Chapter No – 5

Summary and Conclusion
5.0 SUMMARY AND CONCLUSION:

In spite of tremendous strides in modern medicine liver diseases remain one of the most serious health problems. As the liver is an organ of paramount importance and plays an essential role in maintaining the biological equilibrium of vertebrates, liver injury caused by various toxic chemicals and certain drugs has been recognized as a toxicological problem. Herbal drugs play an important role in health care programs worldwide, and there is a resurgence of interest in herbal medicines for treatment of various ailments including hepatopathy. Various indigenous plants are known to play a vital role in the management of liver disorders but the perusal of literature reveals lack of scientific validation for the use of much of the traditional medicine. Hence the present study “Evaluation of Indian medicinal plant (Enicostima lithorale) for hepatoprotective activity” was undertaken to fill the lacuna in this regard. Though the plant used for the treatment of liver disorders in Ayurveda, an ancient system of medicine, a review of literature showed that these plants have not been subjected to systematic investigation to assess their hepatoprotective effects. Hence the present study was undertaken to explore the possible molecular level mechanisms involved in hepatocellular membrane protection of the above-said plants against carbon tetrachloride (CCl₄) and paracetamol-induced hepatic damage in rats.

The World Health Organization currently recommends and encourages traditional herbal remedies in natural health care programs because these drugs are easily available at low cost and are comparatively safe.

WHO recommends the use of herbal medicines safe & potent. Hence most herbal medicines need to be studied scientifically. Jaundice can be identified by the yellow appearance of the skin and mucous membrane due to increased bilirubin concentration in the body fluids. Liver protective drugs are not available. However, herbs play an important role in the treatment of liver disorder. Some hepatoprotection is achieved by herbal medicines belonging to Phyllanthus species. But these plants were declared as endangered by Indian system of medicine.
The plant material used in this study was collected during the month of November in Rajur, Akole, Sangamner Dist Ahmednagar. The herbarium of the plant was prepared and authenticated from the Department of Botanical Survey of India, Pune. Enicostema lithorale is syno name of Enicostemma / Enicostema axillare

The shade dried arial part of Enicostema axillare was extracted with pet ether, chloroform and methanol successively by soxhlation method, water by maceration method at room temperature, concentrated over water bath and evaporated under reduced pressure. The yields of extract were calculated.

Crude drugs (plant material) are usually put in quarantine store and they remain there for long time. During storage, proper ventilation, humidity control, suitable temperature and light condition should be ensured to maintain their original pharmacological action. Proximate analysis is carried out to check the quality of crude material in present research work. Proximate analysis of plant powder was carried out. From analysis it was observed that Foreign organic matter, ethanol soluble extract, water soluble ash, Total ash contents acid insuloble ash, loss on drying and percentage moisture content.

The present work deals with successive extraction of the leaves with solvents of increasing polarity; viz. petroleum ether (60-80° C), chloroform, methanol and water, extractive values found were 2.3 %, 3.1%, 14.6 %, and 26.12 % w/w respectively. Preliminary phytochemicals screening of different extracts was performed to determine the class of phytoconstituents present in the different extracts by using standard qualitative chemical test. Result indicated the steroids and fats present in petroleum ether (60-80°) extract, fats and alkaloids were found in chloroform extract. Saponins, tannins, phenolic compounds, flavonoids, and carbohydrate were found in alcohol (95 %) and water extrats.

HPTLC of the different exacts like pet-ether, chloroform, methanol and water extracts of plant powder was performed with some reference standards.

Experimental animal models of CCl₄ and paracetamol were used to assess the hepatoprotective activity of the selected plant. The marked cellular damage induced by CCl₄ and paracetamol was indicated by inactivation of cellular enzymes, elevation
of serum enzyme and hepatic MDA levels and depleted levels of liver SOD, CAT, GSH, GST and GPX activities.

Treatment of rats for plant powder slurry and different extract of Enicostema axillare and its dose of 1gm/kg and 200-400 mg/kg respectively gave significant protection against CCl₄ and paracetamol-induced hepatotoxicity as indicated by restoration in the activity of serum markers. The hepatoprotection was also associated with a parallel decrease in hepatic MDA and the other antioxidant enzymes viz., SOD, CAT, GSH GPX and GST. Optimal protection shown by the water extract Enicostema axillare was mediated by direct actions such as enzyme inactivation. Biochemical changes caused by water extract of Enicostema axillare treatment may also trigger the time-consuming cellular processes such as enzyme induction and other inter-organ changes in regulatory function.

The possible mechanisms that may be responsible for protection against both CCl₄ and paracetamol-induced hepatocellular damage by water extract of Enicostema axillare root extract may be any of the following (i) water extract Enicostema axillare may act as a free radical scavenger and intercept those radicals involved in CCl₄ and paracetamol metabolism by microsomal enzymes. By trapping oxygen related free radicals water extract Enicostema axillare could hinder their interaction with polyunsaturated fatty acids and hence abolish the enhancement of lipid peroxidative processes leading to MDA formation. (ii) Treatment with water extract of Enicostema axillare significantly increases the liver SOD, GSH, GPX and GST. The higher glutathione content in liver could afford the tissue better protection against oxidative stress, thereby contributing to the abolishment of hepatotoxicity induced by different hepatotoxins.

The water extract sample of Enicostema axillare was tested and confirmed, this exact is hepatoproctective.

The oral drug delivery of water extract of Enicostema axillare was prepared by direct compression method and evaluated for weight variation, drug content, friability, hardness, and thickness for all batches (F1 to F6).

No significant difference was observed in the weight of individual tablets from the average weight. Tablet weights of all bathes were found with in recommended
USP limits. The data of uniformity of content indicated that tablets of all batches had drug content within B.P. limits. The hardness of tablets of all batches are as per I.P. which is acceptable limits. All the formulation showed % of friability less than 1% except F1, F3 that indicates ability of tablets to withstand shocks, which may be encountered. No significant difference was observed in the thickness of individual tablet from the average weight.

The release of water extract of Enicostema axillare from oral specific tablets of the various formulations has shown very significance results.

The extract- excipients compatibility was done at 40°C, 60°C, 80°C and 75% ± 5% relative humidity. Open and closed vial methods were used. The result doesn’t show any physical change to the mixture after 8 weeks. This fact concluded that the extract and excipients are compatible with each other.

From the results of evaluation, it was concluded that all the parameters in present investigation can be used as quality control tool for standardization of Enicostima lithorale extracts.

Preliminary phytochemical screening of the plant revealed the presence of fatty acids, steroids, flavonoids, saponins, carbohydrates, alkaloids, phenolic compounds, tannins and proteins in the leaves.

HPTLC Fingerprint profiles confirm the presence of saponin in water extracts.

Atomic absorption spectroscopy confirms the eighteen elements in this plant powder and Neutron activation analysis confirms the presence of ten elements in plant powder.

The results of our study show that plant powder slurry and the water extract of Enicostemma axillare shows hepatoprotective activity comparable to the standard drug silymarin. Formulation of tablets of hepatoactive potent extract ( water extracts of Enicostemma axillare) showed very stable formulation with different temperatures.

Until now, most herbal drugs have not been widely accepted by “Western” medicine due to the inadequate experimental and clinical data to support their efficacy and safety by “Global Standards”. The present study reveals that the wealth of...
traditional Indian herbs is a “gold mine” for new drug discovery in modern medicine. Detailed pharmacological investigations on compounds isolated from these herbs can make possible the discovery of a new generation of hepatoprotective pharmaceuticals for global health care management. In view, present work on profile and nature of chemical constituents and standardization of Enicostema axillare will provide more information on the bioactive principles responsible for the pharmacological properties and the application of various parameters as has been done in the present work may adequately ensure the quality of medicinal plant and can be used as an analytical tool for the quality control of Enicostima lithorale.
FUTURE SCOPE

- Further fractionation of active extract of *enicostemma axillare* can be done.
- Identification and Characterization of active phytoconstituents responsible for hepatoprotective activity.
- Chromatographic method development for Identification and estimation of saponins, flavonoids and other polyphenolic compounds.
- Structural elucidation of saponins, flavonoids, polyphenolic compound obtained from *enicostema axillare* extracts by using modern analytical instruments viz. FTIR, GC-MS and NMR.
- Further evaluation of different activities of *enicostema axillare* s extracts using different protocol and methods.