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5. Discussion

Use of plants and animals as source of medicine and food is as old as humanity itself. The therapeutic informations from remote past add to the treasures of our medical knowledge. From the large array of hints and claims, the investigator of today has to shift out and verify the best and beneficial, and reject and discard the one not useful. Charaka and Susruta mentioned that medicinal herbs and plants should be recognized and identified with the help of hermits, huntsmen, forest dwellers and those who cull the forest.

Health and disease are coeval with life. Human beings everywhere, at all times and places had to deal with the threat of diseases and illnesses. Health and disease are parameters of the effectiveness with which human groups adapt to their environments. The groups utilized the biological and socio-cultural resources in their way to develop fitness to resist environmental threats and dangers emanating from known and unknown forces and to combat disease, illness and other health hazards arising from a wide range of causative agents and factors. The herbals occupied a distinct place in the life right from the primitive period to today and evidence of rational mastery over these and environment is decipherable, in each period. No wonder if it is said that ethnic populations have their own medical lore, and some of their therapeutic practices have found place in today’s medical knowledge. The therapeutic hints from remote mists of time as well as folk medical lore, even today as in the past, hold key of treasures of medical knowledge.

Age-old drug of Ayurveda, ‘sarpagandha’ used as tranquilizer has become of importance due to spread of tension in our new world. *Vinca rosea*, which was used to make a particular bush-tea in West Indies, is now suggested to have a role in treating diabetes and leukemia. Pre-requisite for such studies is diligent gathering and pooling of the knowledge existing with the tribals and other ethnic groups and also available in the works of various indigenous medical systems.

*Centella asiatica* is commonly used in the Ayurvedic system of medicine and by different tribal populations in India to treat various diseases. Its crude extract has been shown to possess anti-tumor effect which inhibited the proliferation of transformed cell lines and also retarded the development of solid and ascites tumors (Babu *et al.*, 1995).
Red Ginseng is a classical traditional Chinese medicine. Among the Chinese herbs red Ginseng has been considered as one of the tonics. Many studies indicated that red Ginseng could enhance the immune function of the human body. The effects of red Ginseng extracts on transplantable tumors, proliferation of lymphocyte, two stage model and rat liver lipid peroxidation were studied. In a two stage model, red Ginseng extracts have shown a significant cancer chemoprevention. At 50-100 mg/kg body wt., they could inhibit DMBA/croton oil induced skin papilloma, prolong the latent period of tumor occurrence and reduce tumor number per mouse in a dose-dependent manner (Xiaoguang et al., 1998).

Ethnopharmacology is conserved with scientific study of the drugs, traditionally used by small ethnic groups in different parts of the world. In prehistoric era (5000 B.C), the treatment of diseases based upon superstition, religion, magic and witchcraft. This element even today finds its place in treatment, like charm, mantras and other kind of magical remedies for the cure of illness. Therefore, primitive medicine practice by certain group of world population continues.

Ayurveda system is of Indian origin which is practiced throughout India. Charak and Susruta are the celebrated authorities of Ayurvedic medicine, the former being a physician and the latter was a great surgeon. Both were disciples of Atreya. Charak compiled the famous treatise Charak Samhita and Susruta compiled Susruta Samhita, the main source of knowledge of surgery in ancient India. Ayurveda means the knowledge of healthy living. The preventive and curative aspects of Ayurvedic medicine have been given emphasis in it. Therapeutic approaches of Ayurveda are described in four categories which aim to restore the deranged ‘dosha-dathu-mal’ equilibrium to normalcy. This is achieved by the removal of causative factors, dietary manipulation and drug therapy (Ansari and Tiwari, 1998).

The biochemical evidence suggests that oxidative stress caused by accumulation of free radicals are involved in the pathogenesis of several diseases. Appropriate levels of antioxidant nutrients might therefore, be expected to delay or prevent these diseases. Several epidemiological studies have found lower serum levels of antioxidant nutrients in patients with cardiovascular disease, cancer and cataract but there is little evidence that
supplements of antioxidant nutrients prevent disease. More work is needed to establish the role of these antioxidant nutrient supplements in prevention of diseases (Chitale, 1998).

Studies on the hepatoprotective experimental model indicated that carbon tetrachloride is first metabolized by cytochrome P450 in the liver endoplasmic reticulum to the highly reactive 'CCl₃ radical. The free radical, in the presence of oxygen leads to the autooxidation of fatty acids present in the cytoplasmic membrane phospholipids and causes functional and morphological changes in the cell membrane. Furthermore, influx of extracellular calcium ions into cell is claimed to be an important step leading to death. Therefore, the examination of preventive action in liver damage caused by CCl₄ may give an indication of the liver protective action of drugs in general.

Active oxygen species and free radicals are involved in a variety of pathological events, including cancer and aging process. Any compound, natural or synthetic with antioxidant properties that might contribute towards the partial or total alleviation of this damage may have a significant role in maintaining health when continuously taken as components of dietary foods, spices and drugs. Therefore, removing 'O₂⁻ is probably one of the most effective defenses of a living body against diseases.

The roots of *Boerhavia diffusa* commonly known as ‘punarnava’, are used by a large number of tribes in India for the treatment of various hepatic disorders. Its hepatoprotective action has been proved by analyzing the various serum parameters like glutamic oxaloacetic transaminase (GOT) and glutamic pyruvate transaminase (GPT) (Rawat et al., 1997). The aqueous extract of *Boerhavia nivea* exhibited a liver protective effect against CCl₄ induced hepatotoxicity and possessed anti-lipid peroxidative and free radical scavenging activities (Lin et al., 1998).

Nutrients play an important role in the initiation, promotion and progression of cancers although the role of diet in cancer is complex and is continued to be studied actively. Nutrition intervention for the prevention and control of cancer and other chronic diseases are intended to encourage long-term adoption of healthful eating patterns in free-living populations. Micronutrients are chemicals required by the body, again for proper growth, maintenance and functions of the body. They do not directly contribute to the body growth and maintenance but affect the utilization of macronutrients to a significant extent.
Given the increasing evidence of potential for dietary prevention and control of cancer, it is timely to identify avenues for accelerating health promoting nutritional changes through public health policy. Although the exact role of dietary factors and nutritional risk in cancer is still being studied, the evidence is sufficient to warrant concerted efforts to promote healthful dietary behaviour. Although liver is the main organ responsible for drug metabolism, in most species significant activities are present in the extrahepatic tissues including lung, kidney, forestomach, skin and many other tissues. Extrahepatic enzyme induction depends not only on the nature of the inducing agent and the type of tissue but also on the particular test material under investigation.

Induction of drug metabolism may arise as a consequence of increased synthesis, decreased degradation, activation of preexisting components or recombination of these three processes. The inhibition of drug metabolism by xenobiotics or drugs can take place in several ways including the destruction of preexisting enzymes, inhibition of enzyme synthesis or by complexing and thus inactivating the drug metabolizing enzymes. Many xenobiotics (olefinic derivatives such as allobarbital, ethylene, fluoroxene and vinyl chloride; and acetylenic derivatives such as acetylene, ethchlorvynol and norethindrone) have the ability to destroy cytochrome P450 in the liver by a variety of mechanisms including modification of heme. Exposure to environmental xenobiotics may be inadvertent and accidental when they are present as components of air, water and food which is inescapable. Environmental pollutants for e.g. benzo(a)pyrene and other polycyclic aromatic hydrocarbons are present in tobacco smoke and other organic pyrolysis products. These are known to induce cytochrome P450 1A1 and to alter rates of drug metabolism in both experimental animals and in humans. Phenobarbital induces cytochrome P450 2B1; chronic ethanol induces cytochrome 450 2E1; and glucocorticoids and steroids induce cytochrome P450 3A (Monson, 1997).

The use of medicinal plants is based on the experience of many generations of physicians and traditional systems of medicines from different ethnic societies. The use of medicinal plants in modern medicine suffers from the fact that though hundreds of plants are used in the world to prevent or to cure diseases, scientific evidence in terms of modern medicine is lacking in most cases. However, today it is necessary to provide scientific proof as to whether it is justified to use a plant or its active principles (Ammon and Wahl,
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1991). As for as modern drugs are concerned they must be further characterized after their pharmacological screening i.e., pharmacokinetic and pharmacodynamic properties, including toxicity (Kelloff et al., 1994).

Recent upsurge in identifying non-dietary natural products associated with high degree of safety margin as cancer chemopreventive agents, has been hailed by many investigators to be practically beneficial, especially when the carcinogenic insult is mild to moderate. Our present knowledge on chemoprevention of cancer has revealed the presence of a diverse array of naturally occurring bioactive compounds which inhibit carcinogenesis at almost every site (Tanaka, 1994; Morse and Stoner, 1996; Pezzuto, 1997).

_Tinospora cordifolia_ has been known to be beneficial in treating a wide variety of diseases. Many compounds including, alkaloids, bitter compounds, essential oils and a mixture of fatty acids have been shown to contribute to its medicinal properties. _Tinospora_ affords protection against xenobiotic induced liver damage and stimulates liver regeneration (Rege et al., 1984). Furthermore, its immunomodulatory and antitumor activity have been reported by Mathew and Kuttan (1999).

_Andrographis paniculata_ is being used in a number of herbal medicinal preparations. It has been found effective in treating various diseases like jaundice, inflammatory conditions and hepatotoxicity (Deng et al., 1982; Handa and Sharma, 1990; Visen et al., 1991, 1993; Shukla et al., 1992). It has also been shown effective as an immunostimulant (Puri et al., 1993). Reports on induction of infertility in male rats by subchronic doses of this plant have been contradicted by Burgos et al., (1997). Its active principle, andrographolide has been accredited with protective activity against liver disorders and liver damage induced by a number of hepatotoxicants (Bhatt and Bhatt, 1996; Handa and Sharma, 1990).

_Adhatoda vesica_ has been used in treating a wide variety of diseases by folk and Ayurvedic medicine practitioners. This plant is the source of the drug ‘vasaka’, well known in the indigenous system of medicine. A number of different principles like vesicine, vesticinone, vesinol, essential oil (betane), vitamin C, β-carotene and vasakin have been identified as contributing to its medicinal effects. _Adhatoda_ is extensively used for treating cold, whooping cough, asthma and as antihelminthic. Its leaf is also said to cure glandular tumor (Wealth of India, 1989).
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Aloe leaves reportedly have immense medicinal value. It is commonly used as an emollient, purgative, laxative, antibacterial, anaesthetic, antiseptic and for healing the burns and wounds (Chitra et al., 1998). Aloe is suggested to protect the eyes from ultraviolet rays. Recently, Lee et al., (1998) have reported angiogenic activity of Aloe vera gel in vitro. Along with vitamin C supplementation, it was found to reduce the severity of chemical carcinogenesis in rat (Shamaan et al., 1998). In another study, Aloe along with melatonin has been shown to be protective in patients with advanced solid tumors (Lissoni et al., 1998).

Aegle marmelos leaves are used for worship and its edible fruits are valued in indigenous medicine. Besides fruits, its leaf, root, bark and seed are also valued in traditional system of medicine. Its root is an ingredient of the 'dasmula' (ten roots), a medicine commonly used by Ayurvedic practitioners. It is also used as a remedy for ophthalmia, ulcers and beriberi associated with weakness of heart. Aqueous and alcoholic extracts of Aegle possess cardiotonic effects and is found to be effective as insulin in restoration of blood glucose and body weight to normal levels in diabetic rats (Seema et al., 1996). Its essential oil has a broad spectrum of antibacterial and antifungal activity (Pattnaik et al., 1996; Rana et al., 1997).

Clerodendrum inerme is a green shrub and is cultivated as hedge plant. It contains iridoid glycosides and sterols. Two proteins isolated from Clerodendrum leaves, shown to induce systemic resistance against viruses and are also found to have ribosome inactivating properties (Olivieri et al., 1996). This plant remained almost unexplored for its various pharmacological effects.

Lawsonia alba is widely used for colouring palms and feet and dyeing silk and wool. Its leaves are also used against skin diseases, boils, burns, bruises and skin inflammation. The crude ethanolic extract of Lawsonia has been reported to have significant and dose dependent anti-inflammatory, analgesic and antipyretic effects in rats. This extract has also been shown to possess hepatoprotective activity against CCl4-induced liver toxicity (Anand et al., 1992). Tuberculostatic activity of henna has been reported in vitro as well as in vivo in guinea pigs and mice (Sharma, 1990).

Prosopis juliflora is commonly known as mesquite bean, the pollen of which can cause serious pollinosis and allergic asthma (Matheson and Travis, 1998). One of its
alkaloids, julifloricine is shown to be associated with antimicrobial property (Aqeel et al., 1989). Yaniv et al., (1987) has reported that \textit{Prosopis} is used for curing diabetes in Israel. One of its constituents, oleanolic acid has been shown to inhibit HIV-I replication \textit{in vitro} (Kashiwada et al., 1998).

\textit{Decalepis hamiltonii} is considered to be an appetizer and blood purifier. A volatile principle responsible for the aroma and taste of \textit{Decalepis} root is 4-O-methylresorcyraldehyde which also possesses bacteriostatic and toxic properties (Wealth of India, 1989). The root also contains inositols, saponins, tannins, a crystalline resin acid, an amorphous acid, a ketonic substance and sterols (\(\alpha\)- and \(\beta\)-amyris and lupeol both free and as esters) which might contribute to its medicinal properties.

In present study, an attempt to investigate cancer chemopreventive potential of \textit{Tinospora cordifolia}, \textit{Andrographis paniculata}, \textit{Justicia adhatoda}, \textit{Aloe vera}, \textit{Aegle marmelos}, \textit{Clerodendrum inerme}, \textit{Lawsonia alba}, \textit{Prosopis juliflora} and \textit{Decalepis hamiltonii} has been made by evaluating their role, if any, in intervening carcinogenesis process by appreciably inducing detoxification (glutathione S-transferase, DT-diaphorase and antioxidant enzymes) and/or blocking activation enzymes viz., microsomal hemeproteins and cytochrome P450 dependent mixed function oxidases. Extrapolating the results obtained, would highlight mechanistic insight to chemopreventive and hepatoprotective activity associated with these plants.

A growing body of evidences has been implicating oxidative stress, a consequence of normal metabolic function that produces oxygen radical, as an important pathogenic factor in the development of many human diseases including cancer. Chemoprevention strategy to prevent cancer owes its origin to epidemiological observations wherein, high intake of fruits and vegetables have been linked to reduced cancer risk. Cancer chemoprevention is an exciting pharmaceutical cancer research involving the use of either natural or synthetic components to delay, inhibit or reverse the development of cancer in normal or preneoplastic conditions. Thus, additionally, the role of these plant extracts in affording protection, if any, against oxidative damage as evaluated by determining the activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and main non-enzymatic antioxidant-GSH quantification have been pursued.
The findings of the present investigations are based on the examination of inducibility of enzymes involved in the metabolism of xenobiotics including carcinogens. Since inducibility of drug metabolizing enzymes is one of the reliable markers for evaluating the chemopreventive potential of the test materials in murine model system, by measuring their modulatory effects on phase I and phase II enzymes and antioxidative parameters. 80% ethanolic extract was used as test material except for Aloe in which crude leaf extract was used and treatment was given through oral gavage. There were no easily discernible adverse effects on the animals at the given dose levels (dose I and dose II: 50 and 100 mg of extract/kg body weight of mice/day for 14 days; for Aloe 30 and 60 μL of extract/animal wt. 25 gms/day for 14 days) of modulators. BHA was used as positive control to validate the authenticity of assay protocols and response to modulators in our animal model system. It can be deduced from the fact that there was neither any increase in the rate of mortality nor any decrease in the body weight of animals following treatment with all the modulators studied. Furthermore, the liver-somatic index which is the ratio of liver weight to the final body weight of animal was found either increased or remained unaltered indicating mostly favourable effect of modulators on general body metabolism. Also, at cellular level there is no indication of damage as observed by the measurement of lactate dehydrogenase activity as an index of cell damage; in most of the cases its activity was reduced from the control value showing the possibility of protection against damage caused during normal metabolic processes. Even the higher dose (100 mg/kg body wt./day) that we investigated had a safety margin sufficiently distant from toxic range. The information regarding the doses of modulators and their toxicity to animals, are very crucial, since chronic treatment with any agent for achieving chemopreventive prophylaxis should be free from all undesirable side effects.

Microsomal cytochrome P450 system which is a product of the CYP super family of genes, constitutes a major electron transport chain in the membrane of endoplasmic reticulum. It plays a key role in oxidative activation, inactivation and promotion of excretion of most xenobiotic compounds and also in modulating the duration and intensity of their toxicity (Guengerich, 1988; Miller, 1988). Cytochrome P450 catalyzes the oxidation of lipophilic chemicals through the insertion of one atom of molecular oxygen into substrate thereby rendering the latter to either less harmful or totally harmless.
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hydrophilic metabolites for their ultimate removal from living system. Mammalian cytochrome P450s with which we are primarily concerned, have less catalytic specificity and seem to have general function of removing accumulated materials from the body.

In the present study, an increase in cytochrome P450 by Tinospora, Andrographis, Adhatoda, Clerodendrum, Aegle and Decalepis was observed whereas Aloe, Lawsonia and Prosopis exhibited a decrease in total cytochrome P450 activity. Thus, the investigated plant extracts by virtue of their action as inducers of cytochrome P450 may be speculative of acting as blocking agents. They increase the production of activating metabolites at resistant non-target tissues or by enhancing oxidative detoxification at any tissue site. It may be referred in this context that indole-3-carbinol is a potent inducer of cytochrome P450 enzyme and has chemopreventive activity in a number of animal models (Vang et al., 1999). Thus, modulation of cytochrome P450 by plant extracts could well be an environmental determinant in addition to genetic factors for an individual to metabolize and activate carcinogens. Indeed, it is recognized that main function of phase I metabolism is to prepare a compound for phase II metabolism and not to prepare the drug for excretion. Phase II is usually the true detoxification of drugs and xenobiotics which gives products that are generally water soluble and can be easily excreted.

Out of nine plant modulators studied, only Adhatoda significantly increased the levels/activities of all the measured components of cytochrome P450 system (cytochrome P450, cytochrome b5, NADPH-cytochrome P450 reductase and NADH-cytochrome b5 reductase). A non-specific increase in different components of cytochrome P450 system by Tinospora, Andrographis, Aegle, Clerodendrum, and Decalepis was observed. Aloe and Lawsonia significantly decreased the level of both cytochrome P450 and cytochrome b5 while Prosopis decreased the level of cytochrome P450 only. During oxidative metabolism, in the microsomal microenvironment involving the cytochrome P450 system, the electron flows from NADPH or NADH through a flavoprotein cytochrome P450 reductase or cytochrome b5 reductase to different isomorphic forms of cytochrome P450 and cytochrome b5 (Gibson and Skett, 1994). Cytochrome P450 is the terminal oxidase component of an electron transport system present in the endoplasmic reticulum responsible for many drug oxidation reactions. Since both of these electron transport chains (cytochrome P450 and cytochrome b5) are interlinked, the inhibition in any of the
components of a particular chain, either cytochrome P450 or b5 does not influence much the oxidative metabolic processes carried out by this system (Estabrook and Cohen, 1971). Since no inhibition in the level of cytochrome P450 or cytochrome b5 and in the activity of cytochrome P450 reductase or cytochrome b5 reductase by the extract of Tinospora, Adhatoda, Aegle, Clerodendrum and Decalepis was noticeable, it may be speculated that no inhibition in microsomal electron transfer, critical for cytochrome P450 functional capacity, is operational. Andrographis decreased the level of only cytochrome b5 while Prosopis decreased the level of only cytochrome P450. This indicates that in these cases the source of electron (NADPH or NADH) may be the limiting factor in the operation of either of the two electron transport chains. Aloe and Lawsonia inhibited the levels of both cytochrome P450 and cytochrome b5. This observation may be indicative of inhibition of oxidative metabolic processes which may be involved in blocking the activation of xenobiotic compounds including carcinogens.

Under the present experimental conditions, all the modulators studied, showed a significant elevation in the activities of glutathione S-transferase and DT-diaphorase in the liver of mice. Both these enzymes belong to phase II enzyme system. Thus, according to Wattenberg (1985), Tinospora, Andrographis, Adhatoda, Aegle, Clerodendrum and Decalepis can be classified as bifunctional/Type B inducers as they induce both phase I and phase II enzyme systems; whereas Aloe, Lawsonia and Prosopis can be classified as monofunctional/Type A inducers as they induce only phase II enzymes. All modulators induced the activities of glutathione S-transferase and DT-diaphorase in most of the extrahepatic organs (lung, kidney and forestomach) studied. Andrographis and Aegle, in lung; Tinospora and Adhatoda in kidney; and Clerodendrum in forestomach did not induce glutathione S-transferase, while Tinospora in lung and kidney; Clerodendrum in lung and forestomach; and Aloe in lung did not show any significant alteration in the activity of DT-diaphorase.

The action of phase II enzymes on the substrates generated by the action of phase I enzymes on innocuous and/or hazardous chemicals leads to their solubilization and excretion (Gibson and Skett, 1994). Glutathione S-transferase is a critical detoxification enzyme that primarily functions in conjugating ‘functionalized P450 metabolites’ with endogenous ligands (reduced glutathione) favouring their elimination from the body of the
organisms (Hartman and Shankel, 1990). There are persuasive evidences to support the induction of glutathione S-transferase and protection against a wide spectrum of cytotoxic, mutagenic and carcinogenic chemicals (De Flora and Ramel, 1989; Ketterer, 1988; Reed, 1990).

Molecular forms of glutathione S-transferase having different substrate specificities have been identified to be present in the cytosol (Mannervik and Danielson, 1988). We used CDNB as non-specific substrate in our assay for glutathione S-transferase. Thus, the specific activity of the enzyme measured was the sum of all of its isoforms. The protective effect of many naturally occurring chemopreventive agents against carcinogenesis have been ascribed to decreased bioavailability of potential DNA damaging entities and their destruction into excretable metabolites facilitated through induction of glutathione S-transferase (Coles and Ketterer, 1990).

DT-diaphorase is generally induced coordinately with other phase II detoxifying enzymes (Talalay, 1989). Induction of DT-diaphorase has been evaluated as a means for determining the potency of many anticarcinogenic substances (Benson et al., 1980; De Long et al., 1986). This enzyme protects against the toxicity of quinones and their metabolic precursors viz., polycyclic aromatic hydrocarbon, benzene, etc. (Chesis et al., 1984; Smart and Zannoni, 1984; Karczewski et al., 1999). Induction of DT-diaphorase facilitates bioreductive activation metabolism of quinones by two electron oxido-reduction of quinone to hydroquinone, obliterating semiquinone radical and subsequent oxygen radical production. The stable hydroquinone resulted from two electron oxido-reduction of quinone by DT-diaphorase can be conjugated by glucurinide or sulfate and excreted and thus affords protection from reactive intermediates (Lind et al., 1982; De Long et al., 1986; Talalay, 1989).

It is evident from the present findings that Tinospora, Andrographis, Adhatoda, Aegle, Clerodendrum and Decalepis act as bifunctional enzyme inducers as they induce both phase I as well as phase II drug metabolizing enzyme systems. This reinforces the balance of xenobiotic metabolism towards detoxification and therefore, might be attributed to playing a major role in cytoprotection and chemoprevention. Animals and most probably human beings may be extremely sensitive to activation requiring mutagens and
carcinogens, particularly when an increase in phase I enzyme activities is not followed by an increase in phase II enzyme activities.

The study also reveals that all the plant extracts tested, can significantly attenuate oxidative stress by modulating cellular enzymatic and non-enzymatic antioxidant defense system. Antioxidant responsiveness mediated by these plants was judged by their efficacy to modulate the basal level of reduced glutathione and the specific activities of glutathione peroxidase and glutathione reductase in the liver of mice; and superoxide dismutase and catalase in liver as well as extrahepatic organs (lung, kidney and fore-stomach).

The pathophysiological consequences owing to depletion of GSH has been well studied. The depletion of GSH promotes generation of reactive oxygen species and oxidative stress with cascade of effects thereby affecting functional as well as structural integrity of cell and organelle membranes (De Leve et al., 1996). The elevated level of GSH protects cellular proteins against oxidation through glutathione redox cycle and also directly detoxifies reactive oxygen species and/or neutralizes reactive intermediate species generated from exposure to xenobiotics including chemical carcinogens (Ketterer, 1998).

The increased glutathione reductase level as observed with all the modulators except Prosopis, plays a significant role in the reduction of oxidized glutathione to reduced glutathione at the expense of NADPH and regulates GSH-GSSG cycle in the cell (Vanoni et al., 1991). Its inhibition is likely to be deleterious to cells since it contributes in efficiently maintaining the basal level of cellular GSH (Lopez-Barea et al., 1990; Meister, 1994). It has been reported that depletion of GSH up to 20-30% of basal level impairs host defense against toxicants leading to cell injury (Reed, 1990). Thus, GSH has been endowed with an important function in maintaining the reducing milieu of cells, in addition to its conjugating ability owing to nucleophilic center and is involved in detoxification of xenobiotics that cause toxicity and carcinogenicity.

The antioxidant responsiveness mediated by Tinospora, Andrographis, Adhatoda, Aloe, Aegle, Clerodendrum, Lawsonia, Prosopis and Decalepis, as evaluated by their efficacy to modulate basal level enzyme activities of glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase may be anticipated to have biological significance in eliminating reactive free radicals that may otherwise affect the normal cell functioning. The disfunctioning of these antioxidant enzymes has been implicated in
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several disorders including rheumatoid arthritis, reperfusion injury, cardiovascular diseases, immune injury as well as cancer (Gonzalis et al., 1984; Guemouri et al., 1991; Flagg et al., 1993; Saydem et al., 1997). These enzymes have been suggested as playing an important role in maintaining physiological levels of oxygen and hydrogen peroxide by hastening the dismutation of oxygen radicals and eliminating organic peroxides and hydroperoxides generated from inadvertent exposure to xenobiotics and drugs.

It has been proposed that glutathione peroxidase is responsible for the detoxification of hydrogen peroxide in low concentration whereas catalase comes into play when glutathione peroxidase pathway is reaching saturation with the substrate (Gaetani et al., 1989). Furthermore, the decreased lipid peroxidation with all modulators is correlated well in accordance with the induction of antioxidant enzymes above basal level. In addition, the induction of superoxide dismutase activity may attribute to inhibit the generation of active oxygen species from autooxidation of quinones generated from the action of DT-diaphorase. Under normal circumstances in the intracellular environment hydrogen peroxide originates from two electron reduction of molecular oxygen which, otherwise, is enzymatically reduced via four electron transport to water required for sustaining metabolic reactions. The augmented activity of metalloenzyme superoxide dismutase accelerates dismutation of superoxide radicals to hydrogen peroxide which is removed by catalase (Aebi, 1984). In cytosol, the superoxide dismutase activity determined in the present study is mainly CuZn-SOD. Mitochondria generally contains Mn-SOD which is pelleted out during centrifugation along with nuclei. So, it is unlikely that the cytosolic fraction will contain mitochondria and hence Mn-SOD.

Thus, induced superoxide activity in conjunction with catalase and decrease in lipid peroxidation by Tinospora, Andrographis, Adhatoda, Aloe, Aegle, Clerodendrum, Lawsonia, Prosopis and Decalepis antagonises any cellular injury induced by reactive oxygen species. So, in present experimental conditions, these plants may provide sufficient protection against any prooxidant mediated injury including tissue damage.

Aloe, Lawsonia and Prosopis reduced the levels of phase I enzymes and thus may decrease the activation of endobiotic and xenobiotic compounds. Furthermore, the decreased activated electrophilic species will mean reduced risk of damage to the cell’s genetic machinery. At the same time, the levels of glutathione S-transferase and DT-
diaphorase have been increased. So, it might be possible that decrease in phase I enzyme levels may show decrease in the activation of metabolites but may indicate an increase in the detoxification ability of the cells. But, any probable decrease in the detoxification functions would be replenished by the increased levels of phase II enzymes, glutathione S-transferase and DT-diaphorase which help in maintaining the levels of detoxification in the cell.

Interestingly, *Prosopis* administration had shown a decrease in the levels of sulfhydryl group (-SH) which might indicate reduced free radical scavenging functions. Nevertheless, this should not be a case for alarm, as –SH group functions as physiological nucleophile and due to decrease in phase I enzyme levels, there would be a reduced level of electrophiles too and in such conditions, a subsequent decrease in the level of –SH group would not prove detrimental to the cell. However, the increased levels of glutathione peroxidase and catalase and decreased lipid peroxidation strengthened the antioxidant defense system.

In case of *Aloe* and *Lawsonia*, the increased levels of –SH groups and antioxidant enzymes glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase; and inhibition of malondialdehyde formation indicate that these modulators can impart anticarcinogenic property by favourably elevating the hosts’ antioxidant defense mechanisms. Both these modulators are more potent in detoxifying reactive metabolites when compared with *Prosopis* as it failed to induce the glutathione reductase and superoxide dismutase; and also inhibited the level of acid soluble sulfhydryl groups in the cell.

Thus, the findings and explanation regarding *Aloe*, *Lawsonia* and *Prosopis* suggest that these modulators may safely be implicated as cancer chemopreventive agents which may possibly help to enhance the detoxification reaction of the cell and thus may be effective as blocking agents against carcinogenesis (Wattenberg, 1985, 1992).

Administration of *Tinospora, Andrographis, Adhatoda, Aegle, Clerodendrum* and *Decalepis* in the present work has shown significant induction of phase I and phase II enzymes, -SH groups and antioxidative parameters. These overall inductive effects in aggregate may presumably result in enhanced carcinogen detoxification. These blocking agents are complicated in that the microsomal monooxygenase system can both activate
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and detoxify chemical carcinogens. However, concomitant increase in the levels of phase II enzymes and measured antioxidative parameters including induction of -SH groups and inhibition of lipid peroxidation indicates enhanced detoxification reactions. Thus, the increase in cytochrome P450 level results in enhanced activation of metabolites which would further be efficiently detoxified by the elevated levels of glutathione S-transferase, DT-diaphorase, -SH groups, glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase. The induced levels of antioxidant enzymes can also efficiently detoxify the toxic free radicals generated in different normal as well as abnormal cellular metabolism. The main function of glutathione peroxidase and catalase is the protection of the cell from the possible hydroperoxide damage. The hydroperoxide free radicals have been shown to cause damage to the cellular membranes if not eliminated. These peroxide radicals are acted upon by these enzymes rendering them harmless and excretable products. Superoxide free radicals having the capacity to damage various macromolecules, can be effectively detoxified by the superoxide dismutase-catalase enzyme system. Fortunately, all the examined modulators showed significant inhibition in lipid peroxidation which was measured by the formation of malondialdehyde. This observation is suggestive of the potential of the modulators in protection against membrane damage which may be imparted through modulating the various enzyme systems.

The present investigation has clearly shown that the plants used in the study have cancer chemopreventive potential. The next step is to experimentally substantiate the chemopreventive potential using different chemical carcinogenesis models. This is a very important study as this would surely tell us whether the chemopreventive potentials could be used in human situation after proper trials. Our laboratory has already commenced study in this direction employing skin cancer model system, stomach cancer model system, cervical cancer model system and oral cancer model system in appropriate animals.