standardized UV (or UV-vis) spectroscopy has years been used in analyses of flavonoids. The various flavonoid classes can be recognized by their UV spectra and UV spectral characteristics of individual flavonoids including the effects of the number of aglycone hydroxyl groups, glycosidic substitution pattern and nature of aromatic acyl groups have been reviewed (Markham, 1982). All the flavonoids contain at least one aromatic ring and consequently absorb UV light (Mabry et al., 1970). The typical UV-vis spectra of flavonoids include two absorbance bands maximum in the ranges of λ240~280 nm (band II) and λ300~550 nm (band I). Flavonoids are composed of three rings structure (A, B and C) with various substitutions. Changes in the substitution of the A ring tend to be reflected in the band II absorption while alterations in the substitution of the B and C rings tend to be more apparent from band I absorption (Markham, 1982). Additional oxygenation (especially hydroxylation) generally causes a shift of the appropriate band to the longer wavelengths. Based on the UV-visible absorbance spectra, the flavonoid class can be predicted for each chromatographic peak separated.
The UV-VIS spectroscopic studies revealed the presence of peaks in the range 200 to 236nm. The spectra for phenolic compounds and Flavonoids typically lie in the range of 210-290 nm (Neha et al., 2006). The result of UV-VIS spectroscopic analysis confirms the presence of Flavonoids in the *Corallocarpus epigaeus* rhizome extract. Present finding is in agreement with Rajadurai Maruthamuthu and Kumaresan Ramanathan (2016) studies.

Pragna et al. (2012) showed absorption maximum in the range of 200 – 300 nm. Fraction A was found to be having absorption in visible region and it yielded a yellow crystalline powder, suggestive of presence of coloured flavonoids. Rest of all fractions yielded a white crystalline powder. These results supported the study of Yang et al. (2005) who isolated two new aporphine alkaloids from *Litsea bark* and reported one’s absorption maximum at 218, 282 and 306, while the other compound has absorption maximum at 278 and 306.

Neha Sahu and Jyoti Saxena (2013) studied the phytochemical analysis of *Bougainvillea glabra* by FTIR and UV-visible spectroscopic analysis. UV-visible spectrum of this plant extracts has absorption bands at 324 and 290 nm. These absorption bands are characteristic for flavonoids and its derivatives.

**5.1.3 Fourier Transform Infra-Red Spectroscopy analysis of *Corallocarpus epigaeus* rhizome extract**

FTIR has played a vital role in pharmaceutical analysis in recent years (Ellis et al., 2002). FTIR spectroscopy is a physicochemical analytical technique that does not determine concentrations of individual metabolites but provides a snapshot of the
metabolic composition of a tissue at a given time (Hori and Sugiyama, 2003). The FTIR method measures predominantly the vibrations of bonds within chemical functional groups and generates a spectrum that can be regarded as a biochemical or metabolic “fingerprint” of the sample (Griffiths and Haseth, 1986).

FTIR has proven to be a valuable tool for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plant extract (Eberhardt et al., 2007; Hazra et al., 2007). The FTIR spectrum of the Corallocarpus epigaeus rhizome extract was pronounced absorbance was recorded in the region between 4000 and 400 cm⁻¹. They include 3386 (1°,2° amines, amides), 2947 (Alkanes), 2525 (Carboxylic acids), 2215 (Nitriles), 1655 (Alkenes), 1453 (Alkanes), 1415 (Aromatics), 1113 (Alcohols, Carboxylic acids, Esters), 1025 (Alcohols, Carboxylic acids, Esters) and 671 (1°,2° Amines) cm⁻¹. FTIR spectrum of the Corallocarpus epigaeus rhizome extract showed the presence of alcoholic, phenolic, aromatic, Carboxylic acids and Esters. Our result agrees with the earlier report (Sithara et al., 2017).

Yamunadevi et al. (2012) identified the functional groups present in the crude powder of Aerva lanata (L.) Juss. ex Schult. stem, leaves, root and flower through FTIR spectroscopy. The results of Aerva lanata flower FTIR analysis confirmed the presence of amide, alcohols, phenols, alkanes, carboxylic acids, aldehydes, ketones, alkenes, primary amines, aromatics, esters, ethers, alkyl halides and aliphatic amines compounds which shows major peaks at 3675.36, 3618.49, 3587.12, 2918.08,
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2849.76, 1771.81, 1733.59, 1652.96, 1636.03, 1457.06, 1318.57, 1243.66, 1053.77 and 510.63 respectively. The leaves of *A. lanata* FTIR analysis results proved the presence of alcohols, phenols, alkanes, carboxylic acids, aldehydes, alkenes, nitro compounds, alcohols, carboxylic acids, esters, ethers, aliphatic amines and alkyl halides compounds. The FTIR analysis results of *A. lanata* root revealed the presence of amines, amides, alkanes, aldehydes, ketones, esters, carboxylic acids, carbonyls, alkenes, primary amines, nitro compounds, aromatics, alcohols, esters, ethers and alkyl halides compounds. The FTIR analysis results of *A. lanata* stem validated the presence of amide, alcohols, phenols, amines, alkanes, ketones, primary amines, nitro compounds, alcohols, carboxylic acids, esters, ethers, alkyl halides and aliphatic amines.

5.1.4 Identification of bioactive compounds in *Corallocarpus epigaeus* rhizome extract by GC MS analysis

Gas Chromatography–Mass Spectrometry (GC-MS) is a hyphenated analytical technique that combines the separation properties of gas-liquid chromatography with the detection feature of mass spectrometry to identify different substances within a test sample. GC is used to separate the volatile and thermally stable substitutes in a sample whereas GC-MS fragments the analyte to be identified on the basis of its mass (Sahil et al., 2011). GC-MS is widely used in pharmaceutical industries for analytical research and development, quality control, quality assurance, production, pilot plants departments for active pharmaceutical ingredients (API), bulk drugs and formulations. It is used for process and method development,
identification of impurities in API. It is an integral part of research associated with medicinal chemistry (synthesis and characterization of compounds), pharmaceutical analysis (stability testing, impurity profiling), pharmacognosy, pharmaceutical process control, pharmaceutical biotechnology etc. (CDER, 1994, 2004).

In the present study twenty chemical constituents have been identified from extract of *Corallocarpus epigaeus* rhizome by Gas Chromatogram- Mass spectrometry (GC-MS) analysis. The prevailing compounds were 2-Hexadecen-1-ol, 3,7,11,15-tetram, 9-Octadecenoic acid, Octadecanoic acid, methyl esters, 1- (+) - Ascorbic acid 2,6-dihexadecanoate, Oleic Acid, 9,12-Octadecadienoic Acid (Z,Z), Octadecanoic Acid, Methyl Ester, Octadecanoic acid, 1,2-Benzenedicarboxylic acid and Stigmast-5-en-3-ol.

The identified compounds in *Corallocarpus epigaeus* rhizome possess many biological properties. For instance, 2-Hexadecen-1-ol, 3,7,11,15-tetram possesses cancer-preventive, antimicrobial, anti-inflammatory, anti-diuretic and antioxidants. 9-Octadecenoic acid can be an antihypertensive, increase HDL and decrease LDL Cholesterol. Octadecanoic acid methyl ester has anti-tumour activity. 1-(+)-Ascorbic acid 2,6-dihexadecanoate is an Vitamin C, antioxidant, and immunomodulators. Oleic Acid possesses 5-alpha-reductase-inhibitor, allergenic, alpha-reductase inhibitor, anemiagenic, antialopecic, antiandrogenic, antiinflammatory, antileukotriene-D4 (Anti-platelet activating factor), cancer-preventive, choleretic and hypocholesterolemic activity. The presence of various
bioactive compounds justifies the use of the whole plant for various ailments by traditional practitioners.

Agim et al (2017) was designed to determine the phytochemicals present in the seed of *Aframomum melegueta* using preliminary test for secondary metabolites and Gas Chromatography Mass- spectroscopy (GC-MS) method of analysis. The results of the qualitative phytochemical screening indicated that alkaloids and saponins were moderately present; tannins, flavonoids, cardiac glycoside were slightly present while steroids were absent. The quantitative phytochemical screening using GC-MS showed the presence of thirteen (13) compounds among which are Glycerin (R/T 2.568), Caryophylene (R/T 5.858), Humulene (R/T 6.173), Cis-Vaccenic acid (R/T 7.735), Gingerol (R/T 9.848), d-Decanone,1(4-hydroxy-3-methoxyphenyl)- (R/T 11.003), Gingerol (11.527), d Manose (R/T 11.581), DL-Arabinose (R/T 11.998), Hexadecanoic acid, Methyl ester (R/T 12.180), n-Hexadecanoic acid (R/T 12.490), 9,12-Octodecanoic acid (z,z,z)-, 9,12,15-Octodecanoic acid (z,z,z)-. In the analysis the quantity of DL-Arabinose (R/T 11.998) is more with area percentage of (26.36%) while the least is Gingerol (R/T 9.848) with area percentage of (0.80%).

5.1.5 HPLC analysis of leaves extract *Corallocarpus epigaeus* rhizome

Phytochemical analysis is a very important laboratory process or scientific process. This process is used to identified essential components of any plant part such as bark, leaves, stem and root. Standardization and characterization of herbal
drugs is a topic of continuous scientific interest in the herbal drug industry. With the advent of modern chromatographic systems there is an ever increasing intent to produce and develop easy, rapid, convenient and cost effective methods for standardization (Selvamani et al., 2009).

HPLC profiles of *Corallocarpus epigaeus* rhizome were analysed and compared with the respective standard. Two phenolic compounds namely Quercetin, and Gentisic acid having different elution times could be obtained when each compound was analyzed individually using the mobile gradient phase consisting of ethanol and 1% acetic acid in water during 30 minutes run time.

Paranthaman *et al.* (2012) investigated the GC-MS analysis of phytochemicals and simultaneous determination of flavonoids in *Amaranthus caudatus* (Sirukeerai) by RP-HPLC. A sensitive and selective high performance liquid chromatography method (HPLC) for simultaneous analysis of following five flavonoids like gallic acid (GA), caffeic acid (CA), rutin (RU), ferulic acid (FA) and quercetin (QU) in *Amaranthus caudatus* (Sirukeerai) leaves. The results demonstrated that the *Spermacoce hispida* leaves were separately extracted and analyzed using HPLC method. The contents of gallic acid (0.083 \( \mu \)g/gm), Caffeic acid (0.004 \( \mu \)g/gm), Rutin (0.019 \( \mu \)g/gm), Quercetin (0.001 \( \mu \)g/gm) and Ferulic acid (0.001 \( \mu \)g/gm) in *Spermacoce hispida* leaves.

Nadia Alam *et al.* (2011) were carried out to characterize the phenolic acids and flavonoids in methanolic extracts of *Withania somnifera* leaves by HPLC. Five
phenolics (gallic, syringic, benzoic, p-coumaric and vanillic acids) and three flavonoids (catechin, kaempferol and naringenin) have been identified in *Withania somnifera* leaves. Similarly catechol, gallic acid, ellagic acid, and catechin of compounds were identified in *Spermacoce hispida* leaves among the four compounds of this present study.

5.2 Antioxidant activity of *Corallocarpus epigaeus* rhizome

The human body has a complex system of natural enzymatic and non-enzymatic antioxidant defenses which counteract the harmful effects of free radicals and other oxidants (Badarinath *et al*., 2010). Free radicals are responsible for causing a large number of diseases including cancer, cardiovascular disease, neural disorders, Alzheimer’s disease, mild cognitive impairment, Parkinson’s disease, alcohol induced liver disease, ulcerative colitis, aging and atherosclerosis (Velavan, 2011; Smith *et al*., 2000). Protection against free radicals can be enhanced by ample intake of dietary antioxidants. Substantial evidence indicates that foods containing antioxidants and possibly in particular the antioxidant nutrients may be of major importance in disease prevention. There is, however, a growing consensus among scientists that a combination of antioxidants, rather than single entities, may be more effective over the long term (Blokhina *et al*., 2003).

Antioxidants may be of great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases. In addition, they have a potential for substantial savings in the cost of health care delivery. Various methods
are used to investigate the antioxidant property of samples (diets, plant extracts, commercial antioxidants etc.) (Nur Alam et al., 2013). Antioxidant activity should not be concluded based on a single antioxidant test model. And in practice several in vitro test procedures are carried out for evaluating antioxidant activities with the samples of interest. In the present study was to investigate the in vitro antioxidant activity of *Corallocarpus epigaeus* rhizome extract.

### 5.2.1 DPPH radical scavenging activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical. DPPH is gained its stability as free radical molecules due to the delocalization of odd electron throughout the molecules. This more stabilized DPPH produce intense violet colour in ethanol solution. The antioxidant present in the extracts reacts with DPPH free radical solution and converts them into reduced form either by donating hydrogen atom or transferring electron followed by proton. This oxidation reaction is accompanied with loss of violet colour which can be measured quantitatively at 517 nm (Nuutila et al., 2003). DPPH radical scavenging activity of *Corallocarpus epigaeus* rhizome extract and standard as ascorbic acid are presented in Fig 4.6. The half inhibition concentration (IC$_{50}$) of *Corallocarpus epigaeus* rhizome extract and ascorbic acid were 47.16μg/ml$^{-1}$ and 52.01μg/ml$^{-1}$ respectively. The *Corallocarpus epigaeus* rhizome 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.

5.2.2 Total antioxidant activity

Total antioxidant capacity of *Corallocarpus epigaeus* rhizome is expressed as the number of equivalents of ascorbic acid. 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 study reveals that the antioxidant activity of the *Corallocarpus epigaeus* rhizome extract is in the increasing trend with the increasing concentration of the *Corallocarpus epigaeus* rhizome extract (Table 4.10). The half inhibition concentration (IC$_{50}$) of plant extract and ascorbic acid were 34.68$\mu$g/ml$^{-1}$ and 53.35 $\mu$g/ml$^{-1}$ respectively.

5.2.3 Superoxide scavenging activity

The superoxide anion radicals O$_2^\bullet$-scavenging activity of the extract was determined by nitro blue tetrazolium (NBT) method. Phenazine methosulphate (PMS) reacts with Nicotinamide adenine dinucleotide (NADH) to produce superoxide anion radicals. The generated superoxide anion radicals reduce Nitro blue
tetrazolium into formazan. Free radicals scavenger present in the extracts competes with Nitro blue tetrazolium for superoxide anion radicals and slowdown the formation. 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 and Richardson, 1978). The superoxide scavenging activity of *Corallocarpus epigaeus* rhizome was increased markedly with the increase of concentration (Table 4.11). The half inhibition concentration (IC$_{50}$) of *Corallocarpus epigaeus* rhizome was 49.39μg/ml and ascorbic acid was 50.28 μg/ml$^{-1}$ respectively.

5.2.4 The ferrous ion chelating activity of rhizome extract of *Corallocarpus epigaeus* rhizome

Iron is essential for life as it is required for oxygen transport, respiration and for activity of many enzymes. Chelating agents inhibit lipid peroxidation by stabilizing the transition metals (Nabavi *et al.*, 2009) Decrease in the red color ferrozine–Fe$^{2+}$ complex indicates high scavenging activity of the compound. Earlier in 1990, scientists reported that the chelating agents are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion.

The formation of the ferrozine–Fe$^{2+}$ complex is interrupted in the presence of *Corallocarpus epigaeus* rhizome extract, indicating that have chelating activity with an IC$_{50}$of 44.59μg/ml and ascorbic acid was 50.75 μg/ml respectively. The chelating
effect of the extract increases with the increase in the concentration this may be due to the increase in the concentration of the secondary metabolites in the extracts. Thus the results suggest that the leaf extracts are capable of scavenging the free radicals and prevent the initiation of free radicals by stabilizing them to participate in any deleterious reactions. The scavenging activity of the all extracts was comparable to ascorbic acid.

5.2.5 Reducing power activity

This method is based on the principle of increase in the absorbance of the reaction mixtures. Increase in the absorbance indicates an increase in the antioxidant activity. In this method, antioxidant compound forms a colored complex with potassium ferricyanide, trichloro acetic acid and ferric chloride, which is measured at 700 nm. Increase in absorbance of the reaction mixture indicates the reducing power of the samples (Jayaprakash et al., 2001). The reducing power of *Corallocarpus epigaeus* rhizome increased with increasing dosage. All the doses showed significant activities near to the control exhibited greater reducing power, indicating that *Corallocarpus epigaeus* rhizome consist of hydrophilic polyphenolic compounds that cause the greater reducing power.

5.3 Effect of *Corallocarpus epigaeus* rhizome in experimental lung carcinogenesis

Benzo(a)pyrene [B(a)P] as a PAH, potent carcinogen is considered as a marker to the carcinogenic potency of the polycyclic aromatic hydro carbons (PAH)
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by world health organization (Delgado-Saborit et al., 2010). PAH is the main cause of carcinogenesis which induced DNA adducts formation and equally contributes to oxidative DNA damage exposed to ROS (Anandakumar et al., 2009). Under some pathological condition the antioxidant systems of cells cannot withstand the oxidative stress and eventually results in damage of cells an issues due to rigorous accumulation of ROS. This B(a)P metabolically activated into B(a)P-7,8-diol-9,10-epoxide (BPDE) which reacts with DNA predominantly to form a adduct and facilitates the progression of carcinogenesis. The exact mechanism of the development of lung tumor not yet fully investigated, but it is assumed that mechanism of B(a)P generates ROS and initiates proliferative change via an intensive action of tissue marker enzymes leading to decrease in activities of antioxidants in the lung (Tsuji et al., 2011).

Natural antioxidants are capable of inhibiting the ROS production and thereby reducing the associated intracellular oxidative stress (Feng et al., 2001). Lipid peroxidation or the oxidative deterioration of unsaturated fatty acids can occur by exposure to various carcinogens that have been associated with cancer initiation and progression. Reports suggest that lung tissue damage after exposure to B(a)P generating enormous amount of ROS is accompanied by LPO which in turn causes damage to cell membranes with the release of intracellular components, leading to further tissue damage (Gosset et al., 2003). In our observation, B(a)P administration caused significant increases in the MDA in lung tissues; this could be because of oxidative stress mediated generation of free radicals in the form of ROS. Treatment

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(pre and post) with *Corallocarpus epigaeus* rhizome caused significant decreases in the MDA against B(a)P. Results of our study are in support with documented report, which denotes that *Corallocarpus epigaeus* rhizome possesses significant decrease the MDA level against freund’s adjuvant induced arthritis (Subashini Uthrapathy *et al.*, 2011).

Cells have different antioxidant systems to defend themselves against free radical attacks. SOD, GPx, and CAT have been determined to be the most important members of enzymatic antioxidant defenses against ROS and they are closely related to the modulation of cytotoxicity (de Zwart *et al.*, 1999). 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 H$_2$O$_2$ to harmless byproducts. 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).

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. Glutathione-dependent system belongs to the key components of antioxidant defense system that take part in neutralization of lipoperoxides and in regulation of mitochondrial anti-apoptotic proteins (Sorokina et al., 2010). GST has been used as an important parameter for diagnosis and monitoring of lung malignancy, and it protects cells from mutagens and carcinogens as a free radical scavenger along with glutathione (Gupta et al., 2000). GR is an important enzyme for maintaining the intracellular concentration of reduced glutathione. In this study, B(a)P-treated animals showed a significant decrease in the activities of enzymic antioxidant (SOD, CAT and GPx) and non-enzymic antioxidants (GSH, vitamins C and E) depicts the utilization of these molecules against lipid hydroperoxides and in turn shift redox balance to oxidative stress (Anandakumar et al., 2008).

However, treatment with Corallocarpus epigaeus rhizome caused a significant increase in the levels of these antioxidant enzymes which suggest that free radical scavenging ability of Corallocarpus epigaeus rhizome (superoxide, hydroxyl, and alkoxyl radicals) and its potential cytoprotective function against B(a)P-induced lung carcinogenesis. Results of our study are in support with documented report, which denotes that Corallocarpus epigaeus rhizome possesses significant antioxidant action against freund’s adjuvant induced arthritis (Subashini Uthrapathy et al., 2011). These results are also in agreement with those reported by Bindu Sharma et al (2014)
who observed a significant induction of antioxidant defenses in *Terminalia arjuna* stem bark treated rats exposed to Benzo(a)pyrene.

**5.4 Effect of Corallocarpus epigaeus rhizome on tumor markers in experimental lung carcinogenesis**

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 (CEA), Pancreatic oncofetal antigen, fetal sulfoglycoprotein. 2. Tumor associated antigens /Cancer Antigens. Hormones e.g., Beta human chorionic gonadotropin, calcitonin, placental lactogen etc. 3. Hormone receptors e.g., estrogen and progesterone receptors 4. Enzymes and Isoenzymes e.g., prostate specific antigen (PSA), prostatic acid phosphatase (PAP), Adenosine deaminase(ADA), Aryl hydrocarbon hydroxylase (AHH), 5’-Nucleotidase (5’-ND), neuron specific enolase (NSE), glycosyl transferases, placental alkaline phosphatase (PALP), terminal deoxy nucleotidyl transferase (TDT), lysozyme, alpha amylase 5. 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).

The activities of marker enzymes were found to be elevated in tissues of lung carcinoma bearing animals, which could be due to the destruction of the neoplastic tissue. The abnormal variations in the marker enzymes reflect the overall change in metabolism that occurs during malignancy (Stefanini, 1985). The marker enzymes such as Adenosine deaminase (ADA), Aryl hydrocarbon hydroxylase (AHH) 5’-Nucleotidase (5’-ND), Lactate dehydrogenase (LDH) are specific indicators of lung damage (Durak et al., 1993). The increase in the activities of these enzymes may be due to the increased tumour incidence.

The enzyme system specialized for hydroxylation of polycyclic hydrocarbon has been termed as aryl hydrocarbon hydroxylase (AHH) (Heidelberger, 1975). AHH is a promising marker of lung cancer especially for lung squamous carcinoma and it is used in clinical diagnosis, monitoring and prognosis estimation in patients with lung cancer (Chen and Liu, 2000). AHH, a drug metabolizing enzyme, is useful in determining the individual differences in genetic susceptibility to lung carcinogenesis. AHH is reported to be responsible for the activation of carcinogens of benzo(a) pyrene and other aromatic hydrocarbons (Brown et al., 1978; Yamazoe et al., 1984)

AHH is responsible for the initial oxidative step in the metabolism of a variety of polycyclic hydrocarbons. These compounds are potent carcinogens, however, the precise relationship between AHH activity, inducibility of the
DISCUSSION

enzyme in target tissues and susceptibility to chemical carcinogenesis by polycyclic hydrocarbons remains controversial. Benzo[a]pyrene, the most commonly used substrate in AHH studies, is metabolized to highly reactive epoxides (Bast et al., 1976; Kuroki et al., 1987; Finnen et al., 1983) which bind covalently to cellular macromolecules and initiate toxic and possibly carcinogenic responses. It is the balance of this activation and protective detoxification measures such as epoxide hydrolase and glutathione conjugation that determines local toxicity and carcinogenicity. Corallocarpus epigaeus rhizome treatment has strongly shown to inhibit AHH activity.

Increased ADA activity may be a compensatory mechanism against toxic accumulation of its substrates due to accelerated purine and pyrimidine metabolism in the cancerous tissues and cells (Daoud et al., 1978) It has been reported that the patients with lung cancer were shown to have elevated serum ADA levels (Nishihara et al., 1970) In the present study increased ADA activity was observed in lung cancer bearing animals. Upon Corallocarpus epigaeus rhizome treatment, the activity of this enzyme was brought back to near normalcy highlighting the antiproliferative/antitumour property of Corallocarpus epigaeus rhizome.

Increased activity of 5’-ND seems to have originated from the proliferating tumor cells, (Dao et al., 1980) a fast moving 5-nucleotidephosphodiesterase is found to be elevated in metastases to liver from tumor of the lung and breast (Vanisree et al., 1998) In the present study, the elevated activity of 5’-ND was observed in cancer bearing animals and upon administration of Corallocarpus epigaeus rhizome to lung
cancer bearing animals the activity of 5’-ND was brought down to near normal values indicating its antitumour and/or antiproliferative effect on lung cancer.

LDH is a tetrameric enzyme and is recognized as a potential tumor marker in assessing the progression of the proliferating malignant cells. It is a fairly sensitive marker for solid neoplasma and its activities has been found to be raised in tumor bearing animals (Koss and Greengard, 1982). In the present study, significant elevation in all the above tissue marker enzymes were observed in benzo(a)pyrene-administered animals. *Corallocarpus epigaeus* rhizome treatment brought down the levels of these marker enzymes close to normal suggesting its anti-cancer potential. The decrease in the activities of above mentioned marker enzymes on treatment with *Corallocarpus epigaeus* rhizome suggests that terpenoids offers some protection against abnormal cell growth by changing the permeability or affecting cellular growth. This may be due to the antineoplastic property of terpenoids.

Carcinogenic antigen (CA) is a good monitoring marker for the conventional anticancer therapy, since some tumors produce much higher concentration of CA than normal tissues (Thompson *et al.*, 1991). In our present study, we observed reduction in the levels of CA in *Corallocarpus epigaeus* rhizome treated animals presumably due to decrease in the production rates of tumors that reveal the anti-tumor effect of *Corallocarpus epigaeus* rhizome. The reduction in the level of CA was more profound in group III *Corallocarpus epigaeus* rhizome treated animals, this shows that *Corallocarpus*
DISCUSSION

Corallocarpus epigaeus rhizome is a more potent anticancer activity when used as a chemopreventive agent.

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 (Kuroda and Hara, 1999). Present findings are similar to the Pakkir et al., (2011) study. In ethanolic
extract of *Corallocarpus epigaeus* rhizome (500 mg/kg) treated animals, the nucleic acid levels were decreased due to its inhibition of mutagenesis process.

Macrosopic appearance of the lungs of control group 1 animals shows normal morphology. B(a)P alone administered group 2 animals showing enlargement and several number of well-developed grayish-white foci or nodules of lung tumors on the peripheral surface of the lungs. Lung cell shows normal morphology in CERE alone treated group 4 animals. Most of the foci and nodules disappeared in the lung from B(a)P and CERE treated group (group 3) of animal showing the effect of chemoprevention.

Histological examination of the lungs showed normal appearance of alveolus with normal intact architecture, bronchiole lined by single-layered uniform epithelial cells with basement membrane in both normal control group and CERE alone supplemented animal. However, cellular damage with malignancy was obvious in the B(a)P treated lungs. The lungs showed alveolar damage, the histological appearance of lung carcinoma is also extremely variegated and severe hyperplasia in bronchiolar and alveolar region. Many different histologic patterns may be seen from hyperchromatic and irregular nuclei in the cells of alveolar wall. In contrast, B(a)P with CERE co-treatment showed near normal without disruption of the lungs architecture.

In conclusion, restored the altered levels of serum Adenosine deaminase (ADA), Aryl hydrocarbon hydroxylase (AHH), Cancer antigen (CA), 5’-Nucleotidase (5’-ND), Lactate dehydrogenase (LDH), Lungs DNA and RNA on
treatment with B(a)P and CERE. The macroscopic and histopathological studies further supported the anticancer activity of CERE. The protective properties of the ethanolic extract *Corallocarpus epigaeus* rhizome 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 *Corallocarpus epigaeus* rhizome. The tissue-weight/ body-weight and morphological studies also supported the anticancer properties of CERE.

4.5 Effect of *Corallocarpus epigaeus* rhizome on liver markers as Protein, AST, ALT and ALP in control and experimental animal.

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
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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 Corallocarpus epigaeus rhizome extract (CERE) on hepatospecific enzymes such as transaminases AST, ALT, ALP and protein content in B(a)P induced lung carcinoma rats.

The rise in their activities is shown to be in good correlation with the number of transformed cells in cancer conditions (Kumar and Rao, 2012). AST and ALT activities in blood serum are generally accepted as an index of lung damage and this tendency is also known to be distinct in rodents (Bedi et al., 2008). There was a good correlation between the activities of ALT and AST with tumor volume
during therapy. The stable pattern of these enzymes was noticed inpatients with lung carcinoma malignancy after chemotherapy, while patients fail to respond for drug treatment showed progressive increase in the level of these enzymes. Kuznietsova et al. (2015) observed similar results in dimethyl hydrazine induced colon cancer in 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 carcinogenesis. 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 activities in serum of cancer patients. The liver plays a vital role in protein metabolism, including deamination and transamination of amino acids and plasma protein synthesis. B(a)P induced liver injury can affect the concentrations of plasma protein. In concurrence with the above findings an elevated serum aminotransferase activity and decreased protein content was observed in animals bearing cancer with simultaneous restored on treatment with CERE.

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 cancer, 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 CERE in diet.

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

4.6 Effect of *Corallocarpus epigaeus* rhizome on liver antioxidants in
experimental lung carcinogenesis

Benzo[a]pyrene has been identified as a major risk factor for liver and kidney
related cancer. Benzo[a]pyrene when administrated it gets distributed and deposited
in organs such as the lungs, liver, kidney and heart. The reactive metabolite 7,8-diol-
9,10- epoxide-benzo[a]pyrene has been formed from the metabolic conversion of
benzo[a]pyrene by cytochrome P-450 (Gelboin, 1980). As this reactive metabolite
reacts very rapidly with O2 and forms more reactive free radicals (Selvendirsan
*et al.*, 2004). These free radicals cause oxidative damage in vital organs like liver and
kidney. They have the capacity to initiate the peroxidation of membrane
polyunsaturated fatty acids, necrosis of cell, reduced glutathione, damage to
membrane and loss of antioxidant enzyme activity.
DISCUSSION

In this experimental study we investigated the change in the level of biomarkers. These markers are the important indices for the diagnosis of dysfunction in hepatic and kidney (Vijayakumar et al., 1997). From this it is revealed that cells are damaged, cellular leakage and loss of functional integrity of cell membrane in both tested organs. Benzo[a]pyrene has been found to elevate lipid peroxidation in tissues. Lipid peroxidation is an important event to cell death and has been reported to cause serve impairment of membrane functions through increased membrane permeability and membrane damage, cytotoxicity and eventually cell death. The free radicals reacts with lipids and generates LPO which is involved in the formation of tumours (Cigremis et al., 2004). Group III (Corallocarpus epigaeus rhizome treated) animals there was a significantly decreased in the levels of MDA when compared with tumor bearing animals. However, Corallocarpus epigaeus rhizome alone treated animals did not show any significant changes when compared with control animals.

The two important enzymic antioxidants are SOD and CAT that reacts against the free radicals such as superoxide (O₂) and hydroxyl ions (OH⁻). SOD is an enzyme containing copper (Cu²⁺) and zinc (Zn⁺) as cofactors that converts superoxide radical into hydrogen peroxide (Vamajuchi et al., 1994, Anbarasi et al., 2006) and molecular oxygen and thus protects the cells from oxidative damage caused by H₂O₂ and OH⁻(Bolann and Ulvik 1991; Fridovich, 1986). In this study, SOD activity was significantly decreased in both liver of mice who exposed to benzo[a]pyrene.
CAT is a hemoprotein, localized in peroxisomes or microperoxisomes. This also catalyses the decomposition of $\text{H}_2\text{O}_2$ to $\text{H}_2\text{O}$ and $\text{O}_2$, thus protecting the cells from oxidative damage caused by $\text{H}_2\text{O}_2$. In the present experiment we also observed a similar decrease in CAT activities of the liver tissues on benzo[a]pyrene exposure. Thus, both SOD and CAT activities are decreased with exposure to benzo[a]pyrene. (Anbarasi et al., 2006, Ramesh et al., 2008; Ramesh et al., 2007).

GPx is an enzyme containing four selenium has a cofactors that catalyses the breakdown of $\text{H}_2\text{O}_2$ and organic hydroperoxides into $\text{H}_2\text{O}$ and $\text{O}_2$. Thus it plays a significant role in protecting cells against the free radicals and carcinogenic chemicals by scavenging the free radicals. In this study, GPx activity was significantly decreased in the liver of mice exposed to benzo[a]pyrene. (Ramesh et al., 2010).

Glutathione a non enzymic antioxidant is a major low molecular weight non-protein thiol in living organisms. It plays a role in body’s antioxidant defence against free radicals, peroxides and other toxic compounds (Sies, 1992). In our study the glutathione level was significantly lower in the liver of experimental mice exposed to benzo[a]pyrene when compared to control group. This, glutathione depletion increases the sensitivity of cells and leads to tissue disorder and injury, thus causing tumour in liver (Limon Pacheco et al., 2007). This may be the reason for the decrease in vitamin C and vitamin E in tumor bearing mice. But these conditions were found to be reversed in CERE administrated animals. There is no significant change in mice treated with CERE alone when compared to control animals.
DISCUSSION

Significant reversed in the activities of antioxidant enzymes (SOD, CAT, GPx, GSH, vitamin E and vitamin C) to near normal values in *Corallocarpus epigaeus* rhizome treated animals. There is no significant changes were observed on *Corallocarpus epigaeus* rhizome alone treated animals when compared with control animals.

In conclusion, the obtained results showed that benzo[a]pyrene induced oxidative damage on the liver and kidney by enhancing lipid peroxidation and diminishing the enzymic and non-enzymic antioxidant status. Thus, the results of our investigation suggest that liver is more prone to oxidative stress against benzo[a]pyrene induced toxicity. Supplementation of *Corallocarpus epigaeus* rhizome to benzo[a]pyrene induced rats nullified the oxidative stress.

4.7 Effect of *Corallocarpus epigaeus* rhizome on Glycoprotein’s and Membrane-bound enzymes in experimental lung carcinogenesis.

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; Sivagananam 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
DISCUSSION

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; Sivagnanam 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.

The activities of the ATPases in lung tissue have been found to be lowered in B(a)P treated animals. These findings were similar to those reported in various cancers (Randak et al., 1999). The decreased activities of Na+/K+, Mg2+ and Ca2+-ATPases in B(a)P treated animals may be due to increased MDA which occur in cancer conditions. We have also observed increased levels of MDA in cancer animals. Peroxidation of membrane lipids initiates the loss of membrane integrity and membrane bound enzyme activities, which in turn leads to a disruption in
Phytochemical screening and anticancer activity of Corallocarpus epigaeus rhizome in Benzo(a)pyrene induced lung cancer

**DISCUSSION**

Abnormal lipid peroxides affect membrane bound ATPases activities and their levels were decreased due to the excessive production of thio barbituric acid reactive substances (Rauchova *et al.*, 1995). Free intracellular Ca2+, acting as a second messenger, is crucial for a diverse range of biological functions, such as fertilization, neurotransmission, muscle contraction, and gene transcription (Berridge *et al.*, 2000). Intracellular Ca2+ signaling is also a key regulator of proliferation (Carafoli *et al.*, 2001) cell cycle progression (Lipskaia and Lompre, 2004), and apoptosis (Orrenius *et al.*, 2003). Modulation of capacitive Ca2+ entry, a mechanism whereby the influx of extracellular Ca2+ is coupled to the depletion of intracellular Ca2+ stores within the endoplasmic reticulum, is associated with the proliferative phenotype (Ichikawa *et al.*, 2000). Indeed, blockers of Ca2+ entry may be potential anti-proliferative agents (Nie *et al.*, 1997). Alterations in Ca2+ signaling may contribute to tumorigenesis and the mechanism of action of some anticancer drugs. In the present study, decrease in the activities of Na+/K+-ATPase Ca2+-ATPase were found in B(a)P treated animals. The restoration of activities of all the three ATPases to near normal values was observed in Corallocarpus epigaeus rhizome treated animals. This may be due to the enhancement in the status of GSH by Corallocarpus epigaeus rhizomes.

This study shows that CERE administration decrease the glycoprotein synthesis and increased membrane ATPase in tumor cells. This may be due to the inhibitory action of CERE on the initiation of B(a)P activation/detoxification process or alter cell membrane ATPase, glycoprotein synthesis and structure. Thus the
ethanolic extract of *Corallocarpus epigaeus* rhizome shows protective effect on carbohydrates moieties as glycoproteins and restored the membrane ATPAse against B(a)P induced lung carcinoma rats.

**5.8 Effect of *Corallocarpus epigaeus* on glucose metabolism in experimental lung carcinogenesis**

Impairment of energy metabolism in cancer cells has been a recurrent finding for many years. Studies on experimental cancers have shown that metabolic alterations occurring in the tumours are often accompanied by the changes in the activities of various enzymes, including the key enzymes of glucose metabolism (Senthilnathan *et al*., 2006; Sujatha and Sachdanandam, 2002). The cancer cells possess an abnormal pattern of energy metabolism, when compared with the normal cells.

Studies on experimental cancer animals 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).

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 cell 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 Corallocarpus epigaeus rhizome extract has been studied on glucose-metabolizing enzymes in B(a)P induced lung carcinoma in rats.

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
Phytochemical screening and anticancer activity of *Corallocarpus epigaeus* rhizome in Benzo(a)pyrene induced lung cancer

DISCUSSION

of hexokinase reported in Novikoff and Zajdela 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 lung of B(a)P induced lung carcinogenesis rate may be due to its level in malignant tissues (Langeswaran *et al*., 2012). Phosphoglucoisomerase serves as a good index of tumour growth and is significantly elevated in cancerous conditions (Giacchi, *et al*., 1987). It is reported that phosphoglucoisomerase is an indicator of metastatic growth and was elevated in patients with neoplasms, especially after metastasis (Blog, 2003).

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, 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 B(a)P induced rats was in accordance
with the earlier report (Anandakumar et al., 2009). Glucose-6-phosphatase is reduced in residual lung tissue of group III B(a)P -induced rats was in accordance with the earlier report (Anandakumar et al., 2009). Glucose-6-phosphatase is also reduced in liver tissue of B(a)P Induced Lung Carcinoma (Anandakumar et al., 2009). 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. The regulated the activity of glycolytic and gluconeogenic enzymes’ levels during Corallocarpus epigaeus rhizome extract treatment correspond to a return of the tumour towards near normal state. This could be attributed by the potent anticancer activity of Corallocarpus epigaeus rhizome extract.

A sharp drop in the activities of hexokinase and phosphoglucoisimerase and a significant increase in the activities of lungs glucose-6-phosphatase and fructose-1, 6-bisphosphatase observed on oral administration of the extract of Corallocarpus epigaeus rhizome to B(a)P 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 IV 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 Corallocarpus epigaeus rhizome extract has modulatory activity on the carbohydrate metabolism in B(a)P induced cancer 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 Corallocarpus epigaeus rhizome extract may be attributed to the presence of active compounds such as polyphenols and flavonoids. Earlier studies have also shown that Semecarpus anacardium, Hygrophila Vitiginea and Terminalia arjuna, which are rich in flavonoids and polyphenols modulate the glucose-metabolizing enzymes in cancer 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 B(a)P 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, LDH and G-6-PD in lung tumour bearing rats were reverted significantly to near normal with the ethanolic extract of Corallocarpus epigaeus rhizome 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. This finding suggest the CERE has a definite modulating role on the key enzymes of glucose metabolism in lung carcinoma and this may be through its potency in the normalization of abnormal cells behaviour.

4.9 Effect of CERE on Hematological profile in experimental lung carcinogenesis

Hematological parameters, such as hematocrit, hemoglobin, and numbers of erythrocytes and white blood cells, can be used as indicators of toxicity and have a
broad potential application in environmental and occupational monitoring (Sancho et al., 2000; Barcellos et al., 2003). Besides exposure to carcinogens, the process of carcinogenesis itself may lead to several pathologies, including hematological complications (Groopman et al., 1999). In our present study, lung cancer bearing animals showed reduction in hemoglobin percentage and RBC count, which is an indication of anemia. The complications of anemia result from hypoxia of virtually all organs and tumor hypoxia is frequently considered as a potential therapeutic problem (Brown, 1999). Continued hypoxia may result in cellular changes leading to a more aggressive tumor phenotype, as reflected by accelerated malignant progression, increased potential for local invasiveness, and tumor cell spreading (Hockel and Vaupel, 2001). Restoration of Hb contents and RBC count in mice that received B(a)P and CERE indicate that CERE might have reduced the hypoxic condition during lung carcinogenesis, hence the reduction in the extent of tumorigenesis. Increase in WBC count and alterations in differential count have been suggested to be one of the hallmarks of carcinogenesis (Gangar et al., 2010). In our present study, B(a)P-induced lung cancer animals showed altered WBC count, ESR, PCV, MCV and MCHC which was in line with the previous findings. *Corallocarpus epigaeus* rhizome treatment significantly restored WBC, ESR, PCV, MCV and MCHC, revealing its beneficial role against lung carcinogenesis. Our result agrees with the earlier report (Anandakumar et al., 2012).