Conclusion

Ayurveda is gaining prominence as the natural system of health care all over the world. Thousands of herbal products are used in the classical formulations in the Ayurveda system of medicine. The chemical constituents present in herbal drugs are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body. Even the allopathic system of medicine has adopted a number of plant derived drugs which form an important segment of modern pharmacopoeia. The herbal products symbolise safety in contrast to the synthetic drugs that are regarded as unsafe to human and environment. Hence the medicinal plants received considerable use and attention over the past decade. The present study on oral ayurvedic formulation Hippo-08 validates the hepatoprotective and antioxidant activity of the formulation.

Phytochemical Characterization

Phytochemical Characterization of Hippo-08 includes phytochemical screening by qualitative analysis, quantification of secondary metabolites, HPTLC analysis and characterization by GC-MS. Phytochemical screening of Hippo-08 revealed the presence of secondary metabolites namely alkaloids, flavonoids, glycosides, saponins, steroids and tannins. HPTLC analysis of ethanolic extract of Hippo-08 exhibited the presence of phenolic compound Quercetin (Rf value 0.71) and Tannic acid (Rf value 0.87). GC-MS analysis of ethanolic extract Hippo-08 showed the presence of 21 compounds. The predominant compounds identified are oleic acid (51.87%) followed by 2-Furancarboxaldehyde, 5-(hydroxymethyl)- (15.86%), n-Hexadecanoic acid (6.90%), Benzeneacetic acid, 2,5-dihydroxy- (6.34%), Pregna-5,16-dien-20-one,3-(acetyloxy)-16-methyl-(3β)-(4.16%).

In vitro antioxidant activity

In vitro antioxidant activity of Hippo-08 included the evaluation of enzymatic and non-enzymatic antioxidant status and the assessment of free radical scavenging activity. The evaluation of enzymatic antioxidant status of Hippo-08 revealed the significant presence of SOD, CAT, GPx, GST and GR. Among the various antioxidants assessed the level of GPx was found to be predominant in Hippo-08.
The estimation of non-enzymatic antioxidant status of **Hippo-08** showed the presence of GSH, vitamin A, vitamin C and vitamin E. The enhanced level of vitamin E present in Hippo-08 seems to be interesting. **Hippo-08** exhibited free radical scavenging activity against DPPH, ABTS, superoxide radical, nitric oxide radical, hydrogen peroxide and hydroxyl radical. It was also possess reducing capacity and total antioxidant activity. Hippo-08 exhibited better radical scavenging activity against hydrogen peroxide.

**Acute and Sub acute toxicity study**

**Hippo-08** produced no toxicity with the limit dose of 2000mg/kg body weight. The sub acute toxicity results showed the biochemical constituents, haematological parameters, liver marker enzymes noted to be within the normal range. The histology study of liver cells showed no toxic symptoms.

**Chronic study**

The present study demonstrated the hepatoprotective and antioxidant effect of **Hippo-08**. The body weight which was decreased due to ethanol intoxication was reversed by the administration of **Hippo-08**. The increased liver weight was restored to the normalcy on treatment with **Hippo-08**. The enhanced levels of liver marker enzymes (AST, ALT, ALP and GGTP) in AFL rats were reduced to normal when treated with **Hippo-08** confirming its hepatoprotective activity. The altered lipid levels in serum and liver of ethanol intoxicated rats were restored to normal values after treatment with **Hippo-08**. The biochemical constituents which are varied in toxic group were reclaimed to normal range on treatment with **Hippo-08**. The decreased levels of enzymatic and non-enzymatic antioxidants were effectively increased on **Hippo-08** treatment to near normalcy. The enhanced lipid peroxidation due to ethanol intoxication was reduced to normal after treatment with **Hippo-08**. The histopathology study of liver cells showed no deleterious damage, which provides a valid note on the hepatoprotective nature of **Hippo-08**, which is exhibited by the normal histology.

**Molecular Docking Study**

The molecular docking analysis was performed for the 21 compounds of **Hippo-08**. They were docked against two key enzymes namely **CYP2E1** and **CYP3A4**. The good dock score was noted for 7 compounds for CYP2E1 and 5
compounds for CYP3A4. The most interesting fact was that out of 21 compounds docked against the two enzymes 5 compounds namely (9,12-octadecadienoic acid, methyl ester (E,E)-, 4H-pyran-4-one,2,2-dihydro-3,5-dihydroxy-6-methyl, Benzene aceticacid,2,5-dihydroxy-, Phenol,2-methoxy-5-(1-propenyl)-(E)-, and 3 Hexadecanoic acid, ethyl ester) known to be docked by both enzymes CYP2E1 and CYP3A4, which exhibits its structural integrity with the marker enzymes(CYP2E1 and CYP3A4) and Hippo-08.

The efficacy of the oral formulation Hippo-08 in safeguarding the liver and thereby acting as a safe and effective hepatoprotective natural drug to compact liver diseases has been comprehensively and conclusively validated by the results obtained by this present detailed study. The concept of effectiveness and safety are the two prime factors to be considered for the use of any oral formulations. Since the dosage regime which has been standardized authenticates safe and protective effect of Hippo-08. Further molecular docking study provides an insight into the effect exerted by Hippo-08 at molecular level, thereby provides a valid note to exhibit its liver protective function against alcohol intoxication. The herbal option (Hippo-08) holds positive prospects to serve as a safe and effective hepato protectant in future.

Future Recommandations

1. Further studies are recommended for the structural elucidation of the lead compounds.

2. Development of drug designing can be elucidated for the lead compounds.

3. More clinical and pre-clinical trials are recommended for the isolated compounds in order to establish its hepatoprotective and antioxidant effect to combat serious and slowly emerging liver diseases.