Summary and Conclusions
6. Summary and conclusions

Ethnomedicine is a sub-discipline of ethnobotany, which broadly means all aspects of direct relationship of plants with man. Presently, much emphasis is being given on ethnomedicine and search for new therapeutic herbal drugs is in the progress, almost throughout the world. The ethnomedicinal data provide to the scientists, a base to start the search of new compounds with active principles. Followed by the work on phytochemistry, pharmacognosy, pharmacology and clinical trials, experimental sciences take over. The enquiry into the unknown starts and it is the search and research which keeps adding new drugs that may be used as chemopreventive agents.

Possible chemoprevention mechanisms include, carcinogen blocking activities, antioxidant/antiinflammatory activities and antiproliferation/antiprogession activities. Carcinogen blocking activities encompass inhibition of carcinogen formation or activation, deactivation or detoxification of carcinogen binding to DNA and enhancement of the level of DNA repair. Antioxidant activities include scavenging of reactive electrophiles and oxygen radicals.

The aim of the present investigation is to evaluate the cancer chemopreventive properties of *Tinospora cordifolia, Andrographis paniculata, Adhatoda vesica, Aegle marmelos, Aloe vera, Clerodendrum inerme, Lawsonia alba, Prosopis juliflora* and *Decalepis hamiltonii* selected from the ethnomedicinal survey of Madhya Pradesh. Inducibility of drug xenobiotic metabolizing enzymes was taken as a marker to investigate the efficacy of the extract of test materials. Enzyme induction has acquired considerable importance in recent years in pharmacological and toxicological studies. Induced enzyme system accelerates the biotransformation of xenobiotics (including carcinogens and drugs) *in vivo*, and thus alters the duration and intensifies drug action in animal and also in man.

In case of *Tinospora*, all the measured components of cytochrome P450 system were significantly elevated from their basal level at high dose level of treatment (100 mg/kg body weight/day) accompanied by significant increase in the activities of GST and DTD at both doses of treatment (50 and 100 mg/kg body weight/day). Thus, *Tinospora* has increased both phase I and phase II enzymes for which reason it can be classified as bifunctional/Type B inducer. This bifunctional inducing potential of *Tinospora* for
metabolizing xenobiotics is further strengthened by its effects on enhancing the GSH level along with the induction of other antioxidant enzymes (GPX, GR, SOD and CAT). Extrahepatic studies showed that *Tinospora* is more effective in inducing GST and SOD at both dose levels of treatment in lung and forestomach as compared to kidney. Catalase was significantly induced in both lung and kidney, while DTD showed an increase only in forestomach at both dose levels of treatment. These results indicate that, apart from liver, *Tinospora* is also effective in augmenting the conjugating and free radical scavenging ability in extrahepatic organs (lung, kidney and forestomach). Significant elevation in the microsomal protein is indicative of its role in protein synthesis.

The immunomodulatory and antitumor activities of *Tinospora* have been shown by Mathew and Kuttan (1999). They have reported that the methanolic extract of this plant increases the total WBC count, bone marrow cellularity, macrophage activation, humoral immune response and reduces the growth of solid tumors.

*Andrographis paniculata* can be classified as bifunctional/Type B inducer since it induces both phase I and phase II enzymes at both dose levels of treatment. Its effectiveness in eliminating toxic compounds from the body of organisms is further boosted by its favourable modulatory activity on antioxidant enzymes (GPX, GR, SOD and CAT), including GSH level. In extrahepatic organs, *Andrographis* induced GST in kidney and forestomach; DTD in lung, kidney and forestomach; SOD in lung and forestomach; and CAT in kidney. Thus, *Andrographis* proved to be more effective in modulating phase II enzymes in kidney and antioxidant enzymes (SOD) in forestomach at both dose levels of treatment.

*Adhatoda vesica* can also be classified as bifunctional/Type B inducer since it induces both phase I and phase II enzyme systems at both doses of treatment (50 and 100 mg/kg body weight/day). The effect of *Adhatoda* on antioxidant enzymes including GSH showed significant induction at both dose levels of treatment. The microsomal and cytosolic protein was significantly inhibited at higher dose level of treatment indicating the possibility of its involvement in the inhibition of protein synthesis. It is effective in inducing GST and DTD in lung and forestomach; SOD and CAT in kidney. Thus, besides liver, other organs viz., lung, kidney and forestomach were also influenced by *Adhatoda*, in order to detoxify xenobiotic compounds including chemical carcinogens and drugs.
Interestingly, *Aloe vera* reduced the levels of cytochrome P450 and cytochrome b5 whereas it increased the activities of glutathione S-transferase and DT-diaphorase at both doses of treatment. Thus, *Aloe* can be classified as monofunctional/Type A inducer since it induced only phase II enzymes. The higher dose of *Aloe* (60 µl extract/ 25 gms of animal body weight/day) was potent in increasing the activities of antioxidant enzymes glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase including the level of reduced glutathione. Its lower dose was able to induce only superoxide dismutase, catalase and level of reduced glutathione. These findings indicate that the higher dose of *Aloe* is more effective in modulating the activities of drug metabolizing enzymes and free radical scavenging properties. Both microsomal and cytosolic protein contents were significantly increased over their control values, showing the possibility of its role in favouring the protein synthesis. All the enzymes measured in extrahepatic organs (lung, kidney and forestomach) were observed significantly elevated from their basal level activities, except DT-diaphorase in lung. These findings indicate the favourable systemic effect of *Aloe* on biotransformation enzyme systems in the body of the animal.

Treatment of mice with *Aegle marmelos* led to the increase in the levels of cytochrome P450 and cytochrome b5 in lower dose treated group. Cytochrome P450 reductase and cytochrome b5 reductase were induced at both dose levels of treatment (50 and 100 mg/kg body weight/day). Both doses were quite effective in elevating the activities of phase II enzymes glutathione S-transferase and DT-diaphorase in a dose dependent manner. Thus, *Aegle* falls under the category of bifunctional/Type B inducer. Both doses of *Aegle* were also effective in inducing antioxidant enzymes glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase including the level of reduced glutathione in a dose dependent manner. *Aegle* was found more effective in kidney and forestomach as compared to lung with respect to induction of phase II enzymes (GST and DTD) and antioxidant enzymes. The protein content in all the organs investigated, remained unaffected except in forestomach, in which it was reduced by 9 to 12% from the basal level.

*Clerodendrum inerme* was quite effective in inducing the levels of cytochrome P450 and cytochrome b5 at both dose levels of treatment (50 and 100 mg/kg body weight/day). It also induced the activity of cytochrome P450 reductase, but only in lower
dose treated animals. *Clerodendrum* also maximally induced the activities of glutathione S-transferase and DT-diaphorase in a dose dependent manner. Since it induced both phase I and phase II enzymes, it can be classified as bifunctional/Type B inducer. *Clerodendrum* was found more effective in inducing the hepatic antioxidant defense mechanism of mice. Reduced glutathione was maximally induced by 2.12 folds. The antioxidant enzymes glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase were induced in a dose dependent manner. In extrahepatic organs, *Clerodendrum* was most effective in kidney, in enhancing the activities of both phase II enzymes glutathione S-transferase and DT-diaphorase and antioxidant enzymes superoxide dismutase and catalase as compared to that in lung and forestomach, whereas in lung only glutathione S-transferase was observed elevated over that of the basal level. Higher dose of modulator was effective in increasing the protein content of hepatic microsome, lung and forestomach, indicating the possibility of its favourable role in protein synthesis.

Overall effect of *Lawsonia alba* on cytochrome P450 system was depressing. It was found effective in inducing the phase II enzyme glutathione S-transferase, though the magnitude of induction was quite low. Thus, on the basis of its modulatory effect on phase I and phase II enzymes system, it may be classified as monofunctional/Type A inducer. Its effect on antioxidant defense mechanism is strengthening as it induced the reduced glutathione level maximally by 2.52 folds alongwith increasing the activities of antioxidant enzymes glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase. Thus, chemopreventive action of *Lawsonia* might be possible due to its strengthening effect on antioxidant defense mechanism rather than the modulation of phase I and phase II enzymes. High dose treatment of *Lawsonia* resulted in increase in the level of protein in microsomal as well as cytosolic fractions of the liver, indicating its positive role in protein synthesis. In extrahepatic organs studied, it was found more effective in lung in inducing phase II enzymes and antioxidant enzymes. In forestomach, only phase II enzymes were induced significantly.

The effect of *Prosopis juliflora* on phase I enzymes was invariably different. It reduced the level of cytochrome P450 whereas activity of phase II enzymes was increased significantly. So, it can be classified as monofunctional/Type A inducer. It could not effectively induce all the antioxidative parameters measured in the present study. It could
induce only glutathione peroxidase and catalase showing its effectiveness in detoxifying the hydroperoxides and organic peroxides. In extrahepatic organs studied, Prosopis was found very effective in kidney and forestomach in inducing phase II enzymes glutathione S-transferase and DT-diaphorase; and antioxidant enzymes. The protein level was reduced significantly in kidney showing its inhibitory effect on protein synthesis in this organ. Thus, chemopreventive action of Prosopis can be well achieved through the mechanism of conjugating activity of phase II enzymes along with the mechanism of antioxidant defense system.

Decalepis hamiltonii induced the level of cytochrome P450, cytochrome b5 and cytochrome P450 reductase. It also effectively induced the activity of both phase II enzymes glutathione S-transferase and DT-diaphorase at both doses of treatment investigated. Thus, Decalepis can be classified as bifunctional/Type B inducer. It also induced the antioxidant defense mechanism. The level of reduced glutathione was maximally induced by 1.80 folds along with glutathione reductase, superoxide dismutase and catalase. Decalepis significantly increased the level of cytosolic protein at both doses of treatment indicating its role in enhancing the protein synthesis in cytosol.

All the studied modulators reduced lipid peroxidation and the activity of lactate dehydrogenase, therefore, their roles are evident in strengthening the antioxidant defense mechanism and providing protection against cellular damage.

Thus from above findings we can conclude that –

- The cooperation between pharmacologists on one hand and historians and practioners of traditional medicine on the other hand, would link ancient knowledge with modern standards of testing that might result in discovery and isolation of pharmacologically active components from medicinal plants.
- The mass screening of plants in the search for new drugs is vastly expensive and inefficient. It would be cheaper and perhaps more productive to re-examine plant remedies claimed by tribals as well as described in ancient and medieval texts.
- Pharmacological investigations indicate that Tinospora, Andrographis, Adhatoda, Aegle, Clerodendrum, and Decalepis are bifunctional inducers of drug metabolizing enzymes and Aloe, Lawsonia and Prosopis are monofunctional inducers as they induce only phase II enzymes.
Summary and conclusions

- *Tinospora, Andrographis, Adhatoda, Aegle, Clerodendrum* and *Decalepis* could be more potent in metabolizing and detoxifying the xenobiotic compounds including carcinogens, as compared to *Aloe, Lawsonia* and *Prosopis*.

- Among bifunctional inducers, *Tinospora, Clerodendrum* and *Decalepis* seem to be more effective as compared to *Andrographis, Adhatoda* and *Aegle* at presently investigated dose levels.

- *Tinospora* and *Decalepis* were more effective in inducing the levels of cytochrome P450, GST and DTD as compared to other plant modulators investigated.

- All plant modulators (except *Prosopis*) significantly induced the levels of reduced glutathione which is a substrate for GST and a redox cycle involving GPX and GR, at both dose levels of treatment. This is suggestive of the importance of these plants in modulating the xenobiotic as well as endobiotic detoxification machinery.

- All the investigated plant modulators (except *Prosopis*) strengthened the antioxidant enzyme system (GPX, GR, SOD and CAT) of the host. This can be also supported by the fact that all of them significantly inhibited the basal level of lipid peroxidation.

- *Adhatoda, Aloe, Andrographis* and *Lawsonia* favourably induced the SOD-CAT system in liver as compared to other plant modulators, indicating its effectiveness in protecting the cells against reactive oxygen species like superoxide radicals and hydrogen peroxide.

- All the studied plant modulators are effective in inducing either or both of the phase II enzymes (GST and DTD) and antioxidant enzymes (SOD and CAT) measured in extrahepatic organs, lung, kidney and forestomach (with few exceptions).