8. SUMMARY AND CONCLUSION

Summary

In spite of tremendous strides in modern medicine, liver disease remains 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 world wide and there is resurgence in 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 medicines. Hence the present study “Phytochemical and pharmacological evaluation of Acalypha communis mull.Arg. and Lindera communis Hemsl alcoholic extract for their hepatoprotective activity” was undertaken to fill the lacuna in this regard. Though Acalypha communis mull.Arg. and Lindera communis Hemsl are 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 effect. Hence the present study was undertaken to explore the possible molecular level mechanism involved in hepatocellular membrane protection of the above said plants against paracetamol, D-Galactosamine Thioacetamide and Rifampicin induced hepatic damage in rats.

This thesis comprises of 10 chapters. Chapter 1 consists the introduction part dealing with traditional medicine, Indian medicine, introduction to the Indian system of medicine, Herbal medicine developments of phytomedicines for various diseases, Phytotherapeutic approach of drug development and hepatoprotective activities of herbal drugs.

Chapter 2 deals with the review of literature related to liver, hepatotoxins and their mechanism of hepatotoxic actions, liver diseases/disorders, review of the selected plants and studied for their hepatoprotective activity, works done on plants
selected and studied and profile of paracetamol, D-Galactosamine, Thioacetamide, Rifampicin, and silymarin.

Chapter 3 deals with the scope of study. Chapter 4 consists of aim and objectives.

Chapter 5 includes the materials and methods, in which the collection, identification and authentication of plants Acalypha communis mull.arg. and Linderia communis hems1, Loss on drying, physico-chemical parameters, extraction procedure, preparation of the extracts, storage of the extracts of the plants Acalypha communis mull.arg. and Linderia communis hems1, preliminary phytochemical screening of the extracts and HPTLC analysis of the extracts.

Chapter 6 deals with the pharmacological studies in which acute toxicity studies (LD50 values), in-vitro and in-vivo Antioxidant studies of the extracts and In-vitro and In-vivo hepatoprotective studies of the extracts, wet weight of liver, and histopathological studies of liver sections were carried out.

Chapter 7 deals with the summary and conclusion. Chapter 8 deals with the scope for further research. Chapter 9 deals with the References and Chapter 10 deals with the list of publications

Based upon the traditional uses by the tribals (IRULAS) and Traditional Indigenous Medical plants Acalypha communis mull.arg. and Linderia communis hems1 were collected from Malappuram distric, Kzhikode, Kerala, India. identified and authenticated.

The leaves of Acalypha communis mull.arg. and Linderia communis hems1 were separated and shade dried, and they were subjected for their physico-chemical properties.

The dried parts of the plants were granulated and extracted with 95% alcohol by continuous hot percolation using Soxhlet apparatus and their percentage yields were determined.
Both the alcoholic extracts and the preparations were subjected to preliminary phytochemical screening which revealed the presence of flavonoids, tannins glycosides and phenolic compounds qualitatively, which may be responsible for the hepatoprotective action upon toxins (Paracetamol, D-Galactosamine Thioacetamide and Rifampicin) induced liver damage. The hepatoprotective activity may be attributed to the individual or combined effect of phytoconstituents present in the extracts and preparations. Hence, they were selected for the pharmacological studies.

HPTLC analysis of the ethanolic extracts of the two different of plants were carried out. The results revealed the presence of flavonoids qualitatively.

The Acute oral toxicity was carried out as per OECD 423 guidelines using female rats and the LD$_{50}$ was determined as 5000 mg/kg/p.o, individually for all the extracts and the preparations as there was 2 mortality in three animals in each of them. The doses were fixed as 1/10$^{th}$ of the LD$_{50}$ dose, individually and were used for Pharmacological studies.

*In-vitro* antioxidant potential capacity of the leaves of *Acalypha communis* and *Linderacommunis* were subjected for the studies of DPPH method and Nitric oxide scavenging assay by using the standard L-Ascorbic Acid (Vitamin C). The levels were reversed by the extracts that of standard reference, L-Ascorbic Acid. Hence they showed statistically significant antioxidant activity, near than the standard reference, L-Ascorbic Acid.

So the Oxidation reactions can produce free radicals, which start chain reactions that damage cells. An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves.

So the results suggest that alcoholic extracts of both plants possess free radical scavenging activity, these methods will also helpful for quality control tool as over to overcome from adulteration and substitution. However In- vivo studies is required
further to confirm the efficacy of the extracts and with the isolated compounds for knowing the maximum benefit of it.

The Liver homogenates of the treated animals were subjected for the studies of anti-oxidant activity such as LPO, CAT, SOD, GPX, GST and ASH. LPO level was increased and other levels were decreased in the liver homogenates of the toxins (Paracetamol, D-Galactosamine, Thioacetamide and Rifampicin) treated animals. The levels were reversed by the extracts that of standard reference, Silymarin. Hence they showed statistically significant hepatoprotective activity, near than the standard reference, Silymarin.

In vitro study (Viability test) was carried for the ethanolic extracts, the plants against Paracetamol and D-Galactosamine. Silymarin was used as the standard reference. The results showed that all the ethanolic extracts showed significant hepatoprotective activity, since they increased the viability of the isolated hepatocyte cells of the liver of rats of the toxins (Paracetamol D-Galactosamine Thioacetamide and Rifampicin) and treated animals.

Serum enzyme markers like SGOT, SGPT, ALP, total protein, serum bilirubin were elevated by the toxins (paracetamol D-Galactosamine Thioacetamide and Rifampicin) fed animals. They were decreased in the ethanolic extracts of two plants. Hence the results of the enzyme markers showed significant hepatoprotective activity. The combined extracts (AC+LC) showed statistically significant hepatoprotective activity more than the standard reference, Silymarin.

The results of histopathological studies also revealed that the extracts, combined extracts and the standard reference, Silymarin had reversed histopathological changes produced by the toxins (paracetamol D-Galactosamine Thioacetamide and Rifampicin) the results were comparable with the standard reference Silymarin. Hence they showed statistically significant hepatoprotective activity.

Pretreatment with extracts restored the hepatic architecture and protected the liver tissue from fatty changes ultimately leading to necrosis. The various active
ingredients present in the extracts and preparations were helpful in the changes in the membrane in the mitochondria or at the ionic level like calcium. They played a role in the process of regeneration, prevention of fibrosis.

Combined extracts preparations regenerated hepatocytes which were damaged by the toxins (paracetamol D-Galactosamine Thioacetamide and Rifampicin) and thereby they showed their significant dose dependent hepatoprotective activity.

The combined extracts were more significant hepatoprotective activity than the standard reference Silymarin.

The hepatoprotective properties of the extracts, combined extracts of two plants provided useful information in the possible application of them in the treatment of liver diseases/disorders.

A combination of data derived from this study is useful for recognition and delimitation of the species and can help in selecting the plants for pharmacognostic crude drug research without ambiguity.
Conclusion

Medicinal plants are a valuable and readily accessible resource for primary health care. Although two different of plants are consistently been screened for their hepatoprotective activity, many species of plants of medicinal value are yet to be explored. These plants were selected based on their ethno medicinal use.

In conclusion, from the overall results of the biochemical, histopathological examinations and antioxidant studies inferred that the extracts showed their hepatoprotective activity. They are strongly recommended in the herbal treatment of hepatic problems. The present study showed that the extracts, the combined extracts of plants possessed hepatoprotective activity. They afford maximum hepatocellular protection mediated through antioxidant mechanisms comparable to the standard reference, Silymarin. The hepatoprotective property was attributed to the active principles of the plants namely, flavonoids, tannins and other polyphenolic compounds.

The results also show that different two plants assayed here possess different levels of hepatoprotective activities, that ethanol extracts of Acalypha communis mull.arg. Exhibited the highest activity, followed by the leaves of Lindera communis hemsl.

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 can make possible discovery of a new generation of hepatoprotective pharmaceuticals for global health care management.