Discussion
DISCUSSION

In the present study, the anticancer and antioxidant properties of Indigofera aspalathoides on 20-methylcholanthrene (20-MCA) induced fibrosarcoma in rats were investigated.

In the past few decades the increased cancer risks were associated with the exposure of specific chemicals i.e., 20-methylcholanthrene (Polycyclic aromatic hydrocarbon) is most potential carcinogen for both animals and human beings. It is widely distributed in the environment, being produced by the combustion of almost all carbon containing material. They are readily detected in the air we breath and the tobacco we smoke.

Chemical carcinogenesis is usually a multistage process, comprising different stages from the conversion of a normal somatic to a tumour cell (transformation) and ultimately, after a long-latency period, to a clinically manifested malignant tumour. Chemicals cause cancer either directly or more often, after metabolic activation. In addition to a number of organic compounds such as Ni\textsuperscript{2+}, Pb\textsuperscript{2+}, Cd\textsuperscript{2+}, Co\textsuperscript{2+} and Be\textsuperscript{2+} are also included in this category. The organic carcinogens require conversion to an ultimate, more reactive compound. This conversion is enzymatic and are converted from procarcinogens to their active form.

The use of herbs has been reported in traditional system of medicine and it's modern medical applications are receiving wide spread attention day by day. The aqueous and alcoholic extract from the different
parts of plants have been investigated extensively for various pharmacological and biochemical activities including their activity against cancer (Palani et al., 1999).

Medicinal plants are the oldest source of pharmacologically active compounds and provided virtually the only source of medicinally useful compounds for centuries (Cordell, 1981). The use of medicinal plants in treatment regimen is gaining importance. Herbal medicines have been known to man for many centuries. The therapeutic efficacy of many indigenous plants for a variety of diseases and disorders have been widely documented in traditional medicinal literature (Satyavathi and Gupta, 1987).

Herbs are known to have medicinal properties and the need for research is felt to find out efficacious, broad spectrum activity, cheaper and safer natural product. Medicinal plants are natural resources yielding valuable herbal products which are often used in the treatment of various diseases (Kirtikar and Basu, 1935).

A large proportion of the world population especially in developing countries depends on the traditional system of medicine for a variety of diseases. Several hundred genera are used medicinally mainly as herbal preparations in the indigenous system of medicine in different countries and were source of potent and powerful drugs which have stood the test of time and modern chemistry has not been able to replace most of them. Many pharmaceuticals we use today are of botanical origin and are based on herbal remedies from the folk medicine of native peoples. Most
important drugs of the past 50 years or so, were first isolated from plants used ethanomedically. Infact 74\% of the 119 biologically active plant derived compounds at present used were discovered as a result of research on species first identified on ethanobotanical surveys (Fransworth and Soejarto, 1985).

The World Health Organisation (WHO) has recommended all the member countries to actively promote native medicines of their country as well as to initiate steps to conserve and or to cultivate medicinal plants so that genuine raw materials become readily available to a large section of the population. The WHO has also brought a technical report series on the usefulness of traditional medicine and the strategies that are to be adopted to integrate and to rationalize the scientific approach in the cultivation of traditional practices (WHO, 1983).

In India herbal medicines have been the basis of treatment and cure for various diseases and physiological abnormalities in traditional methods under practice such as ayurveda and siddha. Indian folk medicine comprises numerous prescriptions for therapeutic purposes which may be as varied as cancer, venereal diseases, leprosy, ulcers, snake bite, healing wounds, and skin diseases etc.

Although, a number of workers have investigated the biochemical evaluation of antitumour effect of various medicinal plants (Palani et al., 1999; Prashar and Kumar, 1995; Soudamini and Kuttan, 1989; Talalay and Fahey, 2001) but there is a paucity of information regarding the biochemical evaluation of anticancer effect of natural products on
fibrosarcoma. Hence, the present investigation was undertaken to study the anticancer effect of *Indigofera aspalathoides* on 20-MCA induced fibrosarcoma in rats.

In the present investigation it is observed that the food and water intake in different experimental groups were found to be unaltered. This feature is of paramount important because nutritional depreciation causing body weight loss may parallel a decrease in tumour volume (Waitzberg et al., 1989). Thus the observed inhibitory effect of *Indigofera aspalathoides* on tumour appearance and growth is likely to be mediated through the impairment of the nutritional status of the experimental animals.

The aqueous extract of *I. aspalathoides* showed more significant tumour regression suggesting the anticancer effect of *I. aspalathoides* on fibrosarcoma bearing animals. Visible observation itself indicated the tumour regression during *I. aspalathoides* treatment. The reduction of tumour growth may be due to a cytotoxic compound in the *Indigofera aspalathoides*. The survival time of the animals were found to be high in *I. aspalathoides* therapy. These results indicate the positive nature of *I. aspalathoides* as an anticancer agent which acts synergistically against tumours.

Malignancies may engender complex metabolic disturbances in both humans and experimental animals resulting in rapid loss of body weight and tissue wasting. The body weight progressively declined in tumour hosts, by contrast it regularly increased in controls. Body weights
were steadily increased after the treatment of *I. aspalathoides* extract rather than fibrosarcoma bearing animals. Tessitore *et al.* (1987) have shown that, tumour growth elicited marked loss of body weight in growing ascitic hepatoma bearing animals. This may be due to decreased food intake and/or absorption, which contribute to muscle wasting in tumour cachexia (Pain *et al.*, 1984). Tumour may act as a nitrogen trap (Sherman *et al.*, 1950) and the cells are more efficient in utilizing amino acids for gluconeogenesis. Also from 1 mole of glucose, tumour produces 2 moles of ATP and lactic acid for its use. This lactic acid is converted by the host liver to glucose, which the tumour uses, consuming 6 moles of ATP. This results in a loss to the host of 8 ATP molecules/mole of glucose. This mole of glucose used by the tumour, is loss to the host, which might have used it to generate 36 moles of ATP via kreb's cycle (Landel *et al.*, 1985). The host responds to increased tumour load by decreased muscle protein synthesis and muscle break down. Amino acids resulting from break down of proteins are subsequently used by the liver, further increasing the host metabolic burden (Water House *et al.*, 1979).

The liver and kidney weight in fibrosarcoma conditions may largely be due to protein degradation during tumour growth. Protein metabolic perturbations in the host, although causing tissue waste may themselves favour the growth of tumour itself (Tessitore *et al.*, 1987).

The steadily increasing body weight in treatment of aqueous extract of *I. aspalathoides* may be due to the anticancer potency of *I. aspalathoides*. Aqueous extract of *I. aspalathoides* causes disappearance
of some symptoms by giving subjective and objective improvement. Absence of significant variations (body, liver and kidney weights) in drug control animals reveals the non-toxic nature of the plant drug changes in non-protein nitrogenous compounds.

4.1 CHANGES IN NON-PROTEIN NITROGENOUS COMPOUNDS

The blood urea and creatinine levels were not significantly changed in group II fibrosarcoma bearing animals. On the other hand uric acid level was found to be significantly decreased in group II animals. In the present study urea and creatinine levels were found to be significantly increased in group III fibrosarcoma bearing animals treated with aqueous extract of I. aspalathoides.

Elevation in urea concentration is considered to be an indicator of nephrotoxicity in fibrosarcoma bearing animals. The amount of urea excreted depends upon the glomerular filtration rate and when this excretion fails to balance the production, plasma level rises.

Serum creatinine is an index for renal function. It is produced endogenously by tissue creatinine breakdown, and an increase in serum creatinine may be due to the tissue damage. The amount of creatinine excreted depend on the glomerular filtration rate, when this excretion fails to balance the production, serum creatinine rises (Nosaka et al., 1992).
When the animals were given a *Indigofera aspalathoides* treatment, the elevated levels of blood urea and serum creatinine were almost brought back to near normal level, thus proving its beneficial role on cancer chemotherapy. Uric acid, the metabolic and product of purine metabolism has proven to be a selective antioxidant, capable especially of reacting with hydroxyl radicals and hypochlorous acid (Hasugava and Kuroda, 1989). The reduced level of uric acid in fibrosarcoma may be due to increased utilisation of uric acid against lipid peroxidation, which is a characteristic feature of cancer condition. Increased uric acid level observed after the treatment of *I. aspalathoides* may be due to the decreased tumour burden.

### 4.2 CHANGES IN PROTEIN LEVEL

Heidelberger (1975) has cited reduced levels of protein in neoplastic tissues. In the present investigation decreased protein levels in serum, liver and kidney of 20-MCA induced rats were observed. The liver is an important site of protein synthesis and it has the highest rate of synthesis of tissue proteins. Major protein mass of the organism is severely affected in cancer cachexia. Protein waste implies the underlying metabolic imbalance which is being expressed by an elevation in the apparent protein-degradation rate with no changes in the apparent synthesis rate (Tessitore *et al.*, 1987). Reduced liver protein in Morris hepatoma bearing animals and walker 256 carcinoma in other reports (Landel *et al.*, 1985) have also been suggested the increased protein degradation. Recycling of aminoacids has been decreased in tumour
conditions resulting in enhanced efflux of these aminoacids from the tissues. Thus, the host responds to increased tumour lead by increasing tissue protein breakdown (Tessitore et al., 1987). Treatment of I. aspalathoides may prevent protein degradation rate and hence the total protein content was almost near normal level.

4.3 CHANGES IN NUCLEIC ACID LEVEL

Nucleic acids play an important role during neoplastic transformation. Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) synthesis was studied in order to determine the effect of I. aspalathoides macromolecular synthesis in fibrosarcoma. It has been observed that the tumour growth corresponds to the elevated levels of DNA and RNA synthesis.

The levels of DNA and RNA of liver and kidney were found to be progressively increased in fibrosarcoma animals. Among the nucleic acids, DNA exhibited prominent increase than RNA.

The increased nucleic acid synthesis in tumour animals was found to be decreased when the animals were treated with I. aspalathoides controlled the nucleic acid biosynthesis, suggesting the enhanced tumoricidal activity during cancer therapy.

4.4 CHANGES IN MARKER ENZYMES

Marker enzymes are more unique and changes in their activities reflects the effect of proliferation of cells with growth potential and its
metabolic turnover is dramatically different from those of normal cells. The rise in their activities is shown to be in good correlation with the number of transformed cells in cancer conditions (Kamdem et al., 1982).

Transaminases are usually regarded as markers of liver injury (Dang et al., 1985). Stable clinical pattern of these enzymes were noticed in patients with hepatic malignancy after chemotherapy, while patients failing to respond to drugs showed progressive increase in the level of these enzymes (Liss et al., 1985).

The altered activities of ALT and AST in liver and kidney of fibrosarcoma bearing animals were brought back to near normal in Group III drug treated animals. This indicates the therapeutic efficacy of Indigofera aspalathoides in cancer chemotherapy.

Alkaline phosphatase (ALP) is membrane bound, and its alteration is likely to affect the membrane permeability and produce derangement in the transport of metabolites. ALP is abundant in kidney and serves as a marker enzyme for the renal proximal tubular injury. Elevated level of ALP was noticed in liver and kidney of fibrosarcoma bearing animals. Patel et al. (1994) observed that ALP level was raised in the serum of cervical carcinoma patients. The enzyme activity is used as a tumour marker during diagnosis in the early detection of cancer (Kobayashi and Kawakubo, 1994). In Indigofera aspalathoides treatment further decrease in the enzyme level was observed in the animals.
Malignant tumours have greater activity of acid phosphatase than normal mammary tissues, whereas the benign pathologies have intermediate activity (Jorge et al., 1984). Wilson (1974) has reported an elevated level of ACP in 20-methylcholanthrene induced fibrosarcoma tissues. In the present study, fibrosarcoma bearing animals showed increased activity of the enzyme ACP. altered enzyme activities in cancer conditions were brought back to near normal in *Indigofera aspalathoides* treated fibrosarcoma animals. Reversal of these enzyme levels correlates well with tumour response to therapy.

5'-nucleotidase has been reported to be altered in the sera of patients with solid tumours (IP and Daw, 1978). In human lymphoid system, 5'-nucleotidase is anchored to the plasma membrane and has been described as an important marker for differentiation of B-lymphocytes (Thompson et al., 1986). In the present study 5'-nucleotidase has been increased significantly in the liver and kidney of fibrosarcoma bearing animals. Schwartz and Bodansky (1965) have demonstrated elevated activities of 5'-nucleotidase in liver carcinoma, gastro-intestinal tract and pancreatic cancers. During *I. aspalathoides* therapy, the enzyme 5'-nucleotidase activity was decreased significantly on fibrosarcoma bearing animals.

Recoupmnet of some marker enzymes on treatment with *I. aspalathoides* gives some protective mechanisms against abnormal cell growth by changing the permeability of membrane or affecting cellular growth. The chemotherapeutic effect of *I. aspalathoides* may be attributed through its antioxidant properties.
4.5 ALTERATIONS IN LYOSOMAL ENZYMES

Lysosomal enzymes have been implicated as having a role in tissue injury, repair and disease like cancer, arthritis and so on. Increased activities of enzymes in liver and kidney, the tumour tissue may be due to abnormal fragility of lysosome in sarcoma conditions. The elevated levels could reflect increased synthesis and secretion of enzymes by the tumour (Goren et al., 1986). Increased production of free radicals in cancer condition led to determination of membrane which resulted in the leakage of enclosed enzymes from the lysosomal sacs (Geetha, 1993). An increased expression of lysosomal enzymes were observed in various tumours. Increased activity of acid phosphatase may be due to the lysosomal imbalance resulting in the destruction of the intact membrane (Sharma et al., 1995).

β-D-Glucuronidase is a sensitive marker of lysosomal integrity and it is released due to the presence of oxygen free radicals (Kalra et al., 1988). β-D-glucuronidase is a cellular hydrolase, able to degrade cell organelles and digest cell materials. It has been used as a marker for tubular damage by many nephrotoxic agents (Fishman and Bernfield, 1955). N-acetyl-β-D-glucoaminidase (NAG) is a high molecular mass lysosomal enzymes found in the proximal and distal renal tubules. The recoumpment of lysosomal enzymes upon plant drug treatment to fibrosarcoma bearing animals, may be due to the stabilizing property of I. aspalathoides on lysosomal membrane which may protect the rapid leakage of enzymes and obstruct the rise in enzyme activity.
4.6 CHANGES IN GLYCOPROTEINS

Because of the crucial role of cell surface and membrane constituents in neoplastic behaviour, change in serum and tissue glycoconjugates have long been associated with malignancies (Patel et al., 1990). Abnormal increase in the level of plasma glycoprotein components has been related to the changes in hepatic cells during neoplastic transformation. Macbeth and Bakesi (1964) postulated that, the presence of tumour in hepatic cells induces the synthesis of glycoproteins, which subsequently appears in circulation. Large amounts of hexose, hexosamine and sialic acid were reported in hepatoma conditions (Shimizu and Funakoshi, 1970).

Sialic acids are acylated derivatives of neuraminic acid and exists as terminal component of the non-reducing end of carbohydrate chains of glycoproteins. Elevated level of sialic acid can be useful for early detection of cancer, stating prognosis of the disease, degree of metastases and recurrence (Shanmugam and Nagarajan, 1985). Increased activity of sialyl transferase leads to increased expression of sialic acid in cancer conditions which may be one of the necessities of neoplastic cells helping malignancy. The influence of sialic acid on the oncogenicity of tumour cells has been studied by many investigators as the main determinant of the cell surface negative charge, electrophoretic mobility and the loss of contact inhibition.

It also acts as an antigen masking agents and as a component of cell surface involved in the adherence of tumour cells to mesothelial membrane to form metastasis (Prasad, 1986).
The reversal of the glycoprotein levels to almost normal in the treatment of aqueous extract of *I. aspalathoides* may be due to its potent antitumour activity. The plant drug may alter cell membrane glycoprotein synthesis and structure. Thus, the *I. aspalathoides* has the ability to suppress malignancy by modulating cell transformation, proliferation, and differentiation.

4.7 CHANGES IN ELECTROLYTES

Trace elements would extend their action directly or indirectly, on the carcinogenic process, by affecting the permeability of cell membranes (or) by other mechanisms (Drake and Skypol, 1989). Statistically significant differences from the normal distribution of sodium, potassium and magnesium and other essential elements have been reported to occur in patient's of various types of cancer.

Fibrosarcoma bearing animals treated with anticancer agent like cisplatin showed an increase in sodium and potassium levels. Notachin *et al.* (1994) reported that, cisplatin treatment increases sodium content in the renal cortex and decreases in the papilla where as the potassium content in renal cortex was found to be increased. Mitochondrial injury is an important early event in anticancer agent (cisplatin) toxicity to proximal tubule cells that proceeds inhibition of Na⁺, K⁺-ATPase activity and loss of cell potassium.

Magnesium is mainly an intracellular cation involved in many enzymatic reactions. It is an important Co-factor for adenosine
triphosphatases. Serum magnesium concentration is maintained within a narrow range by the kidney and small intestine, since under conditions of magnesium deprivation both organs increases the fractional absorption of magnesium. Magnesium excretion may be due to defective reabsorption process in the medullary nephrons or collecting ducts (Schilsky and Anderson 1979; Mavichak et al., 1985).

Ca$^{2+}$ is a major factor in converting reversible cell damage in to irreversible cell injury and cell death (Farber, 1981). An increase in Ca$^{2+}$ levels can activate endogenous enzymes and also it initiates cell necrosis by interfering with production and utilization of energy (Orrenius et al., 1987). It was also reported that the calcium concentrations increased significantly in kidney of tumour bearing mice (Prasad and Giri, 1999). The increase in renal endoplasmic reticulum calcium pump activity may be responsible for an increase in the cytosolic Ca$^{2+}$ concentration and it could disrupt the homeostasis of the cell and cause toxicity to the kidney (Jones et al., 1985).

These observed electrolyte changes in *Indigofera aspalathoides* treated fibrosarcoma bearing animals were reversed around normal.

### 4.8 ALTERATIONS IN ADENOSINE TRIPHOSPHATASES

Adenosine triphosphatases (ATPases) are membrane bound enzymes and are mostly occur on the baso-lateral membrane. Na$^+$, K$^+$-ATPase activity pumps Na$^+$ out the cell. As a result, the intracellular concentration of Na$^+$ is lowered and an inward proton
gradient develops and Na⁺/H⁺ exchange is established across the brush border membrane which splits up the ATP for energy purpose. The activity of Na⁺,K⁺-ATPase can also be regulated by hormones, proteins and second messengers. The lipid peroxidation was also associated with the inhibition of Na⁺,K⁺-ATPase activity in proximal tubule cell lysate and this occurs secondary to mitochondrial injury (Courijault et al., 1994). In our study also the levels of Na⁺,K⁺-ATPase activity of serum, liver and kidney were found to be decreased significantly.

Mg²⁺-ATPase is distributed in all renal cell components and play a role in endergonic processes other than ion transport. The ion sensitive Mg²⁺-ATPase utilises a pool of ATP that is not directly related to change in free energy for sodium transport. It poised to regulate the flow of potential energy from the mitochondria and from the cytoplasm. The Mg²⁺-ATPase activity increased significantly due to I. aspalathoides treatment when compared with fibrosarcoma bearing animals.

Ca²⁺-ATPase the enzyme responsible for active calcium transport, is extremely sensitive to hydrogen peroxides and this may lead to the inhibition of enzyme activity.

In the present study, Ca²⁺-ATPase activity was found to be decreased in fibrosarcoma bearing animals when compared to control animals. The increase in renal endoplasmic reticulum pump activity may be responsible for an increase in the cytosolic Ca²⁺ concentration and activity could disrupt the normal Ca²⁺ homeostasis of the cell and cause toxicity to the kidney.
Aqueous extract of *I. aspalathoides* administration could modify the altered membrane fluidity and thereby improved the cell membrane integrity by modulating the activity of the membrane ATPases.

### 4.9 CHANGES IN BLOOD GLUCOSE

Glucose is an important metabolic fuel for rapidly growing tumours and hypoglycemia is one of the major complications in cancer condition. Cancer cells have increased rates of glucose catabolism compared with healthy cells and the malignant tumours in experimental fibrosarcoma act as a glucose trap (Shatpot, 1979).

Decreased blood glucose level in tumour condition suggested that the glucose availability is not sufficient to provide for both host and tumour. Glucose is diverted away from host tissues not only for direct utilisation by the tumour but also as an important precursor for the synthesis of substances that may be required for tumour growth. Thus high metabolic demand for glucose is experienced by tumour bearing animals (Mulligan and Tisdale, 1991). Due to the antitumour potency of *I. aspalathoides*, the blood glucose content might have been restored to near normal in *I. aspalathoides* treated animals. Due to decreased tumour burden in this group of animals, the excessive utilization of glucose is normalized.
4.10 CHANGES IN KEY ENZYMES OF GLUCONEOGENIC PATHWAY

Malignant tumours are known to have high rates of glycolytic activity leading to increased production of lactic acid. Utilisation of lactate and aminoacids for glucose synthesis leads to increased, uncontrolled gluconeogenesis from the precursors and this was found in tumour bearing host.

Glucose-6-phosphatase (G6P) and fructose-1,6-diphosphatase (FDP) are key enzymes that regulate gluconeogenesis. It is natural that with the increasing tumour growth rate, lactate production from glucose rises, where as glucose production from pyruvate decreases. The progressive failure of gluconeogenesis, manifested most extensively in the rapidly growing tumours is explained partly by marked decrease or complete absence of glucose-6-phosphatase and fructose-1,6-diphosphatase activities in hepatoma.

4.11 CHANGES IN GLYCOGEN

A decrease in glycogen content in liver and kidney with the progression of sarcomagenesis was observed in the present study. Sato et al. (1978) has demonstrated the increased glycolysis and reduced liver specific marker enzymes of gluconeogenesis (G6P, FDP) and glycogen phosphorylase in 20-MCA treated animals. The two stages of sarcomagenesis can be distinguished by demonstration of accumulation of glycogen. In the first stage, glycogen storage increases which probably
defense system and prevent the damage induced by free radicals. Induction of 20-MCA was generated lipid peroxidation products. In general, significant increases in lipid peroxidation in carcinogenic process may be due to abnormal levels of Reactive Oxygen Species (ROS). ROS production in excess of cellular antioxidant capacity may result in damage to lipid, protein, RNA and DNA or other effects (Ceruthi, 1985; Breimer, 1990).

4.13 CHANGES IN ANTIOXIDANT ENZYMES

Enzymic antioxidants provide a major intracellular antioxidant protection by removing superoxide radicals and hydrogen peroxide ($H_2O_2$). Superoxide radicals may be reduced by the enzyme superoxide dismutase to form $H_2O_2$ and catalase converts $H_2O_2$ to neutral products $O_2$ and $H_2O$. Glutathione peroxidase catalyses destruction of $H_2O_2$ and other lipid hydrogenperoxides using glutathione as electron donor. Cytoprotective enzymes which are located within both hydrophilic and hydrophobic compartments of the cells, and the antioxidants in intra- and extra cellular fluids are involved in the scavenging of free radicals.

Elevation in MDA production in neoplastic condition may in part be attributed to the effective inhibition of free radical scavenging enzymes. Decreased activity of NADPH-dependent glutathione reductase required for the conversion of GSSG-GSH, can be ascertained by the decreased level of GSH. Under conditions of oxidative stress, the NADP/NADPH ratio will switch in favour of NADP indicating decreased Glucose-6-phosphate
dehydrogenase activity. The paucity of NADPH production will, in turn, decrease the catalase activity (Nichollas and Schonbaum, 1963).

Superoxide radicals play an important role in cell physiology. Superoxide dismutase (SOD) is widely distributed in cells with high oxidative metabolism and have been proposed to protect such cells against the deleterious effects of superoxide anion.

Glutathione peroxidase (GPx) is considered to be a most important H$_2$O$_2$ removing enzyme in mammalian cells and is more important than catalase in removing H$_2$O$_2$ (Gaetani et al., 1989). The activity of GPx is dependent on the availability of GSH which in turn, maintained by de nova synthesis.

Decreased level of GSH can be ascribed to depress the activity of NADPH dependent GR which is required for the conversion of GSSG to GSH. Therefore, a decrease in NADP production due to G6PD inhibition, and a decrease in GSH level may be responsible for the impaired functioning of GPx in neoplastic tissues. Decreased GPx activity was also observed in red blood cells of untreated patients with malignant lymphoma (Beewick et al., 1987).

Our findings agree well with this and the activity of GPx in the liver and kidney was significantly decreased in fibrosarcoma bearing animals.
The levels of enzymic antioxidant enzymes such as catalase, SOD and GPx were corrected to near normal in fibrosarcoma bearing animals by treating the aqueous extract of *Indigofera aspalathoides*.

Antioxidants have also been advocated to impart anticancer activities by several other mechanisms (Smith *et al.*, 1995) such as i. Trapping the ultimate carcinogen, ii. Blocking the metabolic activation of carcinogens, iii. Modulating xenobiotic metabolizing enzymes, iv. Scavenging free radicals, v. Inhibiting generation of free radicals, vi. Inhibiting promotion stage of carcinogenesis by inhibiting cell proliferation through blocking lipoxygenase/cyclooxygenase pathway or by lowering ornithine decarboxylase activity and vii. By decreasing the bioavailability of ultimate carcinogen etc.

*Indigofera aspalathoides* treatment showed protective action against reactive oxygen species (ROS) induced by malignant tumour possibly through its ability as an antioxidant in quenching the superoxide anion or free radicals.

### 4.14 CHANGES IN NONENZYMIC ANTIOXIDANTS

The non-enzymic antioxidants such as glutathione, vitamin C, vitamin E, total thiols and serum ceruloplasmin were significantly reduced in fibrosarcoma bearing animals. The antioxidant status was improved during the chemotherapy of aqueous extract of *Indigofera aspalathoides*. 
The activity of non-enzymic antioxidants is presumably essential for the removal of radicals (Allen, 1991). Decreased levels of antioxidants, which further increases the free radical activity, are clearly associated with cancer condition (Barber and Harris, 1994). Free radicals being formed by reaction of superoxide radical with \( \text{H}_2\text{O}_2 \) produces hydroxy radical, a highly destructive radical species that can react with practically any molecule present within the cells. Enzymic antioxidants are inactivated by these radicals (Wiseman and Halliwell, 1993; Roussgn et al., 1996; Marnett, 2000).

GSH, acting through enzymes utilising it as a substrate or cofactor, is thought to be involved in an important defence mechanism. It conjugates with a variety of endogenous and exogenous compounds and detoxifies superoxide and hydroxyl radicals following formation of oxidised glutathione (GSSG). GSH also appears to play acute cellular effects of oxidative injury induced by hydroperoxides.

The water-soluble vitamin C, plays several important roles in vivo (Levine, 1986). It is a good scavenger of most reactive oxygen species (Halliwell, 1990) and protects lipid and plasma membrane (Frei et al., 1989) thereby preventing degenerative diseases including cancer (Block and Mankes, 1989). It demonstrates a synergistic interaction with tocopheroxyl radical, resulting in the regeneration of \( \alpha \)-tocopherol. Ascorbate imparts its protection by undergoing oxidation ultimately forming dehydroascorbate (Grimble and Huges, 1967). GSH is required for the reduction of dehydroascorbate back to ascorbate vitamin E.
(α-tocopherol) acts as a biological antioxidant. As a free radical quencher, vitamin E accounts for much of the lipid-soluble chain breaking antioxidant capacity of the human blood plasma and erythrocyte membrane (Sies et al., 1992). It is the most important free radical scavenger within membranes and lipoproteins. The free radical clearing ability of this fat soluble vitamin is due to the delocalisation of an unpaired electron in its conjugated double bond system.

In biomembranes, vitamin E has been found to have potent antioxidant activity due to its ability to penetrate to a precise site into the membrane which may be the important feature of protection against highly reactive radicals (Packer et al., 1979).

Thiols are water soluble antioxidants associated with membrane proteins and are important for the antioxidant system. Thiols, which are the main components of intracellular nonprotein sulphhydryl groups, and they participate in many cellular functions including metabolism of drugs and detoxification of free radicals (Lai et al., 1991).

Total thiols was decreased in 20-MCA induced fibrosarcoma conditions. LPO and decreased membrane fluidity in cancer conditions decrease the reactivity of thiol groups (Oyashiki et al., 1994).

Decreased activities of ceruloplasmin in fibrosarcoma bearing animals were observed. Ceruloplasmin-bound copper does not accelerate radical reactions, ie., ceruloplasmin inhibits copper-dependent lipid peroxidation. However, a more important antioxidant feature of
ceruloplasmin is shown to depend on its ferroxidase activity. Ceruloplasmin catalyses the oxidation of Fe\(^{2+}\) to Fe\(^{3+}\) with simultaneous reduction of O\(_2\) to H\(_2\)O. Ceruloplasmin is shown to have some superoxide dismutase activity i.e., O\(_2^-\) scavenging activity (Gold Stein et al., 1979).

Administration of *I. aspalathoides* reveals its effectiveness in affording protection to cell membrane by maintaining the non-enzymic antioxidants, namely, GSH, ascorbic acid, \(\alpha\)-tocopherol, thiols, and serum ceruloplasmin. The observation made in this study highlights the antioxidant property of *I. aspalathoides* in fibrosarcoma bearing animals.

4.15 CHANGES IN LIPIDS

In the present study, there was an observed elevated levels of total cholesterol with decrease in phospholipids and free fatty acids in group II cancer bearing animals. Deregulated cholesterogenesis observed in tumours, implicated an over production that could result in the enrichment of tumour cell membrane with cholesterol (Dessi et al., 1992, 1995). This may be capable of inducing cell populations in to greatly enhanced proliferative activity (Coleman, 1986). Rao (1995) has cited that elevated cholesterol level proceeds the observed changes in DNA and protein content, suggesting a link between cholesterol and DNA synthetic pathway.

The low free fatty acids (FFA) content of tumours may also be due to inhibition of lipoprotein lipase (LPL) activity. The plasma levels of FFA reflects a balance between their rates of formation and utilization. Bridon
et al. (1991) reported that the decreased levels also correlate with increasing weight loss in cancer.

Tumour cells differ from normal cells in the structure and functioning of their membrane and the activation of various carcinogenic factors, leading to alterations in membrane systems accompanied by changes in the phospholipid composition of tumour cell membranes (Bergelson et al., 1970). The lowered phospholipid content in this study was similar to that observed by (Ruggieri et al., 1976) have suggested a low content of total phospholipids in yoshida hepatoma cells with a typical, fatty acid pattern in their phospholipids.

Accelerated phospholipid degradation alters the structural and functional integrity of liver cells. Also the damaged cells become unable to repair injured membranes by regenerating new phospholipids (Ruggieri et al., 1976). Gross changes in phospholipid content may have serious consequences on the effective transmission of biological signals across the membrane. The membrane alteration could result in an influx of extracellular calcium ions into the cell. As the concentration rises, the degradation of phospholipid may further disrupt the ability of mitochondria and endoplasmic reticulum to sequester these ions (Moore et al., 1976).

In Indigofera aspalathoides treatment animals, the above lipid profiles were reverted to near control level on time dependent manner. The protective effect of I. aspalathoides may be rendered by through its antioxidant property.
4.16 BIOTRANSFORMATION ENZYMES

The cytochrome $P_{450}$ content was decreased sharply in the tissues of fibrosarcoma bearing animals. Their content were reversed to near normal in *I. aspalathoides* treatment.

The decrease in this hemoprotein was assumed to be caused by LPO induced by 20-MCA, it has been noted that LPO caused the degradation of cytochrome $P_{450}$ (Mori *et al.*, 1992). Reduced hepatic concentration of cytochrome $P_{450}$ was also reported in cancer conditions (Gregus *et al.*, 1982). The mechanism of cytochrome $P_{450}$ driven reactions involves the formation of oxy and subsequently peroxyl intermediates.

Breakdown of those intermediates yield ROS cytochrome $P_{450}$ has functional multiplicity and also acts as peroxidase in which peroxides are used as oxygen donors. The suicidal inactivation process of cytochrome $P_{450}$ involves the generation of $\text{OH}^+$ as an intermediate (Karuzina and Archakov, 1984). This increased LPO leads to further decrease of cytochrome $P_{450}$.

Due to the potent free radicals scavenging action of the drug, the abnormal lipid peroxidation reaction was arrested in group III drug treated animals. This might be the reason for the recoupment of cytochrome $P_{450}$ content in this group of animals.

Cytochrome $P_{450}$ and cytochrome $b_5$ are the principal components of the mixed function oxidase of the phase I drug metabolizing system.
Most of the components of mixed function oxidase enzyme system, including cytochrome P₄₅₀ and cytochrome b₅, were found to be severely impaired in tumour bearing animals. These observations are in accordance with Brown et al. (1971) who have reported a decrease in these components of rats bearing mammary gland tumours. The decrease may be due to the alteration in the activities of key enzymes involved in the regulation of haematin, heme and hemoprotein synthesis and degradation, evidence for which is recently accumulating (Dogra et al., 1985).

The decreased cytochrome P₄₅₀ content may also be due to increase in LPO. Wills (1969) hypothesized that the system involved in LPO partially resemble the drug hydroxylating system and appropriate parallelism was observed between the formation of malondialdehyde and loss of microsomal enzymes and cytochrome P₄₅₀. Enhanced cytochrome P₄₅₀ catalytic activity may result in increased first pass clearance of xenobiotics. Hepatic microsomal cytochrome P₄₅₀ and cytochrome b₅ contents were increased in drug treatment. Which shows its ability to metabolize carcinogen and a sparing effect. This detoxification and clearance of target tissues could account for the observed antitumourigenic activity of the *I. aspalathoides* extract.

The carcinogenicity of many xenobiotics depends on the balance between the activities of phase I and phase II enzymes. The activities of hepatic microsomal cytochrome P₄₅₀ dependent enzymes were found to be depressed in 20-MCA induced fibrosarcoma bearing animals because 20-MCA causes damage not only to cytochrome P₄₅₀, UDPGT and GST but
also to other cytochrome P₄₅₀ associated enzyme systems and this may lead to the accumulation of the carcinogen. Activities of both oxidative (phase I) and conjugative (phase II) enzymes were decreased in many tumours (Rao et al., 1987; Roy and Liehr, 1988). These changes may have significant effect on the pharmacokinetics of specific agents in vitro and may contribute to alterations in the endocrine status of the host (Mouelhi et al., 1987).

The rate of drug metabolism is more closely linked to NADPH-cytochrome P₄₅₀ reductase than to the amount of cytochrome P₄₅₀ present (Testa and Jenner, 1976). This inhibition of this enzyme could also result from binding of the carcinogen, either to the reductase (or) to cytochrome P₄₅₀ itself. O₂⁻ stays tightly bound to reductase when this enzyme is still located in the microsomal membrane (Koster and Slee, 1980). Feuer (1988) has reported a 40% reduction in the enzyme activity in fibrosarcoma. NADPH is unable to replace NADPH efficiently in many microsomal mixed function oxidase reactions but in the presence of NADPH, a synergistic effect of NADH on metabolic process is apparent which cannot be explained by simple summation activity (Testa and Jenner, 1976). The decreased activity of NADPH-cytochrome P₄₅₀ reductase in cancer bearing animals may lead to the inhibition of NADPH-cytochrome b₅ reductase.

Aniline hydroxylase activity is also linked to the amount of cytochrome P₄₅₀ present and to the activity of NADPH-cytochrome P₄₅₀ reductase (Testa and Jenner, 1976). The enzyme activity was reported to be depressed in AFB, treated rats (Raisuddin et al., 1994) and on
administration of carcinogens like 3-methylcholanthrene (Matsubara et al., 1976). The present investigation also showed similar results.

Hepatic microsomal phase II enzymes UDPGT and GST are known to be the important preneoplastic and neoplastic markers. In this investigation these were reduced significantly. Activities of UDPGT and GST were found to be decreased also in lung cancer bearing rats (Dogra et al., 1985).

UDPGT is intimately associated with the structure of the membranes to which it is tightly bound. The formation of glucuronide conjugates of drugs occurs predominantly in the hepatocytes (Testa and Jenner, 1976). UDPGT is constrained to phospholipids of the microsomal membranes (Erickson et al., 1978) and hence the observed decrease in group II animals may be due to the prooxidative damage to the microsomal lipids in cancer conditions. The depression of conjugation enzymes have been reported in primary liver cancer (Kamdem et al., 1982).

The enhancement of these phase I and phase II enzymes in Indigofera aspalathoides treated animals shows the decrement in tumorigenesis. In addition to the impairment of drug metabolism, the decrease in the activities of these enzymes and the content of GSH could impair the overall biotransformation process. The I. aspalathoides extract is found to act as a bifunctional inducer because it induces both phase I and phase II enzymes. Such induction may inhibit the formation of
covalently bound complexes of 20-MCA with DNA, RNA and protein and this in turn might cause inhibition of tumour process. The plant drug is also found to be a better inducer of phase II enzymes and hence it also acts as a potential protective agent against 20-MCA induced fibrosarcoma. Due to the increased activity of GST, the microsome mediated 20-MCA binding to DNA may be reduced in the presence of the I. aspalathoides. The UDPGT was buried deeply behind a permeability barrier, and the enzyme was constrained by some membrane components in a conformation not optional for catalytic activity and this enzyme might have been released from this constraint by the chemotherapy these might be reasons for the increased activity of UDPGT in I. aspalathoides treated animals. The anticarcinogenic effect of the plant I. aspalathoides is due to their ability enhancing the activity of phase I and phase II enzymes.

4.17 TOXICITY EVALUATION OF INDIGOJERA ASPALATHOIDES

Aqueous extract of aerial parts does not show any detrimental effect or mortality up to the dose of 2000 mg/kg body weight given intraperitoneously in laboratory animals. There was no significant effect observed with respect to body gain, feed and water intake except at the highest dose level (1000 mg/kg). No significant adverse change was observed in histopathological studies with liver and kidney did not reveal any significant pathological lesion even when the extract was administered in the highest dose.