Chapter 6

Summary & Conclusions

If you can't explain it simply, you don't understand it well enough.

— Albert Einstein
6. SUMMARY AND CONCLUSION

In Ayurveda, the yakrit (liver) is the root of rakthavaha srotas and ranjaka pitta. Any damage to the liver ultimately disturbs the digestion which is the main causative factor for all diseases according to Ayurveda. There are lots of drugs with hepatocurative activity described in classic Ayurvedic texts. Apart from the drugs mentioned in these Ayurvedic texts, there are many indigenous drugs used to treat liver disorders in India.

The present thesis is an exhaustive compilation of our findings in the development of polyherbal formulation containing indigenous medicinal plants with complete safety and efficacy profile in the animal model for treatment of hepatotoxicity. The work was systematically planned to execute the following:

- Selection and standardization of raw plant materials/active ingredients as per WHO guidelines for the formulation of a hepatoprotective formulation.
- Preparation and standardization of extracts from the selected plants employing suitable extraction techniques.
- In vitro and in vivo evaluation of the standardized extracts for antioxidant, hepatoprotective and toxicity studies in suitable models.
- Preparation of combination of extracts and evaluation of the combined mixture for its compatibility studies and toxicity profile.
- Development of a polyherbal tablet dosage form and evaluate for Physical, chemical and pharmacological parameters.
- Stability testing for prepared polyherbal formulations.
Chapter 6

Summary and Conclusion

Development of polyherbal formulations for the treatment of liver disorders of different origin is the need of today research in the field of phyto-medicine. Many herbal formulations are available for hepatoprotective activity but scientific validation and standardization is very difficult for available formulations.

In present study, a polyherbal formulation was developed using indigenous medicinal plants which are having scientific evidence for therapeutic efficacy and bibliographic traditional support were selected in less number.

In the present study three indigenous medicinal plants were screened for the hepatoprotective activities in rats. The screening was carried out before and after the polyherbal formulation as per the standard procedures. The plants selected were leaves of *Coccinia indica*, leaves of *Sida cordata* and leaves of *Scoparia dulcis*.

The plant materials, *Coccina indica* leaf material was collected from Local Vegetable Market, Sambalpur, Odisha. *Sida cordata* leaf material was collected from Herbal garden, Raipur, Chhattisgarh and *Scoparia dulcis* leaf material was collected from Medicinal Plants Garden, Campus of Gayatri College of Pharmacy, Sambalpur, Odisha.

They were identified and authenticated by office of the Additional Director, Central National Herbarium, Govt. of India, Howrah and specimen samples were preserved in the herbarium section of the Department of Pharmacolgy, VSS Medical College, Burla (Sambalpur) with voucher no. PHARM/PhD/HF/61, 62, 63 for future reference.

Soon after authentication, all the plant parts were dried at room temperature, until they were free from the moisture and subjected to physical evaluation with different parameters. The parameters, which were used for evaluation, are nature, odour, colour, taste, size, shape, width, and length.
All the parts were subjected to size reduction to get coarse powder and then passed through sieve no.40 to get uniform powder. Then the uniform powder was subjected to standardization with different parameters as per WHO guideline. All the evaluated parameters were found to be within the prescribed limits. After physicochemical characterization, all the plant materials were subjected to extraction with Pet ether, Chloroform, Ethanol and Aqueous. Overall there are 12 extracts from 3 plants were obtained. The extract after concentration is first subjected for preliminary physical and phytochemical screening to assess the quality of plant materials and understand the nature of active phytoconstituents present. The extracts were standardized using bioactive marker (β-sitosterol) by HPTLC method.

The standardized extracts were subjected to in vitro antioxidant and in vivo hepatoprotective evaluations in the second step. The evaluation was designed to understand the pharmacological behaviour of each plant material at different dose levels so as to select justified doses of each component for the development of a hepatoprotective formulation. The extracts were tested for possible toxicity as per OECD guidelines and found to be safe.

Result obtained from hepatoprotective activity screening indicate that Ethanollic extract of Coccinia indica (CIEE); Aqueous extract of Sida cordata (SCAE) and Ethanol extract of Scoparia dulcis (SDEE) shows more prominent hepatoprotective activity. Petroleum ether extract of Coccinia indica (CIPE); Ethanol extract of Sida cordata (SCEE); Aqueous extract of Scoparia dulcis (SDAE) and Chloroform extract of Scoparia dulcis (SDCE) shown moderate hepatoprotective activity while Chloroform extract of Coccinia indica (CICE); Aqueous extract of Coccinia indica (CIAE); Pet ether extract of Sida cordata (SCPE); Chloroform extract of Sida cordata (SCCE) and Pet ether extract of Scoparia dulcis (SDPE) did not show
any significant activity from parameter studies i.e. biochemical investigation of enzyme level and histopathological studies of liver tissues.

The extracts which were showing promising activity for hepatoprotective activity were selected for polyherbal tablet formulations. Polyherbal formulations are made to improve the efficacy of the extracts. The mixtures of extracts were assessed for their physical and chemical compatibility with each other and with excipients by HPTLC studies. Three polyherbal tablet formulation were developed. Formulation HF₁ was prepared by using the extracts showing maximum hepatoprotective activity. Formulation HF₂ was a combination of extracts showing moderate hepatoprotective activity. Formulation HF₃ was a combination of extracts of HF₁ & HF₂ both.

Polyherbal tablets were prepared by direct compression method and evaluated the same for various parameters. The tablets were prepared with specialized additives as per the requirements and physical nature of extracts in order to achieve acceptable tablets. Formulated tablets were evaluated for Pre-Compression Parameters like angle of repose, bulk density and compressibility index and post-compression parameters like color and shape of tablets, thickness and diameter, weight variation test, friability test, hardness test, disintegration time test, heavy metal analysis and microbial load tests.

We have tried various additives in our trial batches, finally tablet formulations which have shown acceptability as per Indian Pharmacopoeia and United States Pharmacopoeia methods were selected.

Chemical evaluations of polyherbal tablet formulations (HF₁, HF₂ and HF₃) were carried out by using HPTLC Technique. The drug content study was carried by the estimation of β-sitosterol in the tablet formulation by HPTLC. Each extract has
already been standardized to specific marker (β-sitosterol) by its estimation using HPTLC.

All the three formulation showed significant hepatoprotective activity and was significantly comparable with Liv. 52. However the maximum hepatoprotective activity was found with formulation HF3. The hepatoprotective activity was in the order of HF3 > HF1 > HF2.

The polyherbal formulations also pass the stability testing. Organoleptic properties of the formulations were checked after stability studies. There was no change in the organoleptic properties in respect to colour, odour and appearance of tablets. The formulation had pleasant appearance and acceptable odour, indicating that the formulation is stable at accelerated conditions. There was no significant change observed in the HPTLC finger print graph and drug content (β-sitosterol) of formulation (initial) and after accelerated stability studies. This showed that the phytoconstituents present in formulations (HF1, HF2 and HF3) are stable in nature.

**6.1 Future directions**

The developed polyherbal tablet formulations can be further subjected for intensive drug development programme which leads to promising potent hepatoprotective formulation.

- Hepatoprotective potential of the formulations can be evaluated in some more animal models like paracetamol induced hepatotoxicity, ethanol induced hepatotoxicity etc.

- In the present studies, we have concluded that, the antioxidant potential of the formulation was responsible for its activity; as well as the formulations has enhanced antioxidant enzymes present in the liver tissue. Still more extensive
studies can be envisaged to find the exact mechanism of action and phytochemical(s) responsible for its hepatoprotective effects.

Further *in vitro* studies to gain understanding of the dissolution and release profile of different bio-active markers followed by BA/BE studies in normal healthy volunteers are proposed.

Clinical studies are required to establish its efficacy and safety before it can be recommended for human use.