5. Discussion
The ultimate aim of all research endeavours in the field of cancer is to develop strategies for successful prevention or curation of the disease. A great deal of progress achieved in understanding the nature and causation of vast array of animal as well as human cancers has evidently strengthened our efforts to control this dreadful disease. Nevertheless, primary preventive measures through the mediation of motivation, education and legislation as well as secondary preventive measures by the means of surgery, chemotherapy, hormonal therapy, biotherapy and gene therapy have not offered comprehensive control over the occurrence of and mortality due to many cancers of human kind.

Cancer researchers around the world have been looking for alternative and better strategies for the control of cancer and indeed, chemoprevention has emerged as a very promising strategy for blocking or suppressing the process of cancer development. As of now, laboratory animal studies to a considerable extent and epidemiological/human trial studies to a lesser extent have provided scientific basis to the concept of cancer chemoprevention. The theory of multistep carcinogenesis states that premalignancy is a process of evolving steps or stages that can be halted or reversed before progression to invasive cancer. This premise could be extrapolated assuming that specific agents may act on specific steps of the multistep process to halt or reverse it. Effective cancer prevention can be achieved by identifying and eliminating carcinogenic substances from our environment, by introducing blocking and suppressing agents in our food and by improving living standards and nutrition possibly through food additives (Kellen, 1999). Both identification and elimination of the carcinogenic compounds as well as identification of individuals with already initiated cells are extremely difficult. So, ideal approach to the prevention of cancer seems to be the use of chemopreventive agents to inhibit the carcinogenic process during its early phases. Numerous experimental studies in rodents have identified certain foods, food additives and plants with medicinal value as being effective in eliciting a positive response in site-specific tumor models (Ramesha Rao et al., 1980; Rao, 1984; Block et al., 1992; Hayatsu et al., 1993; Thorling, 1993; Rao and Hashim, 1995; Singh, 1999). Variation in chemical stability, extraction process during food preparation, pharmacologic or metabolic variables affecting absorption, metabolism and patterns of induction of phase I, phase II detoxification and antioxidant enzymes are likely to have immense effects on the efficiency of any agent/chemical in a given food preparation. Thus, a thorough
understanding of the finer mechanisms of carcinogenesis and the mechanisms through which synthetic and naturally occurring compounds mediate protection from cancer is necessary, before the most effective dietary or pharmacologic strategies can be promoted to the general population for cancer prevention (Awasti et al., 1996).

Depending on their time of action during the process of carcinogenesis, inhibitors of carcinogenesis can be classified into three categories. Those agents that prevent carcinogenic compounds from reaching and reacting with their target sites are referred to as 'blocking agents'. A large and diverse group of naturally occurring and synthetic compounds fall into this category of inhibitors. One group of blocking agents acts by inhibiting the activation of a carcinogen into its ultimate form. The other group of agents acts by virtue of its inducing activity of enzyme systems having the capacity to enhance the carcinogen detoxification. Inhibitors of this group are of particular interest as they have the capacity to metabolize a wide range of carcinogens. Carcinogen metabolism in general is carried out by phase I and phase II enzyme systems. Blocking agents that induce both phase I and phase II enzymes are referred to as type B or bifunctional inhibitors and those that induce only the phase II enzymes are referred to as type A or monofunctional inhibitors (Wattenberg, 1985).

The present study, is an attempt to identify the cancer chemopreventive efficacy, if any, of four spices, namely, pepper, nutmeg, cinnamon, ginger and a spice mixture (cocktail of 9 spices, routinely used as 'garam masala'), by screening their potency against DMBA-induced skin papillomagenesis at the peri-initiational level and against B(a)P-induced forestomach papillomagenesis. The above-mentioned spices along with cumin and clove, two commonly used spices were assessed for their capacity in inducing phase I and phase II detoxification enzymes and antioxidant enzymes.

Spices are well-known as appetisers and are considered essential in culinary art all over the world. Some also possess strong antimicrobial and antibiotic activities. Some spices possess antioxidant properties and have a profound effect on human health. *Piper nigrum*, commonly known as black pepper is widely used as a condiment in human food. It is also used as a preservative in the processing of industrialized meats (Evans and Trease, 1991). Pepper has been reported to have bacteriostatic and fungistatic properties (Wealth of India, 1969). Nutmeg is the dried kernel of *Myristica fragrans* Houtt. It is used both as a medicine and a condiment. It is used as a stimulant, carminative, astringent and aphrodisiac, in the field of medicine. It is one of the
constituents in drug preparations prescribed for dysentery, stomachache, flatulence, nausea, malaria, rheumatism, sciatica and early stages of leprosy (Wealth of India, 1988). *Cinnamomum zeylanicum* bark, is extensively used as a spice or condiment. It is aromatic, astringent, stimulant and carminative. It is effective in checking nausea and vomiting. Powdered cinnamon is a constituent of chocolate preparations (Wealth of India, 1988). *Zingiber officinale* (ginger) is widely used for flavouring foods such as pies, puddings, cookies, etc. Ginger is valued as a carminative and stimulant of the gastro-intestinal tract. It is as a household remedy for flatulence and colic. An extract of ginger is used as an adjunct to many tonic and stimulating remedies. Seeds of *Cuminum cyminum* Linn., are extensively used as a condiment in many parts of the world. In indigenous medicine, cumin seeds have long been considered to be stimulant and carminative. They are also stomachic, astringent and useful in diarrhoea and dysplasia. *Caryophyllus aromaticum* (clove), from Indian dietary point of view is used as a seasoning agent and is a chief constituent of betel quid and nut powder. Since these spices find their use in our daily food habits and as there are previous reports on the growth-inhibitory or antimutagenic properties of these agents (Prut~, 1998), it was considered to be appropriate to evaluate their chemomodulatory potential in appropriate site-specific tumor models, such as skin and forestomach.

Skin serves as a barrier against the deleterious effects of environmental factors. Being the largest organ in the body and the easiest target to environmental agents, like radiation and chemicals in the air, plethora of studies have been done on skin related disorders, including cancers such as basal cell carcinomas and squamous cell carcinomas. Work on experimental carcinogenesis in mouse skin was pioneered by Mottram (1944) and Berenblum and Shubik (1947). Establishment of multistage nature of skin carcinogenesis (Slaga, 1984; Perchellet and Perchellet, 1989) has greatly facilitated the development in this area. To study the effect of certain dietary agents on chemically induced skin carcinogenesis has been proved to be a useful model system in this regard.

It has been made clear by the pioneering works (Conney, 1982; Guengerich, 1992) that a variety of carcinogens, especially those belonging to PAH class are chemically inert and need to be metabolically activated, primarily by specific enzyme-catalyzed oxidative reactions to exert their neoplastic effect. 7,12-dimethylbenzanthracene (DMBA) used as an initiator in mouse skin carcinogenesis
belongs to the class of PAHs and needs metabolic activation, which is known to occur through several pathways. In one pathway, DMBA is metabolized to DMBA-3,4-diol, 1,2-epoxide (ultimate carcinogen) by the action of cytochrome P450 monoxygenases and epoxide hydrolase. This epoxide is known to form DNA adducts by binding to the N\textsuperscript{6} position of deoxyadenosine causing A:T-T:A transversions (Dipple \textit{et al.}, 1983). In other pathways either one electron oxidation of the PAH ring system or methylation of unsubstituted PAH takes place.

Among the CYP isoforms, the one most relevant to PAH metabolism is the CYP1A1. It is one of the few CYPs expressed in the skin. CYP1A1, though not constitutively expressed, is induced by a variety of xenobiotics. This binds to the substrate, accepts two electrons from cytochrome P450 reductase and/or cytochrome b5 reductase to release the oxidized substrate. This forms the substrate for the action of phase II enzymes. Recent experimental data suggest that CYP1B1 also plays a key role in the metabolic activation of DMBA (Ahmad \textit{et al.}, 1996). As far as the defense provided by the epidermis against the reactive intermediates is considered, thioredoxin reductase forms the first line of defense against the reactive oxygen species (ROS). Glutathione reductase, another flavoprotein enzyme, reduces two electron moieties of O\textsuperscript{2} (superoxide free radical) to hydrogen peroxide H\textsubscript{2}O\textsubscript{2} and H\textsubscript{2}O, thereby decreasing the likelihood of H\textsubscript{2}O\textsubscript{2} combining with O\textsubscript{2} to form HO'. Glutathione peroxidase rapidly detoxifies H\textsubscript{2}O\textsubscript{2} and simple alkyl or phospholipid hydroperoxides. Detoxification of H\textsubscript{2}O\textsubscript{2} in the cytoplasm is largely accomplished by coupled GPx-GR (Trenam \textit{et al.}, 1992). In the presence of glutathione, catalase aids in the dismutation of H\textsubscript{2}O\textsubscript{2} to form H\textsubscript{2}O and O\textsubscript{2}. Superoxide dismutase (SOD) catalyses the dismutation of O\textsubscript{2} to H\textsubscript{2}O\textsubscript{2} and O\textsubscript{2} and since its level is lower in the skin, it is suggested that thioredoxin reductase and glutathione peroxidase systems are more important in skin to counteract the ROS (Carraro and Pathak, 1988).

Stomach cancer was noted to have a high incidence almost everywhere in the world, at the beginning of 20\textsuperscript{th} century. Since nutritional elements and lifestyle play decisive roles in the causation and prevention of cancer, studies on agents that inhibit cancer incidence gain considerable importance. Benzo(a)pyrene [B(a)P], employed in initiating stomach cancer, is widely distributed in our environment, in cigarette smoke, automobile exhaust, etc. Like many other PAHs, B(a)P also needs metabolic activation before it can exert carcinogenicity. Making use of this property, inhibition of B(a)P
induced carcinogenesis can be accomplished in two ways: either by prevention of activation of B(a)P or by detoxification of activated metabolites of B(a)P. Major metabolites of B(a)P include hydroxy B(a)P, hydroxy B(a)P 4,5-, 7,8-, 9,10-dihydrodiols and B(a)P 4,5-, 7,8-, 9,10- epoxides. This metabolic activation again, is brought about by CYP isozymes. B(a)P is initially converted to 7,8 diol which is then converted to anti 7,8-dihydroxy 9,10-epoxy, 7,8,9,10- tetrahydrobenzapyrene (anti-BPDE), the ultimate carcinogen of B(a)P. Numerous experimental data suggest that the major isozyme involved in B(a)P metabolism is CYP1A1 (Bjelogrlic et al., 1993; Sun et al., 1995; Bowes et al., 1996; Singh et al., 1998), the other isoforms being CYP1A2, CYP2A and CYP3A (Sun et al., 1995; Fukuhara et al., 1999). Metabolites of B(a)P are inactivated by conjugation with glutathione, a reaction catalyzed by glutathione S-transferases. B(a)P 4,5-epoxide and anti-BPDE have been shown to be good substrates of GST (Cooper et al., 1980; Ketterer, 1982). Anti-BPDE has been proved to be readily conjugated with GSH to get inactivated (Hesse et al., 1980). Anti-BPDE is an inducer of DNA damage by virtue of its capacity in forming DNA adducts, predominantly at N² position of guanine to form N²-BPDE-deguanosine adduct. Among the GST isoforms, GST-P1-1 has been accredited to be highly effective in the detoxification of anti-BPDE (Singh et al., 1998).

Since carcinogen deactivation or detoxification is generally regarded as a very important mechanism of inhibition of carcinogenesis, to study the induction pattern of the carcinogen metabolizing enzymes might prove of value as an important strategy for chemoprevention. The chemical/substance that brings about this effect is called as a 'blocking agent' (Wattenberg, 1985). Two metabolic pathways phase I and phase II, are crucial for this detoxification/deactivation. The first is an introduction or exposure of polar groups on xenobiotics/carcinogens via phase I enzymes and the second is conjugation via the phase II enzymes. Activated oxygen species viz., singlet oxygen, peroxo radicals, superoxide anion and the hydroxyl radical that are formed throughout this deactivation/detoxification reactions have been implicated in all three stages of carcinogenesis (Kelloff et al., 1996). Thus, the study of induction pattern of antioxidant enzymes also assumes considerable importance.

The cytochrome P450 monooxygenase system represents a major defense against chemical challenge from the environment, constituting part of an adaptive response mounted by an organism following exposure to xenobiotics/carcinogens.
Cytochrome P450s are products of CYP super-family of genes, having overlapping substrate specificities among different isoforms. P450 enzymes play key roles in the biotransformation of many endogenous compounds and in the detoxification of numerous xenobiotics. The modulation of expression of these enzymes can have a significant effect on carcinogenicity and mutagenicity (Yang et al., 1994; Henderson et al., 2000).

Though certain substances are known to act as inhibitors of carcinogenesis by virtue of their inhibitory effect over P450s, for e. g. diallyl sulfide, some proven chemopreventive substances, for e. g. indole 3- carbinol are known to act via induction of specific isoforms of cytochrome P450 (Manson et al., 1998). Curcumin has been proved to inhibit B(a)P-induced forestomach papillomagenesis by induction of an isoform of P450, CYP1A1 accompanied by the hepatic induction of GST (Singh et al., 1998).

In the present study, all the spices studied, including the spice mixture, were shown to induce cyt P450, in one or both the doses tested. Induction/inhibition of phase I system is assessed by the corresponding induction/inhibition of cyt P450 which is the major component of the cytochrome system. The other components of the cytochrome system viz., cyt b5, cyt P450 R and cyt b5 R function in a synergistic manner, facilitating the transfer of an electron to cyt P450, accepted from NADPH or NADH, thus enabling the proper functioning of the cytochrome system.

Phase II reactions in general facilitate the detoxification of the carcinogens and their excretion. The products of the phase I reaction form the substrate for the phase II enzymes. GSTs and DTD are considered to be the major phase II enzymes. GSTs are multifunctional proteins and multiple forms of GSTs are present in most of the species. The main function of GSTs is believed to catalyze the conjugation of electrophilic xenobiotics/carcinogens to the endogenous nucleophile GSH, for the protection of cellular components from these toxic compounds (Awasti et al., 1994). GSTs are capable of intervening in lipid peroxidation at a number of points and are effective quenchers of oxidative stress. As lipid peroxides generated from reactive oxygen species have been shown to be the substrates for α-GST isoenzymes, they are thus instrumental in the termination of the chain reaction of lipid peroxidation (Singhal et al., 1992). Analysis of lipid peroxidation, as measured by the formation of malondialdehyde revealed that lipid peroxidation is inhibited effectively by those doses that caused the
effective induction of GST. GSTs also play eminent roles in the detoxification of reactive metabolites of carcinogens. GSTs catalyze the conjugation of these metabolites with GSH either by non-covalent (azo dyes, polycyclic aromatic hydrocarbons) or covalent (dimethyl amino azobenzene, 3-methylcholanthracene) binding (Hesse and Jernstrom, 1984). Reduced mutagenic response to 7,12-DMBA and AFB1 in Fisher 344 rat hepatocytes has been correlated to the induction of GST (Rogers et al., 1990).

Among the spices studied, pepper, nutmeg, cinnamon and ginger have induced GST at both dose levels, whereas clove, cumin and the spice mixture have induced GST only at the lower dose. Those spices that have induced both cytochrome P450 and GST could be regarded as bifunctional inducers, since they have induced both the phase I and the phase II enzyme system of xenobiotic detoxification.

DT-diaphorase, another major phase II enzyme, is a flavoprotein that catalyzes the two-electron reduction of quinones, quinone imines, azodyes and other nitrogen oxides (Ernster, 1987; Riley and Workman, 1992). Through redox cycling, quinones contribute to oxidative stress due to generation of ROS. A major metabolic role of this enzyme may be to reduce the formation of reactive oxygen species from redox reaction. The induction of DTD that mediates the two-electron reduction of quinones leading to the formation of hydroquinones is important in attenuating the toxicity of quinone metabolites.

Dithiolthiones and their analogues (Oltipraz), known to be chemopreventive against variety of chemical carcinogens, are effective inducers of DTD (Begleiter et al., 1997). Thus, inducers of DTD can be assumed to play important roles in cancer chemoprevention. In this study, only nutmeg, cumin and clove were efficacious in inducing DTD at both doses tested whereas cinnamon and the spice mixture were effective only in the lower dose level. Pepper and ginger were ineffective in inducing the specific activity of DTD.

An increasing body of evidence from literature on the mechanism of carcinogenesis indicates that oxidative stress is an important element of mutagenesis and that mutagenesis is a basis for carcinogenesis (Ames et al., 1993). Now, it is clear that ROS, generated from either endogenous or exogenous sources, are involved in the multistage process of carcinogenesis. They are mutagenic by oxidizing DNA bases and they also cause DNA strand break, chromosomal deletions and rearrangements. The major cellular defense against ROS generated endogenously or by the electrophilic
metabolites of carcinogens generated during phase I biotransformation, is reduced glutathione. The electrophilic functional groups are conjugated with glutathione to form amphiphilic thioether either spontaneously or through GSTs (Jakoby, 1980). Hydroxyl radical produced as a result of oxidative stress initiates a chain of reactions that lead to the process of lipid peroxidation, thus generating the highly mutagenic singlet oxygen. Glutathione is known to be an effective quencher of this singlet oxygen (Mascio et al., 1990; Devasagayam et al., 1991). Three of the spices tested, pepper, cinnamon and cumin have enhanced GSH content at both the dose levels; nutmeg and ginger only at lower dose level and clove and the spice mixture only at the higher dose level.

The other major cellular defense against oxidative stress is the superoxide dismutase-catalase system. SOD dismutates pairs of superoxide anions by oxidizing one to oxygen and reducing the other to hydrogen peroxide. Hydrogen peroxide, which is also considered to be mutagenic, is degraded to H2O and oxygen by catalase. Metabolism of the carcinogens and the application of tumor promoters have been known to generate activated oxygen species. Since SOD-CAT system is effective in detoxifying these radicals, as well as H2O2, agents that cause enhancement in the activities of SOD-CAT system would be of great use in protection against activated oxygen species and peroxide molecules. Pepper, cinnamon, clove and ginger were potent in inducing the specific activity of SOD, at both the dose levels, tested. Spice mixture and nutmeg at lower dose level and cumin at the higher dose level have induced the activity of SOD. Catalase was induced at both dose levels only by nutmeg and cinnamon; at lower dose level by pepper, spice mixture and clove and at higher dose level by cumin.

Glutathione peroxidase, another enzyme of antioxidant defense is involved in the detoxification of hydrogen peroxide when its cellular concentration is quite low (Cohen and Hochstein, 1963). Xenobiotics, including carcinogens, while being modified by phase I monooxygenases, cause oxidative stress due to redox cycling, resulting in generation of hydrogen peroxide and hydroperoxides. Induction of glutathione peroxidase could provide protection against these hydrogen peroxides and hydroperoxides. (Singhal et al., 1992). Glutathione reductase could be regarded as an 'auxiliary mechanism' that facilitates the regulation of GSH homeostasis, in conjunction with other enzymes like gamma-glutamyl cysteine synthetase and glucose 6-phosphatase. Enhanced de novo synthesis of GSH and enhanced channeling of glucose in pentose shunt pathway, providing NADPH for effective recycling of oxidized
glutathione is important for the maintenance of reduced level of GSH in the cell. It might otherwise be depleted due to oxidative/electrophilic stress, generated due to metabolism of carcinogens. Pepper, ginger and clove induced GPx at both dose levels whereas the spice mixture enhanced GPx activity only at the lower dose. GR was induced at both dose levels by the treatment with pepper, nutmeg and clove and only at one of the dose levels by the treatment with rest of the spices.

Lipid peroxidation, initiated in membrane lipids by hydroxyl radical is self-propagating and yields mutagenic ROS, including singlet oxygen and lipid hydroperoxides. Since these spices have induced one or some of the components of the antioxidant defense mechanism, with the concomitant increase in the specific activity of GST, a reduction in lipid peroxidation is highly expectable. Nutmeg, cumin and the spice mixture significantly inhibited lipid peroxidation at both lower and higher dose levels. Clove and pepper in higher dose and cinnamon at lower dose showed significant reduction in lipid peroxidation.

Based on these results, elucidation of the effect of the spices on chemically induced carcinogenesis becomes comparatively easier. In DMBA-induced skin tumor model system, all the spices and the spice mixture were potent in reducing DMBA-induced skin papillomagenesis, except the higher dose of nutmeg. Among the spices examined, it is of interest to note that the dose that has more effectively elevated cytochrome P450 and GST has been shown to be more effective in alleviating skin papillomagenesis.

Since most of the constituents of the spice mixture (garam masala) have been analyzed and proven individually for their anticarcinogenic potency and also for their capacity in augmenting the enzymes of drug metabolism, a synergistic effect of these spices is highly expectable. Keeping this in mind an attempt is made to evaluate the chemomodulatory effect of the spice mixture. In case of the spice mixture, though the higher dose was ineffective in augmenting the activity of GST it has shown remarkable reduction in tumor burden as well as tumor incidence. Even though an induction of phase I system is not accompanied by induction of phase II system, it can still be regarded as a good chemopreventive agent. A proven chemopreventive agent, indole-3-carbinol is a potent inducer of cytochrome P450 and an effective inhibitor of carcinogenesis in many site-specific tumor models (Dashwood et al., 1989; Morse and Stoner, 1996). Further, the level of GSH is also elevated by treatment with the higher
dose of spice mixture, which is shown to protect against mouse skin tumors induced by DMBA/TPA (Kelloff et al., 1996). Moreover, inhibition of carcinogenesis could be brought about by other mechanisms like modulation of signal transduction, modulation of hormonal growth factor activity, etc.

The alkaloid piperine is considered to be the major constituent of pepper that is responsible for its biting taste. Piperine, was shown to increase hepatic GSH content and to inhibit the rise of serum levels of glutamate pyruvate transaminase and alkaline phosphatase (Koul and Kapil, 1993). Piperine has been reported to modulate the oxidative changes by inhibiting lipid peroxidation and mediating enhanced synthesis or transport of GSH thereby replenishing thiol redox. (Khajuria et al., 1998). Clastogenic potential of pepper has also been reported by Abraham and John, 1989. Genotoxic response induced by pepper is confirmed by micronucleus test also (Colunga et al., 1994). In contradiction to these reports, there are other observations to show the antimutagenic/anticarcinogenic property of pepper (Hashim et al., 1994, Higashimoto et al., 1993). These ambiguous reports on pepper make it an interesting test compound and also enhance the need for further confirmation on the nature of pepper. Present investigations beyond doubt prove the anticarcinogenic nature of pepper, the lower dose appearing to be the more potent than the higher dose, suggesting that the clastogenic or genotoxic effect may be due the usage of still higher toxic doses.

Cinnamon bark contains mainly cinnamaldehyde (60-75%), eugenol, benzaldehyde, methyl amyl ketone, phellandrene, pinene and cymene. *Cinnamomum verum* was shown to enhance the activities of hepatic and cardiac antioxidant enzymes and to counteract the increase in lipid conjugate dienes and hydroperoxides in rats fed with high fat diet (Dhuley, 1999). In the present experiments also cinnamon has induced most of the antioxidant enzymes, which could have played instrumental roles in the anticarcinogenic activity displayed by the higher dose of cinnamon.

According to the earlier reports, chloroform extract of nutmeg has been shown to possess anti-inflammatory, analgesic and antithrombic activities in rodents (Olajide et al., 1999). Though both the doses of nutmeg were potent in inducing cyt P450 and GST, the lower dose was efficacious in inhibiting the tumor burden as well as tumor incidence, though it was not statistically significant. The antiinflammatory and analgesic activities exhibited by nutmeg support the anticarcinogenic effect displayed by it. The higher dose on the other hand was not potent in reducing the tumor burden. It could be
because GSH and other important antioxidant enzymes like SOD and GPx could not be induced by this dose.

Three varieties of ginger, Z. officinale, Z. officinale (red variety) and Z. zerumbet were shown to inhibit TPA-induced Epstein-Barr virus antigen in Raji cells, and were proved to have no cytotoxic effect on the cells tested, thus suggesting their anti-tumor promotional activity (Vimala et al., 1999). 6-gingerol was proved to have inhibitory effects on TPA-induced inflammation, epidermal ornithine decarboxylase activity and skin tumor promotion in ICR mice (Park et al., 1998). Ethanolic extract of ginger was claimed to have inhibitory effect on TPA-caused induction of ODC activity and epidermal edema, cyclooxygenase and lipoxygenase activities in SENCAR mouse, thus inhibiting promotion of skin tumor (Katiyar et al., 1996). The findings of the present investigation reveal that ginger at both doses tested, was effective in inhibiting the tumor burden in skin papillomagenesis. The higher dose was potent in reducing the tumor incidence also. This could be due to capacity of the higher dose to induce cyt P450 as well as GST. In addition, most of the enzymes of the antioxidant defense system were also enhanced by treatment with the higher dose, which readily explains the reduction in tumor burden/tumor incidence, as observed by the treatment with the higher dose.

In B(a)P-induced forestomach papillomagenesis, all the spices and the spice mixture revealed an inhibitory effect, in both the doses tested, except the higher dose of ginger. The most remarkable inhibition in tumor burden as well as tumor incidence was shown by the lower dose of spice mixture, which has shown a highly significant induction in the specific activities of cyt P450 and GST. In addition, DTD and all other enzymes of the antioxidant defense have also been induced. Even though the higher dose was almost equally effective in reducing the tumor multiplicity, tumor incidence was not very effectively altered, which might be due to its inefficacy in inducing GST. It has been reported that a spice mixture containing eight spices, viz., mace, clove, black cumin, black pepper, cinnamon, cardamom, dry ginger and nutmeg was effective in inducing cyt P450, cyt b5, GST and glutathione, when given at a dose level of 0.5, 1 and 1.5% for 10 days (Singh and Rao, 1992). The spice mixture at these dose levels, has also been proved to exhibit chemopreventive effect over DMBA-induced transplacental and translactational carcinogenesis in mice (Rao and Hashim, 1995). In case of other
spices, the extent of potency exhibited by them in augmenting cyt P450 and GST could be directly correlated with the extent of inhibition of tumor burden.

Pepper is known to protect colon in presence of dimethyl hydrazine, by decreasing the activities of β-glucuronidase and mucinase (Nalini et al., 1998). Osawa et al., (1981) reported a mutagenic response in Salmonella system when tested the permitted level of nitrite with pepper. Such an effect was confirmed by Higashimoto et al., (1993). Black pepper has also been shown to elicit carcinogenic response (El Mofty et al., 1991, Shwarib et al., 1990). Present study has proved pepper to be a good chemopreventive agent in the doses tested, where around 60 and 55% reduction in tumor burden has been observed in lower and the higher doses, respectively. Since the lower dose is shown to be more effective than the higher, the dosage of pepper might play a key role in determining the carcinogenic/anticarcinogenic response elicted by pepper.

Nutmeg has elicited an inhibitory response over stomach papillomagenesis, where it has shown nearly 50% and 80% reduction in tumor burden by treatment with lower and higher dose, respectively. The important active principles of nutmeg are myristicin, elemicin, safrole and eugenol. Myristicin isolated from parsley leaf oil is known to inhibit benzo(a)pyrene induced lung carcinogenesis in A/J mice (Zheng et al., 1992). Myristicin might have played a key role in the inhibitory response elicited by nutmeg.

Two kinds of cinnamaldehydes isolated from Cinnamomum cassia strongly inhibited in vitro growth of 29 kinds of human cancer cells and in vivo growth of SW-620 human tumor xenograft in nude mice (Lee et al., 1999). These results are suggestive of the chemotherapeutic efficacy of cinnamon, whereas the present findings are suggestive of the chemopreventive potency; wherein, in B(a)P-induced forestomach papillomagenesis, cinnamon has shown appreciable response by reducing the mean number of tumors/animal, approximately to 50% at both the dose levels tested.

Lower dose of ginger proved to be efficacious in reducing the tumor multiplicity up to 55%. 6-gingerol and 6-paradol, the pungent ingredients of ginger are claimed to have antitumor promotional and antiproliferative and apoptosis inducing effects, when tested in cultured HL-60 cells (Surh, 1999; Lee and Surh, 1998). These pungent principles may be responsible for the inhibitory effect on forestomach papillomagenesis.
and enhancement of the phase I, II and antioxidant system as released by the administration of ginger.

Clove and cumin were studied only with respect to their modulatory effects on the enzyme systems associated with carcinogen metabolism and antioxidant defense mechanisms. Clove (*Caryophyllus aromaticus*), when administered in diet to Swiss albino mice for 20 days, was shown to enhance the activities of cytochrome b5, glutathione S-transferase and GSH (Kumari, 1991). Eugenol (4-allyl-2methoxy phenol) is the major constituent of clove oil (Opdyke, 1975) and it was shown to be effective in scavenging superoxide radicals in xanthine-xanthine oxidase system (Krishnakantha and Lokesh, 1993). Eugenol was capable of suppressing both paw and joint swelling in arthritic rats, suggesting its antiinflammatory role (Sharma et al., 1994). A diet containing 3% eugenol (w/w), when fed for 13 weeks showed significant enhancement in the activities of xenobiotic detoxification enzymes like GST and UDP-glucuronyl transfease in rat liver (Yokoter et al., 1988).

The chief constituent of the volatile oil of cumin is cuminaldehyde. Cuminaldehyde, isolated from cumin, when administered in high concentration was shown to inhibit ascorbate/Fe**2+**-induced lipid peroxidation in rat liver microsomes (Reddy and Lokesh, 1992). Cumin when administered in diet was proved to stimulate the activities of arylhydroxylase, cytochrome P450, cytochrome b5 and N-demethylase in rats (Sambiah and Srinivasan, 1989). Essential oil of cumin has the potency to enhance the activity of glutathione S-transferase in mouse and to suppress the formation of aflatoxin B1-induced DNA adduct formation, *in vitro* (Banerjee et al., 1994; Hashim et al., 1994). In the present study also clove and cumin have been proved to be potent in inducing the phase I and phase II drug metabolizing enzymes as well as antioxidant enzymes. These effects exhibited by cumin and clove suggest the chemopreventive potential of these spices and suggest that they could play significant roles in inhibiting site-specific tumorigenesis.

Measurement of lipid peroxidation levels, reveals that most of the spices tested in the study are effective quenchers of lipid peroxidation, the effect mediated primarily by the induction in antioxidant enzymes and by the enhancement of GST. Lipid peroxidation was effectively attenuated by pepper, clove, cinnamon, nutmeg and cumin. Specific activity of lactate dehydrogenase was measured as an indicator of cell damage. None of the spices in the tested dose level induced the activity of LDH; on the other
hand, spice mixture, nutmeg, ginger and pepper inhibited LDH activity, implying the protective role played by these spices.

Current investigation, clearly implies the chemopreventive role played by these spices against chemically-induced skin and forestomach carcinogenesis models, by virtue of their ability to induce the drug metabolizing enzymes and the antioxidant defense system. Outcome of the present study further warrants to identify and characterize the active principles present in each of these spices and to study their effects at initiation as well as promotion and progression stages of carcinogenesis by investigating associated specific cellular, biochemical and molecular events/targets.