CHAPTER-VIII

8. SUMMARY

From the study it is evident that people prefer herbal medicines rather than conventional medicines. Many indigenous communities/ traditional healers have their own conventional medicine system with diverse medicinal plants for treating many untreatable diseases and may use single or combination of different plant parts.

The present survey of hepatic inducing medicinal plants traditionally utilized by local tribe, a total of 40 plants from 26 families and 40 genus have been documented. The tribe uses 29 types of leaves, 10 types of roots, 2 types of flowers and fruits, and one each type of barks and seed. The Moraceae and Rutaceae were reported highest with 3 plants each.

Phytochemical screening from the selected plants for the study showed presence of various phytochemicals that include phenols, flavonoids, tannins, resins, terpenoids, glycosides and steroids. The extract also showed high contents of total phenolics and flavonoids especially in the root extracts of RoMi-EE and RoAc-EE. The same extract also indicated higher activity in total reducing power and total antioxidant capacity. In the *in-vitro* antioxidant test, increase in radical scavenging activity was observed with increase in concentration. In the radical scavenging activity of DPPH, ABTS and H_2O_2, the lowest IC_{50} values were observed in the extract of RoMi-EE, RoAc-EE and RoAc-EE respectively. In case of ICC, the extract that showed lowest EC_{50} value was observed in RoPt-EE, whereas in FRAP method, highest concentration of $FeSO_4.7H_2O$ µM/mg in extract was observed in RoAc-AE. Apart from the DPPH and ICC, the IC_{50} values of the extracts were comparable with that of the standard used in ABTS (BHT) and H_2O_2 (BHA) radical scavenging activity.

The GC-MS analysis also showed the presence of various bioactive compounds that were reported for the first time from the root extracts of *Morus indica,* and were reported to have various biological activities such as antibacterial, anti-inflammatory, anti-diabetic, anticancer, anti-arthritic, hepatoprotective, block HIV-1 entry and infection, anti-asthma, hypocholesterolemic etc,.

In the present *in-vivo* study conducted on the CCl_4 induced acute liver damage, the RoMi-EE markedly decreased the elevated levels of liver serum enzymes such as ALT, AST and ALP in the control group as compared with the normal group. After the CCl_4 administration, there was significant increase in the concentration of TC, TG, LDL, VLDL

and decrease in the HDL level. However after treatment with test drug RoMi-EE, decrease in the TC, TG, LDL, VLDL and increase of HDL levels were observed.

The creatinine level in the CCl_4 treatment was significantly increased as compared to normal group. But, when the experimental rats were treated with different dosage of RoMi-EE, the creatinine level was decreased and was almost back to the normal in the silymarin treated group.

The protein and albumin levels were found to be low in CCl_4 group. After treatment with RoMi-EE and silymarin, the albumin level showed significant increase whereas the total protein level in the treatment group saw no significant changes. In case of serum bilirubin and GGT level the elevation was observed in CCl_4 treated group as compared to normal group. After the treatment with RoMi-EE and silymarin, there was decrease in the levels of GGT and bilirubin concentration.

While the antioxidant enzyme such as SOD, CAT and GPx were markedly decreased after CCl_4 administration. The significant increase in the antioxidant enzymes was noted in the RoMi-EE and silymarin treated groups. The activity of GSH was also reduced by 25% in the CCl_4 treated group as compared to normal group. Upon treatment with two dose of RoMi-EE, the activity was ameliorated and were comparable with that of silymarin group. The activity of RoMi-EE and silymarin also reduced the MDA content significantly, which was elevated by 48% upon treatment with CCl_4.

The results of *in-vivo* study showed that the RoMi-EE high dose treatment (200 mg) was comparable with the standard drug silymain which was effective in reverting the biochemical parameters in diseased animals.

The histopathological reports also revealed that CCl_4 induced severe hepatocyte necrosis, inflammation, biliary cirrhosis, vacuolation, microvesicular steatosis and infiltration of kupffer cells around the central vein than the normal architecture observed in healthy rats. After treatment with RoMi-EE, the severity of CCl_4 induced liver intoxication was reduced in a dose-dependent manner, although the treatment with silymarin showed much better result. The cross section through cortex kidney of CCl_4 group showed vacuolation, glomerular atrophy, widening of capsule space, cell layer thickening and degeneration of cells. The recovery of glomerular atrophy, decrease in capsule space and less degeneration of cell was

observed in 100 mg RoMi-EE experimental drug (100 mg & 200 mg RoMi-EE). Whereas silymarin showed identical structure and was similar to that of normal group.

The *in-vivo* experiment and the histopathological study were well supported by the *in-silico* molecular docking analysis using Maestro, Schrodinger software. The 1NFK and 3LN1 proteins were selected for docking (against ligands identified in the GC-MS analysis) based on the literature available. The ligands which showed interaction with both 1NFK and 3LN1 proteins without violating Lipinski rule of five are CID: 5368759 (2, 6, 10-Dodecatrien-1-Ol, 3, 7, 11- trimethyl-9- (phenyl sulfonyl) –(E,E)-), CID: 550196 (1-Methylene-2b-Hydroxy methyl-3,3-Dimethyl-4b- (3-Methylbut-2-Enyl)-C); CID: 519794 (1,2-Bis(Trimethylsilyl) Benzene) and CID: 610038 (2,4,6-Cycloheptatrien-1-One, 3,5-Bis-Trimethylsilyl-). However, among the selected ligands, the compound 2, 6, 10-Dodecatrien-1-Ol, 3, 7, 11-trimethyl-9- (phenyl sulfonyl) –(E,E)- showed best docking score of -4.958 having ΔG binding affinity of -45.35 kcal/mol and was comparable with that of silymarin which showed -5.956 docking score with MMGBSA ΔG binding affinity of -54.79 kcal/mol in 1NFK docking. The compound 2, 6, 10-Dodecatrien-1-Ol, 3, 7, 11- trimethyl-9- (phenyl sulfonyl) –(E,E)- also showed best docking score of -9.78 having ΔG binding affinity of -27.8173 kcal/mol in docking with 3LN1 protein, whereas the silymarin didn't show any binding affinity. The ADME properties of ligands revealed that, except for the ligands Octadecanoic acid, ethyl ester (CID: 8122), Alpha.-amyrin (CID: 73170), 9,12-Octadecadienoic Acid (Z,Z)-(3931), 1-Octadecyne (69425), Oleic Acid- (445639) and N-Hexadecanoic Acid (985), all the other ligands were in the acceptable range of Lipinski's rule of five, indicating their potential for use as drug-like molecules.

Overall the study suggest that the *Morus indica* root, which is used traditionally by the local tribe of BTAD, Assam, have shown high contents of antioxidant and *in-vivo* activity can be an alternative for treating liver disorders.