6. Discussion
Part A: Effect of plants under investigation on tumorigenesis and xenobiotic metabolizing and antioxidant enzymes

During past 25 years, there has been a major investment in basic research on cancer. As a result, although the knowledge base is still incomplete, by now there is sufficient understanding of how cancer develops (the process of carcinogenesis) to allow the design of rational new approaches for its prevention and therapy. Considering the very nature of ‘Cancer’ a better approach is the prevention of the disease instead of therapy. This is because, given a genotypic and phenotypic heterogeneity (and extent of the tumor burden characteristic of late stage malignancy) of advanced malignant lesions as they occur in individual patients, one wonders what are the specific molecular and cellular targets for the putative cure. A lesion that is automatically defined in reality will contain many different types of cells, each with its own phenotype and genotype. As a consequence, thoughts that we eventually treat invasive cancer with some sort of single gene therapy, seems naïve. Furthermore, fundamental characteristic of cancer being excessive cell proliferation has led to an over-emphasis of testing and development of cytotoxic drugs used in cancer chemotherapy, these are also highly toxic to a wide spectrum of normal tissues.

As an alternative approach, we need to focus more effort on the control of the disease rather than attempting to cure-end stage disease. Chemoprevention, is a pharmacological approach of intervention in order to arrest or reverse the process of carcinogenesis. However it works at an early stage. If we are to meet the desired goal of wide spread implementation of chemoprevention of cancer, progress will need to occur in four major areas. First we need to continue to enlarge the substrate of basic scientific knowledge of mechanisms of carcinogenesis. Second, we need to conduct more clinical studies to validate specific pharmacological agents for chemoprevention of human cancer. Third, we need to develop new and better agents for eventual use of chemoprevention in men and women. Finally, we need a more broadly based educational effort, directed at both physicians and society as whole, to achieve better understanding and compliance with goals of chemoprevention.
Among the four major areas, the areas of our interest include the first and the
third. Understanding the process of carcinogenesis is the foundation for the science of
chemoprevention and understanding the multi-step nature of carcinogenesis has
evolved through highly controlled carcinogenesis studies. The mechanisms through
which synthetic and naturally occurring compounds mediate protection from cancer is
to be understood, before the effective dietary or pharmacologic strategies can be
promoted to the general population for cancer prevention. Experimental skin and
stomach carcinogenesis model systems in mice are studied extensively. Some of the
medicinal plants and other plant extracts used as food ingredients and beverages are
tested for their preventive potential on the chemical-induced carcinogenesis.
Numerous experimental studies in rodents have identified certain foods and plants
with medicinal value as being effective in eliciting a positive response in site-specific
tumor models (Rao, 1984; Block et al., 1992; Hayatsu et al., 1993; Thorling, 1993;
Rao and Hashim, 1995; Singh, 1999). Central to the defensive armamentarium against
the toxic chemicals, is xenobiotic metabolism. metabolic activation of procarcinogen
by phase I enzymes such as cytochrome P450 dependent mixed function oxidases
which catalyze oxidation, hydroxylation, reduction and hydrolysis. These enzymes
can convert procarcinogen into ultimate carcinogen most of which are reactive
electrophiles. The multistep activation process of procarcinogens by phase I enzymes
is however inhibited by phase II enzymes such as UDP-glucuronyl transferase,
sulfotransferases and glutathione S-transferase which primarily facilitates destruction
of reactive electrophiles and oxidants into innocuous, excretable metabolites.
Oxidative stress ultimately leads to cancer and antioxidant enzymes like SOD, CAT,
GPx and GR play an important role in protecting the cell from reactive oxygen
species. In our present study we have assessed the tumor preventing potential of
Withania somnifera, Piper longum, Hippophae rhamnoides, Decalepis hamiltonii and
Micrococus paniculata taking DMBA induced skin papillomagenesis and B(a)P
induced forestomach papillomagenesis as the two model systems in Swiss albino
mice. In the process of understanding carcinogenesis and the action of these phyto
extracts on the tumorigenesis we have tested the activity of xenobiotic metabolizing
enzymes.
Chemopreventive agents have been divided into two broad groups, depending on whether or not the compound is perceived to act before, or after, the mutagenic steps of the carcinogenic process (Wattenberg, 1985). Compounds that act post mutagenesis have been termed suppressing agents and these include retinoids, indoles, carotenoides and non steroidal anti-inflammatory drugs. Such compounds operate in a diverse manner e.g., by enhancing apoptosis or inhibiting the activation of oncogenes. Compounds that prevent mutagenesis have been termed ‘blocking agents’ and these include flavones, coumarins, phenols and terpenes (Hayes et al., 1998). Effective cancer prevention can be achieved by identifying and eliminating carcinogenic substances from our environment, by introducing blocking and suppressing agents in our food. Bifunctional inducers are capable of inducing both phase I and phase II enzymes whereas monofunctional inducers only increase the expression of phase II enzymes. Dual-acting agents are yet another group that inhibit the expression of phase I enzymes but induce phase II enzymes (Manson et al., 1998). In our present study on the modulator induced activity of xenobiotic metabolizing enzymes, Hippophae rhamnoides, Piper longum, Withania somnifera can be placed under the category of ‘dual acting agents’.

**DMBA induced skin tumorigenesis and inhibitory effect of plants under investigation on tumorigenesis:**

The development of basic strategies for producing optimal intervention studies can be markedly facilitated by the use of animal studies mimicking the neoplastic process occurring in humans. Skin, the largest organ in the body, serves as a protective barrier against the deleterious effects of environmental factors. A plethora of studies have shown that exposure of skin to environmental agents, chemicals such as polycyclic aromatic hydrocarbons, nitrosamines contribute significantly to skin related disorders, specifically nonmelanoma human cancer such as basal cell carcinomas (BCCs) and squamous cell carcinomas (SCCs) (Muktar et al., 1996). As the cases of human cancers are increasing every year, so do the efforts of a number of laboratories to investigate and understand the causative factors and the cellular, biochemical and molecular mechanism(s) involved in human skin cancers. Not only
humans, but mice also get skin cancer fairly easily on exposure to several chemicals; rats however are quite resistant (Lahir et al., 1999).

Experimental carcinogenesis in mouse skin was pioneered by the works of Mottram and Berenblem (Mottram, 1944; Berenblem and Shubik, 1947). The demonstration of multistep nature of chemical carcinogenesis in mouse skin was greatly facilitated by the synthesis of pure polycyclic aromatic hydrocarbons like DMBA and TPA a potent tumor promoter from croton oil. In the experiment conducted at present, to develop skin tumors DMBA was used as the initiator, and croton oil as the promoter. Withania somnifera and Decalepis hamiltonii were found to be effective in decreasing DMBA induced papillomagenesis at both lower and higher doses of treatment. Aselu and pipili were effective in preventing tumor only at one dose of treatment. The glycowithanalides from Withania somnifera have shown to possess antioxidant potential and Withaferin-A, a steroidal lactone was proved to have antitumor and radiosensitizing effects on Ehrich ascites carcinoma in vivo (Bhattacharya et al., 1995; Sharda et al., 1996). In our present study also Withania somnifera and Decalepis hamiltonii significantly induced phase II enzymes, antioxidant enzymes and decreased lipid peroxidation levels which might be one of the reasons contributing to their cancer chemopreventive potential. Hippophae rhamnoides did not decrease DMBA induced skin papillomagenesis though there were quite a number of reports showing it as an effective agent in curing skin wounds. The skin wounds induced by carbopol gel were significantly healed by the application of Hippophae rhamnoides extract. A single subcarcinogenic dose of DMBA produces no tumors but, after metabolic activation of DMBA to its ultimate carcinogenic form (DMBA 3,4-diol-1,2-epoxide) it binds to N\(^6\) position of adenine in DNA, to form N\(^6\)-7,12-DMBA diol epoxide-deoxyadenosine adduct (Agarwal and Mukthar, 1991; Dipple et al., 1983; Miller and Miller, 1977). C-ras\(^{Ha}\) proto-oncogene mutation spectra of carcinogen-DNA adduct showed that N\(^6\)-7,12-DMBA diol epoxide generates A:T→T:A transversions (Perchellet and Perchellet, 1989).

B(a)P induced forestomach papillomagenesis and inhibitory effect of plants under investigation on tumorigenesis:

Benzo(a)pyrene has been used as a model carcinogen from past many years to unravel the mechanism of chemical carcinogenesis. It is widely distributed in the
environment and therefore likely to be relevant to cause cancer in man. B(a)P is used widely in experimental carcinogenesis to induce skin, lung, forestomach tumors (Reddy et al., 1992; Lan and Zheng, 1992). Metabolism of B(a)P in the system converts the procarcinogen form to ultimate carcinogenic form. This metabolic activation occurs by several pathways. In one pathway catalyzed by cytochrome P450 (CYP)-dependent mono-oxygenases and epoxide hydrolase, first a non-K region trans-dihydrodiol (proximate carcinogen) is formed, which is then converted to anti-diol epoxide (ultimate carcinogen), which alkylates DNA. B(a)P is metabolized to an ultimate carcinogen named anti-7,8-di-hydroxy-9,10 epoxy-7,8,9,10-tetrahydro benzopyrene (BDPE) which alkylates the N^2 group of guanosine (Cooper et al., 1983; Thakker et al., 1985). An alternative route of trans dihydrodiol metabolism is that, it is catalyzed by dihydrodiol dehydrogenase, involves the formation of ketols, which enolize to catechols that undergo auto-oxidation to form the corresponding PAH-orthoquinones (Flowers-Geary et al., 1996). The deleterious consequences of the formation of PAH-ortho quinone are the generation of reactive oxygen species and ortho-semiquinone anion radicals (Flowers-Geary et al., 1992). The primary enzyme complex catalyzing the benzo(a)pyrene metabolism is NADPH-dependent CYP mixed function oxidases. Among the CYP isoenzymes, the one most relevant to benzo(a)pyrene metabolism is CYP1A1 (Sun et al., 1995; Singh et al., 1998) and other isoforms being CYP1A2, CYP2A, CYP3A (Fukuhara et al., 1999).

Our results show *Withania somnifera* to be very effective in reducing benzo(a)pyrene induced forestomach papillomagenesis. The lower dose of treatment is found to be more effective as it reduced the tumors by 90% and the higher dose by 76%. Disufiram inhibits dimethylhydrazine induced neoplasia of large bowel by inhibiting the activation of carcinogen (Wattenberg, 1975). Unlike curcumin that has proven to inhibit benzo(a)pyrene induced fore stomach papillomagenesis by an induction of an isoform of P450, CYP1A1 accompanied by hepatic induction of GST (Singh et al., 1998) diallyl sulphide, a potent chemopreventive agent inhibited the Phase I enzymes. Potential of thymoquinone the main constituent of the volatile oil of *Nigella sativa* seed, as a powerful chemopreventive agent against BP-induced forestomach tumours in mice may be through its antioxidant, coupled with enhancement of detoxification processes (Badary et al., 1999). Though there was no
significant change in cytochrome P450 activity in Withania treated animals, a significant induction of GST activity combined with its antitumor effect may be one of the factors contributing to a drastic decrease in forestomach papillomagenesis. Hippophae rhamnoides attenuated tumor burden in a dose dependent manner, lower dose of treatment showed a decrease by 63% and higher dose by 73%. Hippophae rhamnoides is used for the treatment of stomach and duodenal ulcers (Nikitin, 1989). Research in the late 1950’s and early 1960’s reported that 5-hydroxytryptamine (hippphan) isolated from seabuckthorn bark inhibited tumor growth. A significant dose dependent attenuation by 27% and 33% in lower and higher doses of treatment respectively, is observed in the animals treated with pipi in diet. Piperine (piperoylpiperidine) is an alkaloid present in piper species like black and long peppers (Piper nigrum and Piper longum). ‘Kilimari’, which contains Piper longum as one of its ingredients, is widely used as a cure for jaundice (Shirwaiker et al., 1992). Its hepatoprotective effect may be responsible for inducing xenobiotic metabolizing enzymes that metabolize PAHs like B(a)P.

Role of Phase I enzymes on tumor inhibition:

Cytochrome P450 mono-oxygenase system constituting cytochrome P450, cytochrome b5 and respective reductases is the major enzymes system that is involved in the conversion of a procarcinogen to either ultimate or sometimes nontoxic forms. The metabolic activation of DMBA is caused by NADPH and cytochrome P450 dependent microsomal mixed function oxidases that are induced in the epidermis (Jaonnides and Parke, 1987; Bowden et al., 1974). Apart from CYP1A1 which is involved in the metabolism of PAHs, CYP1B1, a member of the ‘B’ family of CYP enzymes have been cloned and shown to play a major role in metabolism of DMBA. Except for the slight increase in the activity of cytochrome P450 in the animals treated with Micrococcus paniculata, all other plant extracts decreased the activity of cytochrome P450 monoxygenase system rather significantly.

In skin tumorigenesis, PAH dihydrodiols are cooxidised by prostaglandin endoperoxide synthetase which plays an important role in the oxidative activation of carcinogenic metabolites in tissue with NADPH-dependent mixed function oxidase activity (Marnett, 1981). Cytochrome P450 dependent epoxidation is not absolutely
required for the metabolic activation of low tumor initiating doses of DMBA in mouse skin (Marnett, 1987). But for B(a)P activation cytochrome P450 monooxygenase system is essentially required. The 7,8-epoxide derivative formed by CYP is converted to its dihydrodiol by the action of epoxide hydrolase. Then it may either directly be eliminated by conjugation to glucuronic acid or sulfate, oxidation to catechol or it may be further oxidized to its higher mutagenic B(a)P 7,8-dihydrodiol 9,10-oxide by CYP or prostaglandin synthase (COX).

Role of GST in tumor inhibition:

All the plant modulators under study showed a significant induction of phase II enzyme -GST and DTD. A significant attenuation of both DMBA induced skin and B(a)P induced forestomach papillomagenesis by Withania somnifera treatment may be attributed to induction in the activity of the phase II enzymes. B(a)P-induced forestomach papillomas were reduced considerably in Piper longum treated animals. Piper longum is used in the treatment of giardiasis and abdominal discomfort (Agarwal et al., 1994). Moreover Piperine, an active principle in many Piper sp prevented the depletion of GSH and total thiols in the intoxicated mice (Koul and Kapil, 1993). At the higher dose of treatment, Micrococcus paniculata and at lower dose of treatment Decalepis hamiltonii were effective in inhibiting both forestomach and skin papillomagenesis. There were no earlier reports proving their chemopreventive potential. Both of them showed a significant induction in GST and DTD activities. Cancer chemoprevention actions of phenolic antioxidants and 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline (ethoxyquin) principally appear to exert their beneficial effects through induction of phase II drug-metabolizing enzymes such as GST (Hayes et al., 2000).

Glutathione S-transferases are important safe guards of the organism against chemical carcinogenesis. GSTs conjugate the endogenous nucleophile GSH to a large array of electrophilic compounds. Many potent antioxidants cause differential and tissue specific induction of GST isoenzymes in rodents and possibly in other species (Sharma et al., 1993; Singh et al., 1985). Butyl hydroxy toluene (BHT), a potent antioxidant is found to induce Ya-type subunits that belong predominantly to α-class of GSTs, in rats (Awasthi et al., 1984). Studies on rat liver GST has shown that rat
liver GSTs have shown binding of Ya subunit to activated metabolites of B(a)P indicating that BHT treatment may result in enhanced detoxification/removal of carcinogen. Studies on the effect of BHA on mouse lung, where its protective effect on B(a)P-induced carcinogenesis is well documented, it has been shown that this antioxidant preferentially induced those GST isozymes that showed substrate preference to B(a)P epoxides (Singh et al., 1987). Reduced mutagenic response to DMBA and AFB1 (aflatoxin B1) in Fischer-344 rat hepatocytes has been correlated to the induction of GST by BHA without any effect on cytochrome P450 (Rogers et al., 1990). In vivo investigations in lung and forestomach of mice have shown that the induction of GST by antioxidants and other compounds is associated with the formation of benzo(a)pyrene diol-epoxide DNA adducts (Anderson et al., 1981) and also with the inhibition of B(a)P induced neoplasia (Sparnins and Wattenberg, 1981). GSTs may noncovalently bind the carcinogenic metabolites of PAHs. Such a noncovalent binding traps electrophiles and decreases their steady state levels (Ketterer, 1982). They catalyze the conjugation between electrophilic metabolites and GSH. B(a)P, 3-hydroxy B(a)P and the dihydrodiols (in the positions 4,5-, 7,8-, 9,10-) bind noncovalently to transferases. Among epoxides tested BP-4,5-epoxide is a good substrate for GST (Smith et al., 1980). The anti isomer of B(a)P-7,8 dihydrodiol, 9,10-epoxide presumed ultimate carcinogen of B(a)P, has also been shown to be a substrate for GSTs (Cooper et al., 1980). In the skin detoxification of carcinogens and other hydrophobic agents bearing electrophilic sites also proceeds via conjugation with GSH catalysed by GSTs. GSTs express peroxidase activity towards lipid and DNA hydroperoxides (Ketterer and Tan, 1987). It is therefore imperative that the remarkable induction of these isozymes by antioxidants that are potent chemopreventive agents should result in a significant enhancement of detoxification of the electrophilic xenobiotics and their activated metabolites, as well as reactive oxygen species and propagators of lipid peroxidation (hydroperoxides) generated during the metabolism of xenobiotics (Awasthi et al., 1996).

Azodyes, B(a)P and other PAHs can be placed under bifunctional inducers, that induce both phase I and phase II enzymes (Prochaska and Talalay, 1988). Bifunctional inducers bind to a cytosolic receptor (Ah receptor) and the resultant complex upregulates the expression of GST isozymes (Rushmore and Pickett, 1993).
The antioxidant element (ARE) in rat and electrophile response element (EpRE) in mouse is implicated in the upregulation of GST through which the monofunctional inducers can directly induce GSTs without being metabolized by cytochrome P450. A relationship between decreased GST expression and increased risk of cancer has been proved in many cases. Blood lymphocytes from individuals with a family and personal history of colon cancer, or a personal history of colon polyps, were found to have significantly lower levels of GST activity than those of healthy controls (Szarka et al., 1995). The μ-class isoenzyme (GSTM1) is absent in 40-60% of the general population due to gene deletion (Seidegard et al., 1986). This polymorphic expression when combined with the ability of M1 to inactivate highly reactive environmental epoxides such as the aflatoxin B1-8, 9-endoperoxide and B(a)P-4,5oxide has prompted a detailed investigation of the role of the null genotype in determining personal susceptibility to various cancers (Raney et al., 1992; Warholm et al., 1983).

Role of DTD in the inhibition of tumorigenesis:

DT-diaphorase protects cells against the deleterious effects of redox cycling of quinones, their ability to deplete GSH and to produce neoplasia (Dinkova-Kostova and Talalay, 2000). This enzyme can detoxify chemically reactive metabolites like quinones, quinone imines and nitrogen oxides. Quinonoids are formed as intermediates during phase I metabolism of various carcinogens. The toxicity of quinones is generally attributed to the formation of semi quinones either enzymatically or non-enzymatically, and their subsequent reaction with molecular oxygen, resulting in the formation of superoxide anions and regeneration of quinones (Begleiter et al., 1997). Endogenous quinones, semiquinones and hydroquinones in both the electron transport chain of mitochondria and microsomal mixed function oxidase system can initiate redox cycling via enzymatic reduction and concomitant auto-oxidation to generate superoxide anion and its dismutation product H2O2, which may trigger cell damage, toxicity and carcinogenesis (Sies, 1988).

Dithiolthiones, occurring naturally in cruciferous vegetables and oltipraz a potent chemopreventive agent is an inducer of DTD and other phase II enzymes too (Kensler et al., 1987). Isothiocyanates, present in broccoli induced DTD and GST activity in mouse cells and tissues in vivo and in vitro (Zhang et al., 1992) and
blocked the formation of DMBA induced mammary tumors in rats (Zhang et al., 1994). Several laboratories have reported the presence of a homozygous C→T transition at position 6.9 of NAD(P)H : quinone oxidoreductase gene (NQO1). This mutation, which occurs in population at a frequency of 6% - 17%, results in the loss of NQO1 protein and its activity. This mutation was originally detected in human colon carcinoma cell lines and also in renal carcinoma cell lines (Rosvold et al., 1995; Elckelmann et al., 1994; Traver et al., 1992).

A large amount of data demonstrate that isothiocyanates act as cancer chemopreventive agents by favorably modifying carcinogen metabolism via inhibition of Phase 1 enzymes and/or induction of Phase 2 enzymes. These effects are quite specific, depending on the structure of the isothiocyanate and carcinogen. One of the most thoroughly studied examples of isothiocyanate inhibition of rodent carcinogenesis is inhibition of 4-ethylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced lung tumorigenesis by phenylisothiocyanate (Hecht, 1999). Hippophae and Aselu showed a dose dependent induction in GSH levels. Withania somnifera and Piper longum showed induction only in a single dose of treatment. A significant induction in GSH levels is observed in animals treated with Decalepis hamiltonii though not dose dependent. There are no earlier reports of Decalepis hamiltonii proving its cancer preventive or therapeutic effects except for one, showing it as a blood purifier (Wealth of India, 1989). Piperine, an active component of Piper longum exerted a significant protective effect against tert-butyl hydroperoxide and CCl4, ethyl alcohol etc., (Cheng et al., 1992). It is important to recognise that induction of GSTs alone may not be sufficient to gear up detoxification of electrophilic metabolites, because the physiological substrate, GSH would also have to be induced to cope with the enhanced conjugation of electrophiles. Several studies have shown that many potent antioxidant chemopreventive agents cause elevation of GSH levels in liver and extrahepatic tissues (Singletary, 1990; Partridge et al., 1983).

Role of antioxidant enzymes and parameters in the inhibition of tumorigenesis:

Physiologic, toxic or infectious events leading to an increased generation of ROS or non-oxidants or electrophilic can lead to an increased rate of accumulation of mutations in DNA (Ames et al., 1993; Newmark, 1984). There are a vast array of
enzymes that either in course of their normal cell functions or sometimes inadvertently generate ROS in vivo. Cellular pro-oxidant states appear to play an important role at clinical steps in the process of carcinogenesis especially skin (Kensler and Taffe, 1986; Nishimura and Ames, 1986). The major intracellular antioxidant defense relies on enzymatically removing superoxide and H$_2$O$_2$ hopefully before they reach the iron catalysts and form hydroxyl and ferryl radicals that are detrimental to the system. The major antioxidant enzymes scavenging the ROSs are SOD, CAT, GPx and GR whose activity is responsible in attenuating lipid peroxidation levels. In the present study Hippophae rhamnoides induced all the four antioxidant enzymes very significantly except for CAT activity in high dose treated animals. Hippophae rhamnoides is an antioxidant by itself due to the presence of vitamins like A, C, E and it induces endogenous antioxidants too. Hippophae rhamnoides like vitamin E was found to be a potent antioxidant and strongly decreased malondialdehyde content in cultured smooth muscle cells and hyperlipidemic rabbit serum and protect the cells from the injury of lipid peroxidation, thus keeps the smooth muscle cells growing and proliferating healthily. One of the important mechanisms of Hippophae rhamnoides in anti-atherosclerosis reported recently may be closely related to the action of anti-lipid peroxidation (Wang et al., 1992). Stress induced in rabbits and mice by lipopolysaccharide (LPS) and proteoglycan (PGN) showed decreased lipid peroxidation levels by the administration of the aqueous extract of Ashwagandha (Dhuley, 1998).

Reactive intermediates leads inescapably to the conclusion that our body tissues, more specifically the skin, are continuously exposed to dangerously reactive free radicals and other intermediates. SOD, catalyses the dismutation of O$_2$ to H$_2$O$_2$. Level of SOD is much lower in skin than in other tissues such as heart, liver and kidney, these data suggest that other antioxidant defense systems like thioredoxin reductase and GPx are more important in skin (Carraw and Pathak, 1988). However SOD activity is reported to decrease in various hyperproliferative keratinocytes including SCC, basal cell epithelioma, and benign hyperproliferative skin disorders such as psoriatic epidermis (Kobayashi et al., 1991). GPx, a Se-dependent enzyme with a cysteine residue at its catalytic site rapidly detoxifies H$_2$O$_2$ and simple alkyl or phospholipid hydroperoxides. Detoxification of H$_2$O$_2$ in cytoplasm is accomplished to
be largely coupled to GPx-GR. Intracellular H$_2$O$_2$ is dismutated to form H$_2$O and O$_2$ by catalase in the presence of glutathione. This enzyme is present in the skin and is depleted by UV exposure and in several skin disorders including vitiligo (Schallreuter et al., 1991).

All the plant extracts under investigation did not show any increase in the lipid peroxidation levels. This is correlating with a simultaneous elevation in the activity of all antioxidant enzymes SOD, CAT, GPx and GR or at least some of them. *Hippophae rhamnoides* showed a dose dependent decrease in lipid peroxidation levels with a significant increase in all antioxidant enzymes. This may be one of the reasons contributing for a significant reduction by 63% and 73% of B(a)P induced forestomach papillomagenesis in lower dose and higher dose treated animals by *Hippophae rhamnoides*. There is no significant change in lipid peroxidation levels of animals treated with pipili but a very significant increase in catalase activity by approximately 2 folds is observed. Aselu showed a very significant decrease in lipid peroxidation levels almost 45% and 42% in low dose and high dose treated animals respectively. *Withania somnifera* and *Decalepis hamiltonii* showed lower lipid peroxidation levels only at higher dose of treatment. The chemopreventive potential of selenium in different forms like sodium selenite, selenocysteine and selenomethionine is observed to be due to induction of various antioxidant enzymes like GSH and GPx (Mukhopadyay et al., 2000).

Either a significant decrease or no change is seen in the activity of LDH. LDH is an indicator of cell damage and an increase in LDH level by any phytochemical treatment shows that the particular dose given may be cytotoxic. Prolonged administration at lower dose levels of *Hippophae rhamnoides*, *Withania somnifera*, *Piper longum*, *Micrococcus paniculata* and *Decalepis hamiltonii* did not induce any toxicity. Toxicity studies of subacute doses of *Withania somnifera* proved to be nontoxic on prolonged administration (Arundhati et al., 1998).

All the plants under investigation showed chemopreventive potential on either DMBA induced skin papillomagenesis or B(a)P induced forestomach papillomagenesis or both. The induction of various xenobiotic and antioxidant enzymes by these plants further supports their chemopreventive efficacy in inhibiting chemical carcinogenesis. So it is necessary to isolate the active principles of all the...
plants now proven to have chemopreventive potential and their inhibitory effects at various stages of carcinogenesis and, their effect at cellular and molecular level should be studied.

**Part B: Effect of Hippophae rhamnoides on transcription factors- NF-κB and IRF-1**

The effect of a large number of phytoextracts and their active principles in prevention of cancer is a recent topic of investigation. The underlying mechanisms through which they act remain still obscure. So in the present study we tried to see the effect of *Hippophae* on B(a)P induced forestromach papillomagenesis on two important transcription factors NF-κB and IRF-1. The logic of selecting of NF-κB for our studies is that NF-κB and AP-1 are two eukaryotic transcription factors that regulate genes implicated in ROS-induced responses and both factors are targets of oxidative stimuli (Toledauo *et al.*, 1991, Muller *et al.*, 1997). Many antioxidants and chemopreventive agents are found to inhibit N-κB activation. For example Pyrrolidine dithiocarbamate (PDTC) has been shown to inhibit TNF-alpha induced NF-κB activation in transformed human endothelial cells (Boweik *et al.*, 1997). N-acetyl cysteine (NAC), another antioxidant, was also reported to suppress NF-κB activation induced by TNF- alpha, LPS or UV in Jurkat and Hela cells (Suzuki *et al.*, 1994). Over expression of Mn-SOD, CAT or GPx reduced NF-κB activation induced by TPA, LPS, TNF-alpha or hydrogenperoxide in human melanoma cells (Manna *et al.*, 1998; Kretz-Renny *et al.*, 1996). Based on the above background information and taking the fact in to consideration that *Hippophae rhamnoides* is rich in vitamins A, E, C and flavanoids, it also induced antioxidant enzymes SOD, CAT, GPx and decreased lipid peroxidation levels. It also induced Phase II enzymes like GST that plays an important role in scavenging free radicals by its peroxidase activity and DTD in reducing semiquinone toxicity.

However in the present study carcinogen, i.e, B(a)P administration lead to the inhibition of NF-kB activity and *Hippophae rhamnoides* treatment induced NF-kB activity. The plausible reasons for this might be
(a) All the previous reports which state NF-kB is inhibited by chemopreventive agents and induced by oxidative stress showed that the experiments have been done in *invitro* condition wherein the present experiment is conducted *in vivo*.

(b) In many earlier reports, the cells were exposed to stress inducing agents for a short period of time lasting for few hours. But the present experiment lasted for 14 days. Modulation of transcription factors exhibits an early and a late response and hence the complex mechanisms involved in its upregulation are yet to be studied.

Secondly we have studied IRF-1, that has been reported to be antioncogenic (Taniguchi et al., 1997). Ectopic overexpression of IRF-1 strongly inhibits cell proliferation in several cell types (Yamada et al., 1990). Induction of IRF-1 by chemopreventive agents *in vivo* is not reported till date. We attempted to see the effect of *Hippophae rhamnoides* on the DNA binding activity of this transcription factor. *Hippophae rhamnoides* treatment caused an increase in the activity of IRF-1 higher complexes. So this increase may be attributed to the involvement of IRF-1 higher complexes in the transcription of some important genes that may be involved in the antioxidant pathway.