DISCUSSION
In recent years, there has been a worldwide trend towards the use of the natural phytochemicals present in berry crops, teas, herbs, oilseeds, beans, fruits and vegetables (Kitts et al., 2000; Lee and Shibamoto, 2000). Free radicals, from both endogenous and exogenous sources, are implicated in the etiology of several degenerative diseases, such as coronary artery disease, stroke, rheumatoid arthritis, diabetes and cancer (Halliwell et al., 1992). High consumption of fruits and vegetables is associated with low risk for these diseases, which is attributed to the antioxidant vitamins and other phytochemicals (Ames et al., 1993; Prior, 2003; Weisburger, 1999). There is a great deal of interest in edible plants that contain antioxidants and health promoting phytochemicals.

It was reported earlier from our laboratory that mitochondrial dysfunction caused by the administration of alcohol (Sebastian and Setty, 1999) or thioacetamide (Padma and Setty, 1997) or carbon tetra chloride (Padma and Setty, 1999) could be prevented by prior administration of aqueous extract of *P. fraternus*. This study mainly involved on mitochondrial function and lipid peroxidation. Hepatotoxins like alcohol, carbon tetra chloride, thioacetamide and allyl alcohol induce toxicity and the mechanism by which they induce is different for each agent. For example: alcohol is known to induce ROS by decreasing the oxidation rates by electron transport chain and also it depletes SH groups. Carbon tetra chloride is converted to tri chloro methyl radical which is responsible for hepatotoxicity. Allyl alcohol used in this study is known to deplete GSH and induces oxidative stress. The mechanism by which the administration of plant extracts (*P. fraternus* or *H. indicus*) protect against oxidative stress is the important aspect of this study.

In mitochondria, the respiratory chain is stoichiometrically related to that of ATP synthesis (Pozzan et al., 1979). If the reducing substrate is glutamate or malate 3mol of ATP per pair of electrons are synthesized. If the substrate is succinate 2mol of ATP are synthesized. NADH oxidase which uses the electron carriers of all three sites of electron transport chain was inhibited (fig-1 and 29) in allyl alcohol administered rats. This indicates that there is an inhibition in the transfer of electrons through electron transport chain. RCR and P/O were also significantly decreased by the administration of allyl alcohol. Administration of the extract of *P. fraternus* or *H. indicus* prior to allyl alcohol relieved the inhibition significantly on NADH oxidase, RCR and P/O (fig-1 and 29). Succinate oxidase which uses the electron carriers of site 2 and site 3 of electron transport chain was significantly inhibited (fig-2 and 30) in allyl alcohol administered rats. RCR and P/O were also significantly decreased by the administration of allyl alcohol (fig-2 and 30) showing that mitochondria are uncoupled. Uncoupling of mitochondria normally stimulates respiration, but in this case (using glutamate+malate or succinate as substrates) an inhibition rather than stimulation is seen in the rate of respiration. This clearly indicated that there are blocks/inhibition in electron transport chain for the transfer of electrons due to the administration of allyl alcohol. The block/inhibition could be due to a limitation in the availability of functional electron carriers. Intraperitoneal injection of allyl alcohol to rats depressed state-3 respiration (Jacobs et al., 1987). Administration of *P. fraternus* or *H. indicus* prior to allyl alcohol provided significant protection of 72 and 53% respectively with glutamate+malate as substrate (fig-1 and 29) and
77 and 61% respectively with succinate as substrate (fig-2 and 30). The mitochondrial respiration is tightly coupled to oxidative phosphorylation in intact cells of normal tissues (Tzagoloff and Meyers, 1986). Tightly coupled mitochondria always have high RCR and P/O ratio. Decreased RCR and P/O ratio indicate the damage of mitochondrial membrane, there by rendering the membrane leaky to the ions and other biomolecules that leads to uncoupling of mitochondria. Increased permeability of the mitochondrial membrane leads to uncoupling of oxidative phosphorylation (Soussi et al., 1990). In isolated liver mitochondria fatty acid hydroperoxides, intermediate products of lipid peroxidation (LPO) cause permeability changes in the mitochondria leading to an uncoupling of oxidative phosphorylation (Masini et al., 1994).

Measurement of MDA (secondary product of lipid peroxidation), a useful method for determination of lipid peroxidation products (Sawicki et al., 1963; Placer et al., 1966) showed a significant increase in both liver homogenate and mitochondria by allyl alcohol administration. Lipid peroxidation is a free radical phenomenon and induces a series of alterations in the structure and function of cellular membranes. Prior administration of P.fraternus or H.indicus brought down the lipid peroxide level in rats administered with allyl alcohol (fig-6 and 34).

Membrane lipid peroxidation is an important pathophysiological event in a variety of diseases and stress conditions. Lipid peroxidation (LPO) results in a cascade of degenerative process from membrane denaturation to tissue damage. Biological membranes that are rich in poly unsaturated fatty acids are highly susceptible to free radical catalysed oxidation reactions. Lipid peroxidation has been demonstrated to occur in isolated mitochondria, lysosomes and microsomes (Tappel, 1972). In mitochondria about 90% of the fatty acids in the phospholipids are PUFAs, present mainly in phosphotidyl choline and Phosphotidyl ethanolamine, which account for approximately 80% of the total phospholipids and are susceptible to lipid peroxidation (Daum, 1985). The cell is equipped with an impressive set of system for protecting the integrity of the membrane by having antioxidants and antioxidant enzymes to scavenge free radicals. Vitamin E is the major natural lipophilic antioxidant in the membranes. However, under pathological conditions these systems can be overwhelmed and non-toxic drugs would be required for preventing the deleterious effects of lipid peroxidation. In the present study the two extracts (P.fraternus or H.indicus) showed that they have good potential to protect the membranes against lipid peroxidation (fig-6 and 34). Administration of the aqueous extract of the P.fraternus significantly decreased the thioacetamide (Padma and Setty, 1997) or carbon tetra chloride (Padma and Setty, 1999) induced lipid peroxidation in vivo and protected the liver from the hepatotoxin induced toxicity.

Transaminases are used as marker enzymes of liver damage. Aspartate aminotransferase mediates the reversible transfer of α-aminogroup from aspartate to α-ketoglutarate resulting in the production of glutamate and oxaloacetate. Alanine amino transferase mediates the transfer of α-amino group from alanine to α-ketoglutarate forming pyruvate and glutamate. Administration of allyl alcohol resulted in a significant decrease in the activities of these enzymes in the liver (fig-3 and 31). This decrease is
probably due to the leakage of the enzymes from liver into the extracellular compartment. If this extracellular compartment is in dynamic exchange with the blood, then the enzyme activities will increase in blood (Mukherjee, 1990). It was observed that on administration of allyl alcohol the levels of these enzymes were increased in the serum (fig-4 and 32). This is probably due to the leakage of enzymes from the liver cell due to the toxicity induced by the administration of allyl alcohol. Prior administration of the plant extracts (P.fraternus or H.indicus) along with allyl alcohol exerted significant protection against the liver toxicity induced by allyl alcohol (fig-3, 4, 31 and 32).

Membrane potential is the main component of the proton motive force (Mitchell and Moyle, 1969) and has significant control over mitochondrial respiration (Brand, 1990a, 1990b; Murphy, 1990). It is a sensitive index of the integrity of mitochondrial membranes and altered by changes in protein and lipid composition (Hoch, 1988; Hafner et al., 1988; Murphy, 1990). Membrane potential was decreased in allyl alcohol administered rats (fig-5 and 33). Under normal conditions, the transfer of electrons through the respiratory chain leads to the vectorial translocation of protons from the matrix to the cytosolic side. Any changes in the generation of proton gradient will have adverse effects on mitochondrial energy production, in particular on ATP synthesis, because this proton gradient is the driving force for the formation of ATP from ADP. The present study shows that the driving force for ATP synthesis decreases significantly by the administration of allyl alcohol and prior administration of P.fraternus or H.indicus could protect the membranes and raised the membrane potential significantly (fig-5 and 33). This data also suggest that allyl alcohol disrupts the integrity of mitochondrial membranes and prior administration of plant extracts could protect the integrity of these membranes. The decreased P/O ratio due to allyl alcohol administration (fig-1, 2, 29 and 30) could be partly due to decreased driving force i.e membrane potential.

Allyl alcohol is metabolized by cytosolic alcohol dehydrogenase to acrolein, an unsaturated aldehyde (Rees and Tarlow, 1967). Alcohol dehydrogenase activity is increased in allyl alcohol administered rats which indicates the formation of more amount of acrolein. Acrolein is a powerful electrophile and reacts with nucleophiles, such as sulphydryl groups. The thiol group of glutathione is a favored target and so glutathione is primarily involved in the reaction which leads to GSH depletion. The reaction is markedly accelerated by the activity of cytosolic glutathione S-transferase. The nucleophilic glutathione is an important protective factor of hepatic cells in the detoxification of acrolein. That the alkylation of nucleophilic groups of cellular macromolecules affected by acrolein after glutathione depletion is the event that actually leads to cell injury (Ohno et al., 1985). Increased activity of alcohol dehydrogenase that occurs due to the administration of allyl alcohol (fig-15 and 43) leads to the generation of more acrolein and thus depletes GSH at faster rate and leads to oxidative stress. The administration of the plant extracts (P.fraternus or H.indicus) significantly decreased (fig-15 and 43) the alcohol dehydrogenase activity which leads to less acrolein and depletes GSH slowly and induce less oxidative stress. This partly explain the protective effect of plant extracts against the oxidative stress induced by allyl alcohol.
The carbonyl content is significantly increased (118%) due to the administration of allyl alcohol (fig-7 and 35). Typically, cellular nucleophiles target acrolein’s β-carbon, generating carbonyl-retaining adducts (Esterbauer et al., 1991; Uchida et al., 1998b; Burcham and Fontaine, 2001). The reactive carbonyl may then react with neighboring nucleophiles to form inter or intramolecular cross-links (Esterbauer et al., 1991; Permana and Snapka, 1994; Kurtz and Lloyd, 2003). During reactions with protein, acrolein readily carboxylates lysine, cysteine and histidine side chains. It is shown that lysine modification involves sequential addition of two acrolein molecules to a given residue, followed by ring fusion and dehydration to form a six-membered heterocycle, N-(3-formyl-3,4-dehydropiperidino) lysine (FDP-lysine). There is increasing evidence for an involvement of toxic carbonyls in number of human diseases Calingasan et al., 1999; Tanaka et al., 2001). The prior administration of aqueous extract of P. fraternus or H.indicus offered protection of 99 and 44% on carbonyl content respectively (fig-7 and 35) and prevented the significant increase due to the administration of allyl alcohol.

Oxidants, inhibitors of enzymes containing an iron-sulfur center, free radicals and other reactive species cause the oxidation of biomolecules (e.g., protein, amino acids, lipid, and DNA), which leads to cell injury and death (McCord, 2000; Freidovich, 1999). Following initiation of lipid peroxidation, protein thiols of the membrane can be attacked by lipid derived radicals or reactive, lipid soluble aldehydes like 4-hydroxy nonenol and other hydroxyl alkenals originated within the lipid core of the cell membranes. This results in a membrane protein thiol loss which in turn is associated with the development of hepato cellular injury (Pomppella et al., 1991). Significant decrease on the total sulphhydryl groups due to the administration of allyl alcohol was protected to an extent of 59 and 70% (fig-8 and 36) by prior administration of aqueous extract of P. fraternus or H.indicus respectively.

Oxidative stress is associated with increased formation of ROS that modifies phospholipids and proteins leading to peroxidation and oxidation of thiol groups (Molavi & Mehta, 2004). The assaults by ROS lead to changes in membrane permeability, membrane lipid bilayer disruption and functional modification of various cellular proteins.

Superoxide is generated from O2 by multiple pathways (Gilbert, 2000; Freidovich, 1999; Wu and Morris, 1998; Evans and Halliwell, 2001). Under physiological conditions, there is a well-managed balance between formation and neutralization of ROS. Oxidative stress can occur when ROS production is accelerated or when the mechanisms involved in scavenging ROS are impaired. Increased production of ROS is thought to occur more frequently than diminished antioxidant defence, and is postulated to play a role in the pathogenesis of several diseases (Halliwell and Gutteridge, 1999). Epinephrine is converted to adrenochrome by superoxide and using this principle, the adrenochrome can be followed spectrophotometrically and thus superoxide level can be estimated. The prior administration of aqueous extract of P. fraternus or H.indicus offered protection of 53 and 51% respectively on superoxide level (fig-9 and 37) and prevented the significant increase due to the administration of allyl alcohol.
ESR spin trapping offer sensitive method for superoxide determination. Spin trap is a compound which forms a stable free radical by reacting covalently with an unstable radical. Thus the radical trapped in a long-lived form which can be observed at room temperature using ESR. The hyperfine splitting of the adduct provides information which can aid in the identification of the radical. The addition of glutamate and malate resulted in a low intensity ESR signal characterisic of the DMPO-OH spin adduct (quartet signal with intensity ratios of 1:2:2:1). The DMPO-OH signal is formed by spontaneous decay of the DMPO-superoxide adduct (DMPO-OOH) in mitoplasts. Healthy mitochondria under normal conditions will not generate detectable free radicals coupled to substrate oxidation. The major sites in the electron transport chain for the generation of free radicals are complex I and complex III. The electron transport chain is to be inhibited with suitable inhibitors to see the maximum capacity of electron transport chain to generate free radicals. Inhibition in the flow of electrons will force them to go on to oxygen to generate superoxide. This process is minimum when electrons are flowing freely through the electron transport chain. When electron transport chain is inhibited by an inhibitor of complex 3 (eg:- antimycin), maximum amount of free radicals are generated and now one cannot see the difference between the normal mitochondria and mitochondria that have blocks in the electron transport chain due to experimental treatment. Fig 25, 26, 53 and 54 shows the ESR signal intensities of group-A (control), group-B (allyl alcohol administered) and group-D (allyl alcohol+plant extract administered). The change in the signal intensities of these 3 groups is not statistically significant in the presence of antimycin. When the same experiment was done in the absence of antimycin, the ESR signal intensity was statistically increased due to the administration of allyl alcohol (fig 25 and 53) and there was a significant protection (47 and 57%) due to the prior administration of P.fratetnus or H.indicus. This shows that there is a significant block in the electron transport chain in group-B (allyl alcohol administered) which was responsible for generation of high amount of superoxide compared to group-A (control). When succinate was used as a substrate there was no statistically significant difference in ESR signal intensities due to the administration of allyl alcohol when compared with control, even in the presence (fig 54) or absence of antimycin (Fig-55). This demonstrates that there is no significant block in electron transport chain from succinate to oxygen for the generation of excess of free radicals. These results clearly indicate that the block is at site 1 and the significant amount of superoxide that is generated due to the administration of allyl alcohol is from complex I. Addition of SOD, abolishes the ESR signal completely, showing that the superoxide is the source for hydroxyl radical (fig 24 iv and 52 iv). In congestive heart failure mitochondria produce more superoxide than normal mitochondria in the presence of NADH but not succinate showing that and that complex I is the predominant source of such superoxide production (Idle et al., 1999).

The protective roles of glutathione against oxidative stress are (Masella et al., 2005): (i) glutathione is a cofactor of several detoxifying enzymes against oxidative stress, e.g. glutathione peroxidase (GSHPx), glutathione transferase etc. (ii) GSH participates in amino acid transport through the plasma membrane; (iii) GSH scavenges hydroxyl radical and singlet
oxygen directly, detoxifies hydrogen peroxide and lipid peroxides by the catalytic action of glutathione peroxidase; (iv) glutathione regenerates vitamins C and E, back to their active forms (glutathione can reduce the tocopherol radical of Vitamin E directly, or indirectly, via reduction of semidehydroascorbate to ascorbate).

Under conditions of enhanced oxidative stress, GSH content decreases, this in turn increases the content of protein mixed disulphides. A significant number of proteins involved in signaling have critical thiols, such as receptors, protein kinases and some transcription factors can be altered in their function by formation of mixed disulphides. Acrolein formed from allyl alcohol (Miccadei et al., 1988) produces an abrupt depletion of GSH which leads to lipid peroxidation and cell death. In the present study GSH levels were significantly decreased due to the administration of allyl alcohol (fig 14 and 42). Prior administration of plant extracts (P.fraternus or H.indicus) significantly increased the GSH levels.

Administration of P.fraternus or H.indicus promoted the conversion of GSSG (oxidised glutathione) into GSH (fig 14 and 42) by the reactivation of hepatic glutathione reductase enzyme in allyl alcohol-administered rats. The availability of sufficient amount of GSH thus increased the detoxification of active metabolites of allyl alcohol through the involvement of glutathione peroxidase. The restoration of GSH level after the endurament of the plant extracts to such allyl alcohol-treated rats account for the protective efficacy of the extract.

Antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase are important as part of an organism’s defence mechanisms against free radical production and the damage by these molecules (Wispe et al., 1992; Rothstein et al., 1994; Keyer and Imlay et al, 1996). Due to the defensive roles of these enzymes, the potential for antioxidant enzyme therapy is becoming more and more important in number of diseases.

Presumably, a decrease in catalase activity could be attributed to cross-linking and inactivation of the enzyme protein in the lipid peroxides. Decreased catalase activity is linked up to exhaustion of the enzyme as a result of oxidative stress caused by allyl alcohol. In the present study catalase activity was significantly decreased due to the administration of allyl alcohol (fig 10 and 38). Prior administration of plant extracts (P.fraternus or H.indicus) provided significant protection (fig 10 and 38). The catalase activity was restored to normal after treatment with extracts evidently shows the antioxidant property of the extracts against oxygen free radicals. H. indicus reversed cumene hydroperoxide-mediated inhibition of the activities of antioxidant enzymes such as catalase, glutathione peroxidase and glutathione reductase. Thus H.indicus diminishes cumene hydroperoxide-induced cutaneous oxidative stress, which plays an important role in tumor promotion (Sultana et al., 2003). The exact mechanism by which H.indicus exhibits antioxidative activity in allyl alcohol induced toxicity is not well known, although this evidence suggests its action and efficacy in interception of the free radicals and protection of cellular macromolecule from oxidant damage. GSH may be depleted either by a conjugation reaction with electrophiles or by inhibition of GSH regeneration from the oxidized glutathione and/or its biosynthesis.
The increased GSH (allyl alcohol+plant extract)) associated with glutathione peroxidase might help in reducing the formation of peroxidative stress as observed by the decrease in MDA due to the administration of these plant extracts. Treatment with allyl alcohol depletes selenium-dependent glutathione peroxidase, an enzyme responsible for detoxifying oxygen derived toxic species. Glutathione peroxidase catalyzes the reduction of hydrogen peroxide and organic hydroperoxides by using GSH. GSSG derived from glutathione peroxidase activity is then reduced to GSH by the reaction catalyzed by glutathione reductase. Glutathione peroxidase activity was significantly decreased due to the administration of allyl alcohol (fig 12 and 40). Gutathione reductase activity was also significantly decreased due to the administration of allyl alcohol (fig 13 and 41). In the present study, decreased glutathione peroxidase and glutathione reductase activities may be due to the low level of GSH in allyl alcohol treated rats. Prior administration of plant extracts (P.fraterrnus or H.indicus) provided significant protection on glutathione peroxidase activity (fig 12 and 40) and glutathione reductase activity (fig 13 and 41). Although glutathione peroxidase is a relatively stable enzyme, it has been reported that glutathione peroxidase may be inactivated under conditions of severe oxidative stress [Condell and Tappel, 1983].

In the present study superoxide dismutase activity was significantly decreased due to the administration of allyl alcohol. Prior administration of plant extracts (P.fraterrnus or H.indicus) provided significant increase in SOD activity when compared to rats administered only allyl alcohol (fig 11 and 39).

Studies on the overexpression of Cu,Zn SOD which occurs as a result of the trisomy in Down’s syndrome suggest a role of this enzyme in the neuropathology of the disease (Iannello et al., 1999). Studies on end stage heart failure have shown that upregulation of myocardial catalase appears to be a compensatory response to the increased oxidative stress, although the other key antioxidant enzymes, SOD and glutathione peroxidase are not upregulated (Dieterich et al., 2000). Various stress factors may alter the levels of antioxidant enzyme expression in tissues (Sen and Packer, 1996; Allen and Tresini, 2000; Otieno et al., 2000).Coordinated expression of antioxidant enzymes does not necessarily occur in response to stimuli (Ma and Johnson, 1999; Rohrdanz et al., 2000; Wilson and Johnson, 2000). Transcriptional regulation appears to be the most common form of regulation over antioxidant enzyme expression (Harris., 1992; Nanji et al., 1995), and there is extensive investigation on genetic elements and mechanisms which are responsible for transcriptional regulation of antioxidant enzyme gene expression. The redox state probably plays a significant role in the expression of Mn-SOD activity in mitochondria (Wright and Reichenbecker., 1999).

It has been reported that oxidative stress modifies Mn SOD, Cu,Zn SOD and glutathione reductase gene expression at the transcription, post-transcriptional translational and post-translational levels(Cryne et al., 2003). It is reported that menadione-induced oxidative stress not only decreases translational efficiency for Cu,Zn SOD but also increases the proteolysis rate(Cryne et al., 2003). Cellular injury from ROS has been implicated in the development and progression of several diseases. Increases in the
levels of ROS during periods of oxidative stress, are detected by redox-sensitive regulatory molecules in the cell and trigger a homeostatic response to prevent cellular injury called the oxidative stress response (Camhi et al., 1995). Included in the cellular oxidative stress response is the regulation of antioxidant enzymes gene expression, leading to increased antioxidant enzymes activities and, therefore, to a faster removal of the oxidants by the cell, protecting the cell against oxidative stress (Shull et., 1991; Ho et al., 1996; Rohardanz and Kahl, 1998; Franco et al., 1999). However, the cell response has a complex regulation and an increase in antioxidant enzymes mRNAs during oxidative stress do not always correlate with increased activities or protein content of these enzymes in mammalian cells and tissues (Ho et al., 1996; Rohardanz and Kahl, 1998; Franco et al., 1999, Clerch et al., 1998; Jackson et al., 1998).

Some studies on the effect of oxidative stress in mammalian cells have found increases in the activities of antioxidant enzymes and protein level that follow increases in mRNA level (Shull et., 1991; Ho et al., 1996; Rohardanz and Kahl, 1998; Franco et al., 1999; Clerch et al., 1998; Jackson et al., 1998; Yoshioka et al., 1994). However there are other studies with mammalian cells and tissues that show no correlation between the increase found in antioxidant enzyme mRNA and activities or protein content of these under oxidative stress. It is suggested that there is a translational block for the synthesis of these enzymes during oxidative stress (Ho et al., 1996; Rohardanz and Kahl, 1998; Franco et al., 1999; Clerch et al., 1998; Jackson et al., 1998). The increase in mRNA levels found during oxidative stress may occur to compensate for the decrease of translational efficiency and to maintain the protein levels and enzyme activities.

Thus, allyl alcohol not only increases free radical production in the liver, but also decreases its ability to detoxify reactive oxygen species. Possible reasons for the decreased SOD and glutathione peroxidase activities might be due to the inhibition of enzyme protein synthesis, inhibition of enzymes by allyl alcohol or some lipid peroxidation products. Recently, it was shown that depletion of mitochondrial SOD by about 50% results in a functional decline of oxidative phosphorylation, an increase in oxidative stress, an increase in rates of apoptosis in an age-dependent manner (Kokoszka et al., 2001), and depletion of mitochondrial GSH (Williams et al., 1998), suggesting that Mn-SOD is important to maintain the balance of mitochondrial redox state. Moreover, these data suggest that minor changes in Mn-SOD may have a significant impact on the antioxidant status of mitochondria and support the hypothesis that overexpression of Mn-SOD may be protective mechanism against mitochondrial oxidative stress.

GSTs represent one of the major cellular defence mechanisms against electrophilic xenobiotics and their metabolites. It was shown that GSTs use 4-hydroxy nonenol and malonaldehyde (Awasthi et al., 1995) as substrates and that among all GST classes alpha-class shows high selenium independent glutathione peroxidase activity (Awasthi et al., 1975). Since GSTs play an important role in detoxification and physiological functions, effect of allyl alcohol administration on cytosolic GSTs, where majority of allyl alcohol is metabolized was studied. In the current study, the overall
GST activity was measured using CDNB, as substrate show significant decrease in the allyl alcohol treated rat liver cytosol compared to control. Prior administration of plant extracts (P. fraternus or H. indicus) increased the GST activity which indicates the induction of a detoxifying system to enhance the conjugational capacity of GST for inactivation of electrophiles. The GST enzyme system consists of several isozymes. In rat liver, the GST isozymes are mainly made up of three major subunits, the Ya (Mwt-25600), Yb (Mwt-27000) and Yc (Mwt-28000). Each subunit has a specific function. Ya and Yc catalyse the reduction of hydro peroxides, isomerisation of prostaglandin. Yb subunit involved in the formation of leukotrienes. In the present study SDS-PAGE shows Ya, Yb and Yc subunits of GST (fig 22A and 50A). Significant decrease in the Ya subunit was observed by the administration of allyl alcohol (fig 23i and 51i). Prior administration of plant extracts (P. fraternus or H. indicus) provided significant protection (fig 23i and 51i). Alpha class GST isoenzymes, hGSTA1-1 and hGSTA2-2 are involved in the protection mechanisms against lipid peroxidation (Yang et al., 2001).

The histological changes induced by allyl alcohol is evidenced by degenerative changes of liver (fig-57ii). Histopathological findings also support the protective effect of the extract (H. indicus or P. fraternus) as evidenced by the liver with mild degenerative changes (fig-58ii and 59ii).

Petrol extract of whole plant (Bhatnagar et al., 1961) and the leaf extract (Bhowmick and Chaudhary, 1982) of P. fraternus reported to have antifungal activity. Ethanolic extract of whole plant showed anticancer activity in the mouse and antispasmodic activity on isolated ginea pig ileum (Dhar et al., 1968). The aqueous extract from the leaves of it reported to have hypoglycaemic effect in normal as well as alloxan-diabetic rabbits (Ramakrishnan et al., 1982). The hydroalcoholic extract (HE) given intraperitoneally, produced significant inhibition of acetic acid-induced abdominal constrictions (Adair et al., 2000). Ahmed et al., 2002 have reported that the methanolic fraction was most active among all fractions studied in protecting liver against carbon tetra chloride induced toxicity.

Chemical studies of this plant show that the leaves of P. fraternus contain a number of lignans and alkaloids. Phyllanthin (a bitter constituent) and hypophyllanthin (a non-bitter compound) were isolated from the leaves of P. fraternus and identified as lignans. The hexane extract of the leaves gave three additional lignans, niranthin, nirtetralin and phyltetralin. Among phyllanthin, hypophyllanthin, triacantanal and tricantanol isolated from a hexane extract of P. fraternus, phyllanthin and hypophyllanthin showed a protective effect against carbon tetra chloride and galactosamine induced cytotoxicity in primary cultured rat hepatocytes, while triacantanal was protective only against galactosamine induced toxicity (Syamasunder et al., 1985). Two alkamides (E, E-2,4-octadienamide and E,Z-2,4-decadienamide) have been isolated from P. fraternus, a plant that is used in Ghanaian traditional medicine to treat malaria. The compounds possess an alpha, beta, gamma, delta-unsaturated conjugated amide, a feature believed to enhance antiplasmodial activity. In vitro assay of the two alkamides showed to possess moderate antiplasmodial activity (Sittia et al., 1998).
A diet rich in brussels sprouts (300 g/d) markedly decreases the urinary excretion of 8-hydroxydeoxyguanosine in humans, indicating a reduction of DNA oxidation (Verhagen et al., 1995). Similarly, dietary supplementation of cabbage and broccoli extracts to rats decreases free radical-induced tissue damage brought about by irradiation (Fang et al., 1987). Moreover, phytic acid has a high chelation potential and can be supplemented to diets for suppressing iron-catalyzed oxidative reactions and potentially for reducing the incidence of colonic cancer and inflammatory bowel disease (Graf and Eaton, 1990). Collectively, these studies suggest that phytochemicals may be used as effective antioxidants for improving human health.

*Hemidesmus indicus* is employed in traditional medicine for gastric ailments. It mainly consists of essential oils and phytosterols like hemidesmol, hemidesterol, and saponins. Phytochemical investigations have shown the presence of coumarino-lignoids, flavonoids and triterpenoids (Mandal et al., 1991; Rastogi and Mehrotra, 1995). It was reported earlier that nine pregnane glycosides viz. Desinine (Oberai, 1985), Indicine, Hemidine (Prakash et al., 1991), Indi-cusin (Deepak et al., 1995), Hemidescine, Emidine (Chandra et al., 1994), medesimesine, Hemisine and Demicine (Deepak et al., 1997b) were isolated from *Hemidesmus indicus*. Phytochemical studies on the roots of *Hemidesmus indicus* resulted in the isolation of six new pentacyclic triterpenes including two oleanenes, three ursenes and a lupene formulated us lup-1, 12-dien-3-on-21-ol including a known compound, beta-amyrin acetate, on the basis of spectroscopic techniques and chemical means (Roy et al., 2001).

Ethanolic extract of *Hemidesmus indicus* at a dose level of 1.5 and 3.0 mg/kg body weight in acetone prior to that of cumene hydroperoxide treatment reported to inhibit cumene hydroperoxide-induced cutaneous oxidative stress (Sultana et al., 2003). It was reported that oral treatment with the ethanol extract of *Hemidesmus indicus* roots at dose of 100 mg/kg, for 15 days significantly prevented rifampicin and isoniazid-induced hepatotoxicity in rats (Prabakan et al., 2000).

Animal studies have shown that dietary phytochemical antioxidants are capable of removing free radicals. Among them, phenolic and polyphenolic compounds, such as flavonoids and catechin in edible plants, exhibit potent antioxidant activities (Decker, 1995). A large body of the literature has documented the beneficial effects of tea polyphenolic compounds on scavenging superoxide and hydroxyl radicals (Fang et al., 1998), and on their role in the prevention and therapy of disease. These polyphenols also enhance Cu,Zn-SOD activity and decrease malondialdehyde concentrations (Cui et al., 2000).

From all these studies it appears that the oxidative stress induced by allyl alcohol is mainly attributed to the free radical production due to GSH depletion which is responsible for oxidation of membrane proteins and increased lipid peroxidation which in turn responsible for the observed mitochondrial dysfunction. The mode of action of both these plant extracts in exerting the hepatoprotective activity against allyl alcohol may be due to the increased mitochondrial membrane integrity, increased glutathione (reduced) levels and activation of antioxidative enzymes such as glutathione reductase, glutathione peroxidase, superoxide dismutase and catalase which intern scavenge free radicals.