DISCUSSION
Cancer in simple terms is equated with uncontrolled growth of cells throughout the life. The various cells in the body interact with external environment to undergo regular turnover. When the cells turnover exceeds the limits of requirement of growth, repair and reproduction it manifests as cancer (Alberts et al., 1989).

Epidemiological and experimental studies have provided sufficient data which suggest that cancer is a multifactorial, multi-staged, multi-mechanistic complex process and multifaceted disease in which several environmental and host factors play an important role. The development of cancer may be correlated with the degree or extent of alterations in the internal and external environment of host. The major environment factors associated with cancer are physical, chemical and biological factors which are known to damage the DNA, a pre-event before it manifestation as cancer.

Apart from the exposure of carcinogenic chemicals found in the environment, diet as a life style factor play an important role in cancer of various origins as it can modify the process of carcinogenesis. (Tomatis, 1990). Hence, considerable attention is now being directed in the study of substances which over a period of time can help in mitigating the effects of carcinogens or act as blocking agents that can decrease risk of cancer.

Thus the continuing magnitude, severity and complexity of the disease provides a strong rational for a preventive approach to its control.

Food contains many components such as carbohydrates, protein, fats as macronutrients, vitamins and minerals as micronutrients. These are of
biological significance in relation to initiation, promotion or inhibition of cancer. (NRC, 1989)

To this a whole array of non-nutrient components are now added. None nutrients appear to be as important as nutrients in prevention of cancer and are gaining wide recognition.

The bioactive compounds which have been investigated for their plausible or putative cancer preventing properties called as chemopreventive agents.

This new strategy of cancer prevention began with evidence that chemical carcinogenesis in rodents is inhibited by administration of minor dietary constituents. (Troll et al., 1970; Wattenberg and Leong, 1970). In addition understanding of molecular carcinogenesis in human, has increased interest in the use of cancer chemoprevention.

Chemoprevention has emerged in the last few decades as the most promising strategy for cancer control. It has been suggested that chemoprevention should be considered as an inexpensive, easily applicable approach to cancer control (Morse and Stoner, 1993).

Chemoprevention may be defined as inhibition and reversal of carcinogenesis a process that starts with cell of normal physiology and ends with invasive growth, by administration of one or more naturally occurring synthetic and chemical agents which may have multiple biological mechanisms of its action (Kelloff et al., 1994; Wattenberg, 1985; Malone, 1991).

The possible mechanisms of chemopreventive activity include carcinogen blocking activities such as inhibition of carcinogen intake (calcium), inhibition of formation or activation of carcinogens (arylalkyl
isothiocyanates, DHEA, NSAIDs, polyphenols) deactivation of detoxification of carcinogens (Indole-3-carbinol, oltipraz, other GSH enhancing agents) enhancement of the level or fidelity of DNA repair (protease inhibitors). Antioxidant/anti-inflammatory activities include scavenging of reactive electrophiles (GSH-enhancing agents) scavenging oxygen radicals (polyphenols / vitamin E) inhibition of arachidonic acid metabolism (Curcumin, glycyrhetinic acid. Tamoxifen). Antiproliferation / antiprogession activities such as modulation of signal transduction (Glycyrrhetinic acid. retinoids). modulation of hormonal/growth factor activity (DHEA, NSAIDs, retinoids). inhibition of oncogene activity (DHEA, genistein. monoterpenes) inhibition of polyamine metabolism (DFMO. retinoids). induction of terminal differentiation (calcium. retinoids. vitamin D3), restoration of immune response (Selenium. vitamin E). enhancement of intercellular communication (carotenoids. retinoids). restoration of tumor suppressor function. induction of programmed cell death (apoptosis) (genistein, retinoids, tamoxifen). inhibition of telomerase / correction of DNA methylation imbalances (folic acid). inhibition of angiogenesis (genistein, retinoids, tamoxifen). inhibition of basement membrane degradation (protease inhibitors) and activation of anti metastasis genes (Kelloff et al., 1996b).

In the present study the chemopreventive property of an ethanolic seed extract of mustard seeds (*Brassica campestris* var sarason) has been observed on 7, 12-dimethyl benz (a) anthracene induced skin papillomagenesis. The hepatic and extrahepatic carcinogen metabolising enzymes as well as hepatic antioxidant enzymes have been studied.

On the skin tumor model system, mustard seed extract, at the dose 800 mg/kg body weight showed significant chemopreventive potential given at pre. peri, initiational and promotional stages of skin carcinogenesis.
The ethanolic extract of mustard seed significantly reduced the total number of papillomas, average weight of tumors, tumor incidence and tumor mean per mouse as compared to the control animals. The average latent period was significantly longer in the group treated orally with the mustard seed extract.

All these observations are reflection of the chemopreventive action of mustard seed, which was markedly seen in the group where mustard seed extract treatment was given continuously at all the stages of carcinogenesis.

Hence, it seems that a continuous treatment of mustard seed extract not only lowers the carcinogenic ability of 7, 12-dimethyl benz(a) anthracene but also modulate the effects of promotion i.e. the croton oil.

It is well known, that tumor promotion requires several years in humans and hence it is the longest step involved in the carcinogenesis process. Therefore although complete reversal would imply a continuous anti promoting treatment due to the highly reversibility of promotion and anti promotion, especially at the early stages even some delay at this stage would result in a significance decrease in cancer incidence (Bertram et al., 1987).

Hence, there is long interval available to prevent progression from initiation to invasive cancer, if the appropriate pharmacological agents could be developed and used during this promotional stage of cancer which is considered as latent period.

Since the mustard seed extract could reduced not only the number of tumor and the mean number of tumor per effective mouse but also the percentage of animals having tumors. it seems that treatment of mustard
extract when started at pre/peri initiational stages lower the carcinogenicity ability of 7, 12 dimethyl benz (a) anthracene which lead to initiation. It has been reported that male wistar rats fed with mustard diet of seed powder of Brassica campestris var sarason showed a decrease in the benzo(a)pyrene binding hepatic DNA (Rajpurohit and Krishnaswamy, 1994). This indicates chemopreventive potential of mustard seed extract during pharmaco kinetic phase of chemical carcinogenesis.

It appears also that mustard seed extract modulate the effects of promoter i.e. the croton oil. This is well observed from the highly significant reduction in the total number of tumor developed in the treatment group and significant increase in latent period in the treatment group compared to control animals.

Various active phyto chemicals have been found in several members of the Brassicaceae family that lead credence to chemoprevention. These include indole glucosinolates, aromatic isothiocyanates, dithiolthiones and phenols (Nugon-Baudon and Rabot, 1994).

Many experimental studies on commonly consumed varieties of Brassica vegetables and chemoprevention of cancer have focused on purified indolyl glucosinolates and their derivatives omitting the presence of other potentially confounding substances (Wattenberg and Loub, 1978, Wattenberg, 1992, Nugon-Baudon and Rabot, 1994, Zhang and Talalay, 1994).

Indole 3-carbinol, benzyl isothiocyanate and indole glucosinolates, given to rats before administration of 9, 10 dimethyl -1, 2-benz (a) anthracene (DMBA) significantly reduced the tumor multiplicity and incidence of mammary tumors (Wattenberg and Loub, 1978, Wattenberg, 1980).
Indole-3 carbinol has also been shown to inhibit hepato carcinogenesis induced by diethyl nitrosamine when administered concurrently (Tanaka et al., 1990, Jang et al., 1991).

In rainbow trout, dietary indole-3-carbinol substantially affects the distribution metabolism and elimination of aflatoxin B1, leading to significantly reduced hepatic DNA damage (Nixon et al., 1984, Goeger et al., 1986).

Benzyl isothiocyanate administered 15 minutes before carcinogen treatment reduces in a dose dependent manner both pulmonary adenomas and fore stomach tumors in A/J mice and forestomach tumors in ICR/Ha mice (Sparnins and Wattenberg, 1981, Wattenberg, 1987). Other reports include dietary phenethyl isothiocyanates and a reduced incidence of lung tumors in rats and mice (Morse et al., 1989, Morse et al., 1991, Rodwell et al., 1993) and N-nitroso methylbenzylamine - induced oesophageal tumors in rats (Stoner et al., 1991).

Since the ethanolic extract of mustard seed strong chemopreventive potential in skin tumor model system, the underlying mechanism of chemopreventive action needed to be elaborated. Therefore, the present study was also designed to evaluate the modulatory influence of ethanolic extract of mustard seed on mouse liver phase I (cytochrome P-450, cytochrome b5) and liver lung fore stomach kidney and skin phase II (glutathione-S-transferase, DT-diaphorase, glutathione reductase) as well as liver antioxidant enzymes (SOD, catalase glutathione peroxidase) and also the glutathione status content measured as non-protein sulphhydryl content.

In addition, the phenolic antioxidants BHA, which protect against tumor induction via modulation of these enzyme activities (Cha and Beuding, 1979) was also taken in consideration.
Mustard seed extract administered orally in double distilled water to the mouse for 15 days at the low dose level of 400 mg/kg body weight did not show significant changes in the hepatic detoxification system profile, however it elevates significantly the DTD enzyme activity from the basal level in fore stomach as well as the glutathione level in lung.

There was marked changed in the hepatic enzymatic parameters when the mustard extract was administered for 15 days, at the high dose level of 80 mg/kg body weight. There was significant elevation of cytochrome p-450, cytochrome b5 with an associated increase in glutathione S-Transferase and DTD.

Indeed, modulation of the metabolism of carcinogens is one of the most effective and well established strategies for protection of cells against the toxic and neoplastic effects of chemical carcinogens (Talalay et al., 1995). Modulation in these parameters in our study can change the host response to xenobiotics owing to their ability to introduce a polar group in xenobiotic compound, there by providing a means by which subsequent conjugation reaction can take place resulting in inhibition of carcinogenesis.

The enzymes altered are of significance relevance to the DMBA detoxification in the liver of an organism. Metabolic activation of the carcinogen DMBA is the first essential step in initiation of cancer.

DMBA is converted to its epoxide by cytochrome p-450 (CYP). The epoxide is then hydrated via the action of epoxide hydratase (EH) to the proximate carcinogen, the 3,4 -dihydrodiol. The proximate carcinogen is then again modified by CYP to the bay region diol epoxide, the ultimate carcinogen.

The concomitant elevation in conjugative enzyme activity in phase II results in deoxification and elimination of the ultimate carcinogens.
Diets augmented with cruciferous vegetables have been found to very significantly altering the xenobiotic metabolizing enzymes both in liver as well as in the intestine. Thus accelerating the disposal of chemical carcinogens and thereby destroying their ability to damage DNA (Whitty and Bjeldanes, 1987, Guo et al., 1992).

Indoles-derived compounds naturally occurring in edible cruciferous vegetables such as indole-3-carbinol often induce phase I specific enzymes in addition phase II enzymes (Dashwood et al., 1990; Bailey et al., 1991). This has led to some concerns about the use of Indole-3-carbinol as cancer chemopreventive agent.

In the present study, the observed elevated levels of cyt b5 and cyt p450 may leads toward overall protection from potent carcinogenic forms in conjunction with phase II enzymes.

The present study also revealed that mustard seed extract could effectively elevate the GST enzyme activity from basal level in the liver, lung and forestomach.

Glutathione-S-transferase has been reported to be present in multi molecular forms common to several mammalian species (Habig et al., 1974). In view of differences in substrate specificities of glutathione-S-transferase isoenzymes, using 1-chloro-2, 4-dinitro benzene (CDNB) as a substrate in our assay mixture, the enzyme activity measured in the assay solution was thus a measure of total GST enzyme activity inclusive of all the isoforms.

The glutathione S-transferase has been proposed as a marker for evaluating the anti carcinogenic potential of certain inhibitors (Wattenberg, 1983).
The National Research Council in the United States in fact has directed research to identify substances which can stimulate GST, the most versatile enzyme system that can mop up electrophilic substances otherwise initiate carcinogenesis (NRC, 1989).

As most of the mutagenic and carcinogenic species of chemicals are electrophilic the conjugation of electrophiles with glutathione (one of the major endogenous antioxidants) catalysed by GST represent an important detoxification mechanism for withstanding the neoplastic effects of various chemicals (Wattenberg, 1980a).

So, the enhanced GST activity in our study would favour conjugation of reactive molecules of ultimate carcinogens generated by the increased activity of phase I enzymes, with the thiol group of reduced glutathione (Chasseud, 1979). This would possibly block and prevent the formation of genetic lesions or any other cellular damage.

The Swiss albino strain of mice employed in our study also responded to the induction of glutathione-S-transferase by BHA, a synthetic antioxidant, which has been approved to have protective effect against tumor induction by chemical carcinogens of diverse nature (Hoeman, 1988).

The protective nature of BHA has been attributed to the reduction of intracellular concentration of reactive carcinogen metabolites through enhancement of glutathione-S-transferase enzyme activity and intracellular glutathione concentration (Benson et al., 1978; Cha-Beuding, 1979).

The present study also revealed that mustard seed extract could elevate the hepatic and extra hepatic contents of reduced glutathione.

Glutathione is one of the major endogenous antioxidants present in
all the mammalian cells in substantial concentrations. It is considered an important defense mechanism of protecting cells against oxygen derived free radicals and also from cellular lethality following exposure to anti cancer drugs or ionizing radiations (Orrneius and Moldeus, 1984; Biaglow et al., 1987).

The anti carcinogenic properties of many antioxidants and other dietary constituents have at least partly been attributed to their ability to enhance GSH levels (Benson et al., 1979; Sparnins et al., 1982). Thus in the present study the enhancement level of glutathione would trap and scavenge electrophilic carcinogenic moieties generated by phase I enzyme and favour cellular antioxidant defense by providing glutathione-S-transferase activity which in turn weaken the process of carcinogenesis.

The present study also revealed that the mustard seed extract could significantly elevate the level of quinone reductase which is also known as DT-diaphorase in liver, forestomach and skin. This is another widely distributed enzyme that protect cells against the toxicities of quinones and their metabolic precursors (e.g. polycyclic aromatic hydrocarbons, benzene) and is considered phase II enzyme because it does not introduce new functional groups and is generally induced coordinately with other phase II enzyme (Talalay and Prochaska, 1987).

It is also notable that several laboratories in the world now effectively use the induction of phase II enzymes like Quinone reductase to guide new classes of naturally occurring anti carcinogens.

Quinones and their phenol precursors are widely present in the human diet (Ames, 1983). However, they are quite toxic as they can act as electrophiles or accept a single electron to yield the semi quinone free
radicals which can participate in oxidation-reduction cycles at the expense of molecular oxygen to generate superoxide radical and other reactive oxygen species. These react directly with DNA leading to DNA damage (Kappus and Sies, 1981; Smith et al., 1985).

NADPH quinone reductase promote obligatory two-electron reductions of quinones (Powis and Appel, 1980) and thereby diverts these substances from oxidative cycling and render them susceptible to conjugation with glucuronic acid (Lind et al., 1978; Lind, 1985).

The oxidative stress of quinone cycling can be a meliorated by elevating the levels of quinone reductase (Wefers et al., 1984). Furthermore, quinone reductase is inducible by a wide range of compounds including polycyclic aromatic, azo-dyes, 2(3)-tert-butyl-4-hydroxanisol (BHA) and 2, 3, 7, 8-tetra chloro dibenzo-p-dioxin, all of which can protect-against carcinogenesis (Talalay and Benson, 1982).

Isothiocyanates one of active principle's naturally occurring in Brassica species of cruciferous plants has been shown to block chemical carcinogenesis of DMBA induced mammary tumor in rats (Wattenberg, 1977, 1981) and inhibit forestomach tumors induced by benzo(a)pyrene (Wattenberg, 1978) as well as induce phase II enzymes like quinone reductase (Wattenberg, 1992; Tawfiq et al., 1995).

Tumor blocking activity of glucosinolates has been attributed to quinone reductase in murine hepa 1C1C7 cell line (Tawfiq et al., 1995).

In our study quinone reductase also increased significantly by BHA, a synthetic antioxidant, which has been found to block the formation of tumors in several rodent organs induced by different carcinogens (Hocman, 1988).
The natural cellular antioxidant enzymes of the body such as superoxide dismutase and catalase are believed to be the major cellular constituents involved in the defense system against the toxic oxygen free radicals produced during normal metabolism and often oxidative insult.

Recently, substantial evidence has suggested that free radicals particularly oxygen radicals, which include superoxide anion ($O_2^-$), hydrogen peroxide ($H_2O_2$) and hydroxyl radicals (OH) play an important role in the complex course of multiple step carcinogenesis (Cerutti, 1985; Troll et al., 1984; Oberley and Oberley, 1986; Doroshow, 1986).

Much of the evidence has come from the fact that antioxidants that scavenge free radicals directly, or that interfere with the generation of free radicals - associated events, inhibit the neoplastic process.

Superoxide dismutase is an enzyme responsible for dismutation of highly reactive and potentially toxic superoxide radicals ($O_2^-$) to $H_2O_2$. A reduced activity of this enzyme may reduce its cellular efficacy to detoxify these potentially toxic oxyradicals which lead to an increase in the levels of lipid peroxidation causing cellular damage, tissue damage and DNA modification. (Chance et al., 1979; Fridovich, 1983). However, the free radical scavenging activity of SOD is highly effective when it is followed up by increase in the activity of CAT.

Catalase is another important antioxidant enzyme which is responsible for scavenging $H_2O_2$ produced by SOD as metabolite which is very toxic to cells. $H_2O_2$, an active oxygen species, has been associated with the induction of cancer in animals (Shamberger, 1972; Ito et al., 1981). This has been found to be able to induce molecular damage leading to mutation and cell transformation in vitro (Kennedy et al., 1984, Mac Cann et al., 1972).
Fig. XVIII: Formation of reactive oxygen species and antioxidant mechanisms in cell (Sun, 1990).
It has been shown that the decreased susceptibility to chemically induced cancer in rat mammary gland and mouse duodenum were correlated with increased levels of SOD and CAT activities (Werts and Gould, 1986; Ito et al., 1984).

Thus, mustard seed extract may presumably render the murine system more resistant to harmful oxygen species and chemical carcinogenic action by enhancing significantly the antioxidant enzyme SOD and catalase levels.

Thus the present study suggest that mustard seed extract has the potential to block or suppress the events associated with chemical carcinogenesis.