CHAPTER 6
DISCUSSION

The limited treatment options and poor treatment success make hepatocellular carcinoma (HCC) one of the leading causes of death in developing countries. Hepatocellular carcinoma (HCC) is one of the most common cancers with poor prognosis. However, various exogenous and endogenous factors are known to affect the normal pattern of cell growth, by which cell becomes a cancerous. N-nitrosodiethylamine (NDEA) is the most important carcinogen among nitrosamines and primarily induces tumour of liver. The liver is one of the largest organs in the body. It plays an important role as a neutralizer of exo and endotoxins. Recent studies during the last few years have shown new hopes in the treatment of this dreadful diseases by measures aimed at the close relationship between free radical production and hepatic cancer (Prince et al., 2004). Among other functions it serves as an interface that processes absorbed nutrients into chemicals that are nontoxic for the organism and can safely be utilized by other tissues and organs. Indeed, curative treatment such as tumour resection and orthotopic liver transplantation are not feasible in advanced stages of Hepatocellular carcinoma (HCC). Therefore, searching for effective chemotherapeutic agents is important to improve the survival rate of patients with advanced or recurrent HCC.

In addition, HCC is well known for multi-drug resistance and its poor response to current chemotherapeutic agents (Geng et al., 2003). On the other hand, Ayurveda, an indigenous system of medicine has long been used for treating liver disorders based on traditional knowledge (De et al., 1993). However, traditional medicine, especially the herbal medicine plays a vital role in the management of various liver disorders. The relative importance of Fumaria indica (FIE) and Tephrosia purpurea (TPE) is considered useful in
the treatment of various ailment as well as liver disorders and tumour. Hence, it is usually intensive to chemotherapeutic drugs currently used in clinical setting, and there is an urgent need for the evaluation of new active drugs against HCC.

Several studies have shown that certain naturally occurring plant phenols possess substantial antimutagenic and anticarcinogenic effects against variety of chemicals (Huang et al, 1983). In recent years, attention has been focused on the identification of naturally occurring plant active compounds as possible chemopreventive agents (Wattenberg, 1997). Epidemiological studies have shown that fruits, vegetables, beverages, spices, tea and medicinal herbs rich in antioxidants and other micronutrients protect against diverse forms of chemically-induced hepatic damage, carcinogenesis, mutagenesis, DNA-damage and lipid peroxidation (Wattenberg, 1990).

A number of drugs employed in traditional system of medicine for liver affections. In recent years, there has been a shift towards the therapeutic evaluation of herbal products in liver diseases by carefully confluences the strength of traditional knowledge with that of the modern concept of evidence based medicinal evaluation using scientific tools (Oliveira et al., 2005). Management of liver disorders by a simple and precise herbal drug is still an intriguing problem. With the increase of our understanding of the pathologic processes in the liver, and as our knowledge about disease-induced changes in liver transcriptome and proteome advances, new drugs and new treatment modalities for acute and chronic liver disease will likely emerge in the near future.

Natural products have long been used to prevent and treat many diseases, including cancer and thus they are good candidates for the development of anti-cancer drugs. The large population use ayurvedic
medicine worldwide. Different in vivo and in vitro screening models are available for anticancer activity. Hence, an attempt has been made in scientifically validated experimental animal models to investigate a novel herbal drug based anticancer agent from plants viz. Fumaria indica and Tephrosia purpurea against hepatocellular carcinoma which is induced by N-nitrosodiethylamine (NDEA) and CCl₄ in experimental rats.

Despite the wide use of Fumaria indica and Tephrosia purpurea in folk medicine, no study has been published in the scientific literature about its toxicological profile. Therefore, efforts are now being made in our laboratories to define its preclinical activity and safety profile necessary for further development of the extract. The present investigation summarizes the acute and subacute oral toxicity of the 50% aqueous ethanolic extract of Fumaria indica and Tephrosia purpurea in mice and rats at different dose levels.

In the oral acute toxicity study no mortality was observed during initial 4h and 14 days after treatment with Fumaria indica and Tephrosia purpurea extract in either sex as well as did not show any kind of abnormal behaviour (Table 5.4 and 5.20). Thus the acute gavage dosing to mice of both sexes was 2000 mg/kg due to absence of toxic effect in the acute toxicity test for both plants. Therefore, one-tenth (1/10th) and one-fifth (1/5th) of the maximum tolerated dose of the extract (2000 mg/kg) tested for acute toxicity was selected for further subacute toxicity study and evaluation of chemopreventive effect of the titled plant.

The 50% aqueous ethanolic extract of Fumaria indica and Tephrosia purpurea at the doses used did not produce any marked changes in experimental groups of rats, as evidenced by the absence of toxic symptoms. Whereas the doses selected in sub acute studies have shown that, during the experimental period of 28 days, the increase in body weight, food
consumption and water intake in all test groups are not significantly different when compare with control group. The body weight, daily food consumption and water intake were not altered by the treatment with the test drug at various dose levels. Analysis of blood parameters is relevant to risk evaluation as the changes in the haematological system have a higher predictive value for human toxicity, when the data are translated from animal studies (Olson et al., 2000).

At the end of the study period, no statically significant differences are seen in the mean RBC, Hb, Ht, MCV MCH, MCHC, PLT, WBC and differential cell counts of all test groups when compared to control group. The normal values of the renal biochemical parameters such as blood urea nitrogen and creatinine suggest that the extract does not produce any sort of disturbance in the renal function, as has been found in case of various plant extracts and hence is safe on its chronic use in various diseases.

The subacute administration of FIE shows no change in the serum biochemical markers viz., glucose level, TC, TB, TP, ALB, AST, ALT, ALP level in all test groups when compared to control group. Further subacute administration of TPE did not cause any significant change in serum glucose level TC, TB, TP and ALB. However, liver enzymes (AST, ALT and ALP) were decreased at higher dose but the magnitude is too small to have biological relevance. The increase levels of AST, ALT and ALP in the blood are associated with structural and functional dysfunction of hepatocellular membrane damage of hepatic cells (Deshpande et al., 2003). These observations of decrease in the levels of liver enzymes might be due to the presence of hepatoprotective agents in the extract. These results are in support of the previous studies (Trivedi, 2007) and decrease of hepatic enzymes levels need to be further investigated. Therefore our ongoing research work is directed towards identifying an herbal
Moreover, histopathological observations correlate the other results showing the normal cellular architectures in the TPE treated (200 and 400 mg/kg) group of animals, without any necrosis or fatty infiltration, which can substantiate the safety profile of the extract at this dose level. Present observations indicate for the first time that 50% hydroalcoholic extract of *Tephrosia purpurea* have a broad safety margin in experimental animals commonly used in *in-vivo* experimental and preclinical pharmacological studies.

The weight of the important organs also showed that, there were no statistically significant differences observed in FIE and TPE tests groups when compared to control group. The outcome of urinalysis does not show any abnormalities in excretion pattern when compare with normal control group rats.

The histopathological studies with liver and kidney did not reveal any pathological changes and they were found normal. Several researchers have reported that plant drugs are safe and effective in treatment of incurable diseases (Rao et al., 2003: Rao et al., 2005). Moreover, the selected plant *Fumaria indica* has been reported as successful and safe hepatoprotective activity (Rao and Mishra., 1998). Therefore, the 50% ethanolic extracts of the selected plants were further assessed to understand the safety and efficacy in the treatment of Hepatocellular carcinoma (HCC).

Natural products have long been used to prevent and treat many diseases, including cancer and thus they are good candidates for the development of anti-cancer drugs. The large population use Ayurvedic medicine worldwide. Different *in vivo* and *in vitro* screening models are available for anticancer activity. The present investigation showed that oral administration of FIE and TPE counteracts lipid peroxidation (LPO) and
Discussion


prevents the development of hepatocellular carcinoma (HCC) that is usually induced by N-nitrosodiethylamine (NDEA) and CCl₄ in experimental rats. On metabolic biotransformation of NDEA produces promutagenic products, O⁶-ethyldeoxyguanosine and O⁴ and O⁶-ethyldeoxythymidine in liver which are responsible for their carcinogenic effects (Verna et al., 1996). It is well established that CCl₄ induces hepatotoxicity by metabolic activation and therefore selectively causes toxicity in liver cells maintaining a semi-normal metabolic function. CCl₄ is bio-transformed by cytochrome P₄₅₀ (CYP) enzyme system in the endoplasmic reticulum to produce trichloromethyl free radicals. Trichloromethyl free radicals (CCl₃⁻) then combine with cellular lipids and proteins in the presence of oxygen to form trichloromethyl peroxy radical, which further attack lipids on the membrane of endoplasmic reticulum faster than trichloromethyl free radical. Thus, trichloromethyl peroxy free radical leads to elicitation of lipid peroxidation (LPO) and destruction of Ca²⁺ homeostasis, resulting in cell death (Rao et al., 2006).

In the present study, the effect of 50% ethanolic extract of *Fumaria indica* and *Tephrosia purpurea* on body weight and liver weight changes in HCC were carried and showed a significant result in treatment. Malignancies may engender complex metabolic disturbances in both human and experimental animals resulting in rapid loss of body weight and tissue wasting. There is an appreciable loss in body weight in hepatoma bearing rat as compared to control rats and the reduction in body weight correlates well with the decreased food intake. Tessitore et al. (1987) and Sreepriya and Bali, (2005) have also shown that, tumour growth elicited marked loss of body weight indicate declining in the hepatic function on exposure to hepatocarcinogen (Pain et al., 1984). According to Sherman et al., (1950) tumour may act as nitrogen trap and most efficient in utilizing amino acids for gluconeogenesis. Tumour cell produces two moles of ATP and lactic acid.
from one mole of glucose for its use. This lactic acid is converted to glucose by the host liver, which the tumour uses and consume six moles of ATP. This result in a loss of eight ATP molecules/mole of glucose to the normal cells. This mole of glucose used by the tumour is a loss to host, which might have been used it to generate thirty-six moles of ATP via Kreb cycle (Landel et al., 1985). The host responds to increased tumour load by decreased protein synthesis. The liver subsequently uses amino acids resulting from breakdown of proteins, further increasing the host metabolic burden (Waterhouse et al., 1979).

The results of the present study seem to provide support for the chemopreventive effects of FIE and TPE against NDEA-induced hepatocarcinogenesis in rats. There is an appreciable reduction in body weight and increase in liver weight observed in HCC bearing group II rats as compared to control group I rats. Decreased appetite and food intake contribute to the weight loss which could be an indirect indication of the declining hepatic function, an increase in the liver weight of the animals, which could be attributed to the formation of nodules and tumors in the liver following carcinogen exposure. Sreepriya and Bali, (2005) have also reported marked loss of body weight and increase in liver weights. The steadily increase in body weight and decrease in liver weight after FIE and TPE treatment (groups III and IV) indicate that, increased appetite and reduced tumor incidence shows its anticancer effectiveness (Table 5.12 and 5.28). Administration of NDEA is reported to produce neoplastic nodules in experimental animals (Sreepriya and Bali, 2005). Group II rats given NDEA showed an incidence of 100% in the presence of nodules. Administration of FIE and TPE was able to reduce the percentage incidence of nodules as compared to group II. The effect of FIE and TPE to prevent the multiplicity of neoplastic nodules gives substantial support to the chemopreventive
effects of plant extract.

Further we have subjected these plants to assess the anticarcinogenic effects on tissue defence system on N-nitrosodiethylamine (NDEA) induced hepatocellular carcinoma and CCl₄, tumour promoter in rats. It is well established from the earlier studies that Diethylnitrosamine (DEN) is a well-known liver carcinogen in rats, forming DNA adducts in the liver and inducing hepatocellular carcinomas without cirrhosis through the development of putative preneoplastic enzyme-altered focal lesions (Mitali Basu et al., 2004). An NDEA model was used because nitrite and nitrosamine synthesis is increased in viral hepatitis (Liu et al., 1992). N-Nitrosodiethylamine is widely accepted for induction of preneoplastic lesions and hepatic tumors in rats and it is initiated by perturbations of nuclear enzymes involved in DNA repair or replication (Khan et al., 2011). Investigations have provided evidence that NDEA causes a wide range of tumors in all animal species and such compounds are hazardous to human health (Ramakrishnan et al., 2006). NDEA-induced lesions as well as tumors in rodents show marked biochemical, histological and molecular similarity to the progression of HCC in humans (Feo et al., 2000). It is also reported that the generation of reactive oxygen species (ROS) is apparent during the metabolic biotransformation of NDEA resulting in oxidative stress. Oxidative stress leads to carcinogenesis by several mechanisms including DNA, lipid and protein damage, change in intracellular signaling pathways and even changes in gene expression. Together, these oxidative modifications promote abnormal cell growth and carcinogenesis (Klaunig and Kamendulis, 2004). A single injection of the hepatocarcinogen N-nitrosodiethylamine (NDEA) (200 mg/kg) induces phenotypically altered hepatic foci (PAHF) during the early stages of carcinogenesis without any noticeable involvement of other cell types. Enzymes of the mixed-function
cytochrome P-450 dependent monooxidase systems catalyse N-nitrosodiethylamine (NDEA) metabolism and its metabolic activation is responsible for the onset of the toxic effects (Zimmerman, 1993). Intermediate reactive compounds originating from DEN bioactivation have little affinity for the catalytic sites of conjugating enzymes and therefore, instead of being excreted through the urinary route they may form covalent bond with important cell constituents, thus inducing the onset of mutations, cancer, and necrosis (Schmitt et al., 1993; Swenberg et al., 1991).

In the present study the chemotherapeutic effect of FIE and TPE on NDEA and CCl₄ induced HCC were studied. Biochemical tumour marker enzymes are used to screen particularly tumors conditions for differential diagnosis, prognosis, monitoring the progress and for assessing the response to therapy (Mc-Intyre and Rosalki, 1992). These enzymes are more unique and changes in their activities reflect the effect of proliferation of cells with growth potential and its metabolic turnover. The rise in their activities is shown to be in good correlation with the number of transformed cells in cancer conditions (Kandem et al., 1982). The role of transmination in biological system is well known. These enzymes serve as an index of liver cell injury and can be used to identify or conforms liver diseases. The stable clinical and enzymatic pattern of these enzymes was noticed in patients with hepatic malignancy after chemotherapy, while patients failing to respond to drug showed progressive increase in the level of these enzymes. Transminases are distributed widely in animal tissue. Both AST and ALT normally are present in human plasma, bile, cerebrospinal fluid (CSF) and saliva, none is found in urine unless a kidney lesion is present. Transminase activities occur in various tissues, relative to those in serum. In case of infectious hepatitis and other inflammatory conditions affecting the liver, ALT is characteristically as high as or higher than AST. The relatively
similar elevation of AST and ALT in hepatitis cases have been attributed to the release of only the cytoplasmic isoenzyme of AST into the circulation from reversibly damaged parenchymal cells.

Hepatic damage caused by NDEA and CCl₄ generally reflects instability of liver cell metabolism which leads to distinctive changes in the serum enzyme activities (Wolf et al., 1980). Serum transaminases (AST and ALT), ALP, TBL and γ-GT are representative of liver function and their increased levels are sensitive indicators of hepatic injury (Singh et al., 2009). Elevated activities of serum AST and ALT in NDEA and CCl₄ treated rats may be due to NDEA induced hepatic damage and subsequent leakage of these enzymes from the neoplastic cell into circulation (Dakshayani et al., 2005) or may be due to the release of enzymes from normal tissue by tumour or may be due to possible effect of tumour on remote tissue leading to the loss of its enzyme and release into the blood (Schwartz and Bodansky, 1965). In accordance with the above report we also observed an increase in liver marker enzyme as AST and ALT in NDEA and CCl₄ treated group II when compared to respective control group I rats. On the other hand in the present investigation, treatment with FIE and TPE significantly and dose dependently lowered the enhanced level of activities of these enzymes or recuped back to near normal in HCC bearing animals. It is suggested that FIE and TPE aids in parenchymal cell regeneration in liver and thereby protects membrane integrity by decreased enzyme leakage against carcinogenic effect of NDEA (Table 5.14 and 5.30).

Similarly, increase in ALP reflects pathological alteration in biliary flow and discharge of TBL reflects a non-specific alteration in the plasma membrane integrity and/or permeability (Singh et al., 2009). Phosphatase is actually a group of enzymes that hydrolyze monophosphate ester at an alkaline pH. Alkaline phosphatase (ALP) has been identified in most body
tissues and is generally localized in the membranes of cells. ALP is highest in the liver, bone, intestine, kidney, and placenta. Measurement of SALP is useful in differentiating hepatobiliary disease from osteogenic bone disease. Alkaline phosphatase, a membrane bound enzyme and its alteration is likely to affect the membrane permeability and produce derangement in the transport of metabolites. This enzyme activity is used as a specific tumor marker during diagnosis in the early detection of cancer (Kobayashi and Kawakubo, 1994). In the present investigation, the elevation in levels of ALP was observed in animal of hepatoma (NDEA and CCl₄) induced hepatocellular carcinoma in the serum. Rise in phosphatase activity in HCC bearing animals may be due to the disturbance in the secretory activity or in transport of metabolites or may be due to altered synthesis of certain enzymes as in other hepatotoxic conditions (Sharma et al., 1995). In liver, ALP activity is localized in the bile canalicular plasma membrane (Fredericks et al., 1990) and it may be shed into the surrounding milieu at increasing rate when cells replicate more rapidly (Stefanini, 1985). In rats carrying neoplasma, there is a rise in this enzyme activity in several tissues (Koss and Greengard, 1982). In the present study, 50% ethanolic extract of the plants tested showed significant decrease in the levels of ALP or in other words it shows the potentiating the level of ALP back to normal and also likely show the level of this enzymes near to the level when treated with the standard drug silymarin which indicates, the anticarcinogenic effects of plant in (NDEA and CCl₄) treated hepatoma rats.

Serum bilirubin (BR) which is a biomarker for liver damage, are intracellular enzymes present abundantly in the liver, under normal conditions, In the case of hepatocellular damage caused by xenobiotics, carcinogenic, these enzymes will leak out from the damaged hepatocytes, causing an increase in serum enzyme activities. The accumulation of
bilirubin in the serum is the result of decreased biliary excretion after the conjugation of bilirubin in the liver rather than the result of an increased bilirubin load caused by hemolysis. In hepatic tumours haemolysis plus deranged liver function leads to hyperbilirubinemia (Issabacher, 1991). In the present examination, in (NDEA and CCl₄) hepatoma bearing animals there was an elevation in levels of serum bilirubin (Joy et al., 1999) which may be due to the leakage of plasma membrane and loss of functional integrity of cell membranes in liver (Singh et a., 1999). In groups treated with 50% ethanolic extract of the plants (200 and 400 mg/kg) showed significant drop in the levels of these elevated levels in a dose dependent manner, as well as restoring serum marker enzymes back to normal suggested that FIE and TPE protects membrane integrity by decreased enzyme leakage against carcinogenic effect of NDEA and CCl₄.

γ-GT is an enzyme embedded in the hepatocyte plasma membrane, mainly in the canalicular domain and its liberation into serum indicates damage of the cells and thus injury to liver (Sivaramakrishnan et al., 2008). It is important to point out that serum γ-GT activity is considered to be one of the best indicators of liver damage (Jeena et al., 1999). It is considered as an onco fetal protein because it’s activity changes during development of cancer (Fugiwara et al., 1982). γ-GT gets bound predominantly to the outer surface of the cell membranes with a high rate of secretory and absorptive activity. It 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). γ-GT present in serum and in all cells except those in muscle appears to originate primarily from the hepatobiliary system, and γ-GT activity has been reported to be elevated in various cancers and experimentally –induced malignant lesions (Mock et al., 1987). An increase in γ-GT activity in serum of hepatoma bearing animals (NDEA and CCl₄)
induced carcinogenesis in this study suggests its potential role as an indicator of carcinogen exposure and also reflects the toxic effects of drug on microsomal structures in liver cells. High level of γ-GT is frequently an early event in experimental hepatocarcinogenesis and also in human carcinoma including primary hepatic carcinoma (Bannasch, 1986). Recouping of tumour marker enzyme (γ-GT) upon treatment with plants extract of 50% ethanol showed a significant dose dependent decrease in the levels of γ-GT in comparisons with hepatoma bearing animals and it also shows the result same as in comparisons with the standard drug silymarine suggest a combinatorial therapy gives protective mechanism against abnormal cell growth by changing the permeability of membrane or affecting cellular growth (Table 5.14 and 5.30).

α-feto protein (AFP) is widely used as tumor marker for diagnosis of HCC, which is a unique immunomoduatory glycoprotein (65 kDa) normally made by the immature liver cells in the fetus (Sell and Beckar, 1978). Its detection during monitoring of HCC treatment is well accepted in patients with increased AFP levels prior to therapy, and is recommended by the European Association for the Study of the Liver (EASL). It has long been recognized that exposure of rats to certain carcinogens like NDEA increases the circulating AFP levels. This corroborates the results showing significant rise in levels of AFP obtained in NDEA-induced rats (Sivaramakrishnan et al., 2008) and AFP levels were found to be significantly reduced in FIE and TPE treated (groups III and IV) rats. Carcinoembryonic antigen (CEA), a member of the immunoglobulin supergene family, is a 180–200 kDa heavily glycosylated protein. Frequently it is detected in a high concentration in the serum of individuals with malignancy in the liver (Macnab et al., 1978; Maeda et al., 1988). It functions as an adhesion molecule that can form both homotypic and heterotypic aggregates between cells. CEA is cleared from
the circulation by the liver with significant traces taken up by the spleen and lungs. In this study, an increase in serum CEA levels following NDEA treatment was presumably associated with production rates of tumor, its location, stage, size, differentiation and vascularity. The significant reductions in the levels of CEA in FIE and TPE treated groups III and IV rats were presumably due to decreased production rates of tumors.

Antioxidants are defined as “any substance that even when present at low concentrations compared with those of an oxidizable substrate significantly delays or prevents oxidation of that substrate”. Uncontrolled oxidation in aerobic organisms produces oxidative stress, cell damage and eventually cell death. Antioxidant defense systems protect the aerobic organism from the deleterious effects of reactive oxygen metabolites. Active oxygen species and free radicals are involved in a variety of pathological events. In addition to ROS, nitric oxide is also implicated in inflammation, cancer and other pathological conditions (Halliwell and Gutteridge, 1999). A potential mechanism of oxidative damage is the nitration of tyrosine residues of proteins, peroxidation of lipids, and degradation of DNA and oligonucleosomal fragments (Hemnani and Parihar, 1998). These free radical scavenging enzymes serve as a first line of defense against oxidative injury within the cell and are known as preventive antioxidants (Rao et al., 2005; Singh et al., 1999). They remove the reactants involved in initiation of free radical chain reaction (Buettner, 1993). Many plant secondary metabolites act as potent antioxidants have shown that free radical scavenger/antioxidant such as superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPX), glutathione S transferase (GST) etc. reduce and prevent the tissue damage induced by different hepatotoxins (Vimal and Devika, 1993).

The first line of defence against superoxide anion (O$_2^-$), H$_2$O$_2$ and
(OH\(^\cdot\)), are the major ROS, which induce, cell degeneration by increasing LPO of cell membrane lipids are SOD, CAT GPX are the family of metalloenzymes that convert \( \text{O}_2^- \) to \( \text{H}_2\text{O}_2 \). The toxic end products of peroxidation induce damage of the structural and functional integrity of cell membranes, break DNA strands and denature cellular proteins. The natural cellular antioxidant enzymes include SOD, is an important enzyme as because it is found virtually in all aerobic organisms. \( \text{O}_2^- \) is the only known substrate for SOD and it is considered to be a stress protein, which is synthesized in response to oxidative stress. It scavenges superoxide radicals by speeding up their dismutation. SOD can act as anticarcinogen and inhibitor of initiation and promotion/transformation stage in carcinogenesis.

In the present study, anomalously the levels of SOD were remarkably decrease in hepatoma bearing animals whereas these status were increased significantly after treatment with extracts in a dose dependent manner, which shows an antioxidant potency of plant extract (Table 5.16 and 5.32). Measurement of tissue malondialdehyde (MDA), one of the end products of lipid peroxidation, is commonly used as an indirect index for assessing the extent of lipid peroxidation in tissues.

Table 5.16 and 5.32 illustrated the lipid peroxidation and the enzymic and non enzymic antioxidant levels in liver of experimental animals. Lipid peroxidation (LPO) is a useful marker of oxidative stress because it is linked to increased production of ROS when CYP metabolizes NDEA (Vásquez-Garzón et al., 2009). The level of LPO increases with the administration of NDEA during hepatocarcinogenesis has been reported by Sivaramakrishnan et al., (2008). Interestingly, we also found that the level of LPO increases in group II rats when compared to the control group I rats, the rats treated with FIE and TPE exhibited significantly reduced LPO levels induced by NDEA, suggesting that counteracting this oxidative step is decisive in avoiding the
development of HCC. The products of LPO are considered mutagenic and carcinogenic as they cause damage to cellular macromolecules by generating ROS (Rao et al., 2006; Vásquez-Garzón et al., 2009). The present results are in conformity with previous studies on Tephrosia purpurea extracts which showed the inhibition of lipid peroxidation (Khatria et al., 2009).

Reduced glutathione (GSH) are the well-known non-enzymatic antioxidant defense system of cells. Glutathione is an important endogenous antioxidant system that is found in particularly high concentration in the liver and has key functions in protective processes by protection against free radicals, peroxides and other toxic components. In this study, we observed that FIE and TPE treatments affected all the components of the antioxidant defense system analyzed in the same ‘direction’, namely towards counteracting oxidative stress. GSH is an important factor in this system, which is required to maintain the normal reduced state of cells and to counteract the deleterious effects of oxidative stress (Ramakrishnan et al., 2006). GSH is said to be involved in many cellular processes including detoxification of endogenous and exogenous compounds (Vásquez-Garzón et al., 2009). NDEA, an electrophilic carcinogen may interact with the large nucleophilic pool of GSH thereby reducing the macromolecules and carcinogen interaction (Singh et al., 2009). In the present observation, GSH level has been decreased in hepatoma bearing animals group II. Earlier report (Thirunavukkarasu et al., 2002), the GSH, non-enzymatic antioxidant enzymes were decreased in hepatoma bearing animals. In FIE and TPE treated rats (200 and 400 mg/kg), a significantly high level of hepatic GSH was observed when compared to NDEA-induced rats consistent with the idea of reduced DNA–carcinogen interaction and thereby averting a favorable environment for carcinogenesis.

SOD acts as the first line of defense against superoxide radicals, which
dismutates two superoxide radicals to \( \text{H}_2\text{O}_2 \) and \( \text{O}_2 \). Catalase (CAT) is an enzymic antioxidant which catalyses the decomposition of hydrogen peroxide to water and oxygen, which removes \( \text{H}_2\text{O}_2 \). In addition, CAT and GPx act as supporting antioxidant enzymes by converting \( \text{H}_2\text{O}_2 \) to \( \text{H}_2\text{O} \), thereby providing protection against ROS (Vásquez-Garzón et al., 2009). Detoxification of the superoxide anion is not a terminating step in free radical scavenging, since the enzyme catalysed dismutation results in the production of endogenous peroxides, which accumulate in the mitochondria and cytosol. It was reported that catalase, prevent chromosomal aberration caused by hypoxanthine in Chinese hamster cells (Iwata et al., 1984). The reduction in activity of these enzymes may be caused by the increase in radical production during NDEA and \( \text{CCl}_4 \) metabolism. In the present investigation, an increase in MDA formation was presumably associated with increased ROS, consistent with the observation that these free radicals reduce the activity of hepatic SOD (Robak and Glyglewsi, 1988). Biochemical results of hepatic SOD and CAT, showed a decrease in activity of SOD and CAT in NDEA-induced group II rats compared to control group I and FIE and TPE treated group III and IV rats. The levels of SOD and CAT in treatment with plant extract in different dose concentration significantly increase which suggest the antioxidant capabilities to scavenge radical production produce by NDEA and \( \text{CCl}_4 \) metabolism (Table 5.16 and 5.32).

GPx is another endogenous antioxidant selenoprotein present in the cytosol and mitochondrial matrix that participates in the defense mechanism. Glutathione peroxidase (GPx) is considered to be a most important \( \text{H}_2\text{O}_2 \) removing enzymes in mammalian cells and is more important that catalase for removing \( \text{H}_2\text{O}_2 \) (Gaetani et al., 1989). The activity of GPx is dependent on the availability of GSH, which in turn, maintained by \textit{de novo} synthesis. It is generally activated before the initiation of chronic oxidative stress and...
catalyzes the reduction of lipid and non-lipid hydroperoxides using two molecules of GSH and thereby curtails the quantity of biomolecules having destructive properties (Michiels et al., 1994). In the present study, depletion of GPx level was owed by an enhanced free radical production during NDEA and CCl₄ metabolism (Prince et al., 2004) and also due to free radical-mediated enzyme inactivation. This is in accordance with the finding of Peskin et al., (1977) who have reported decreased levels of GPx in hepatoma condition. Reduction of GPx in hepatoma conditions would be expected to have dire consequences. However reduction in GPx is found to be more deleterious than SOD. The greater relative importance of GPx over SOD can be attributed to the ability of GPx to detoxify hydrogen peroxide by SOD (Simmons and Jamall, 1988). Upon treatment of extracts, shown a dose dependent increase in activity of GPx, which signify a radical scavenging activity by increasing the level of GPx (Table 5.16 and 5.32).

GST measurement is one of the useful markers in liver cancer. Glutathione-S-Transferase (GST) is one of the groups of selenium dependent enzymes that inhibit GPx activity with fatty acid peroxides. Similarly, GST is a soluble protein located in cytosol and plays an important role in detoxification and excretion of xenobiotics (Bansal et al., 2005). GST catalyzes the conjugation of the thiol functional groups of GSH to electrophilic xenobiotics and results in increasing solubility. The xenobiotic–GSH conjugate is then either eliminated or converted to mercapturic acid (Rao et al., 2006). Since GST increases solubility of hydrophobic substances, it plays an important role in storage and excretion of xenobiotics. Induction of xenobiotic detoxifying enzymes is an additional mechanism by which antioxidant rich extracts may act as anticarcinogens as they compete with steps in xenobiotic activation and metabolize toxic compounds to non-toxic ones (Singh et al., 2009). In the present study, the decrease in the level of
GST was observed in HCC bearing rats when compare with the group I rats. As the activity of GST significantly increased in plants treated extract group III and IV rats at a dose concentration (200 and 400 mg/kg) when compared with reduced level of the enzyme activity in the hepatoma bearing animals of group II. It appears that the drug induces greater coupling of electrophilic intermediates with GSH. To verify the anticancer activity of *Fumaria indica* and *Tephrosia purpurea* histopathological studies were carried out. In this investigation, marked changes were observed in the architecture of liver of cancer bearing animals. These indicate the presence of neoplastic conditions following NDEA and CCl₄ administration. In extract treated animals, the NDEA and CCl₄ damage was recovered due to anticancer potency of *Fumaria indica* and *Tephrosia purpurea* extract. *Fumaria indica* is known to contain ferulic acid and caffeic acid. Studies have shown that ferulic acid exhibits anticarcinogenic effects against azoxymethane-induced colon carcinogenesis in F344 rats (*Kawabata et al., 2000*). It has also been reported to depress 12-O-tetradecanoylphorbol-13-acetate (TPA)-promotion of skin tumorigenesis (*Asanoma et al., 1993*) as well as significant decrease in urinary N-nitrosoproline levels in humans on treatment with ferulic acid (*Stich et al., 1983*). Caffeic acid exhibits potent anticancer effect in HT-1080 cell line and that it may be used as an anticancer agent (*Rajendra et al., 2011*). *Tephrosea purpurea* is also known to contain flavonoids and rotenoid such as rutin, quercetin and deguelin. Flavonoids normally scavenge the free radicals and play an essential role in prevention and therapy of cancer (*Singh et al., 2009*). Earlier study revealed that in HepG2 human hepatoma cells, quercetin blocks cell-cycle progression at the G1 phase, and exerts this effect through the increase of p21 and p27 and p53 (*Mu et al., 2007*). Rutin has been reported to inhibit the proliferation of murine leukemia WEHI-3 cells in vivo and promotes immune response in vivo (*Jing-Pin et al., 2009*)
Rotenoids have been shown to exhibit extremely potent cytotoxicity against six human cancer cell lines and deguelin exerts tumour inhibitory activity (Udeani et al., 1997). Hence, the regression of the hepatocarcinogenesis may be due to the protective effect of *Fumaria indica* and *Tephrosia purpurea*. 