SCIENTIFIC VALIDATION AND EVALUATION OF HEPATOPROTECTIVE POTENTIAL OF LEAF EXTRACT OF *Hiptage benghalensis* (L.) Kurz

ABSTRACT

The utilization of medicinal plants in the treatment of human diseases is evidence of man’s ingenuity. The contribution of these plants to the therapeutic arsenal in the fight against disease dates back several centuries. Liver disease is one of the major causes of morbidity and mortality in public, affecting humans of all ages. Some of the commonly known disorders are viral hepatitis, alcohol liver disease, non-alcoholic fatty liver disease, autoimmune liver disease, metabolic liver disease, drug induced liver injury and gallstones. According to WHO estimates, globally 170 million people are chronically infected with hepatitis C alone and every year 3–4 millions are newly added into the list. The value of current therapies is unequivocal, yet inadequate. Insufficiency of current therapies for the treatment of liver disorders, combined with both a lack of trust in conventional medical treatment and an inability of the economy to absorb the cost of pharmaceuticals, have created a growing interest towards botanicals. This prompted the choice of *H. benghalensis*, belonging to the family of malpighiaceae with many reported pharmacological potentials, yet the hepatoprotective efficacy of this plant is still less explored and hence the present investigation focuses on screening and validating its hepatoprotective effect against CCl₄ induced hepatic damage. A key obstacle which has hindered the acceptance of the alternative medicines in the developed countries is the lack of proper documentation, stringent quality control and standardization. Hence, in the present study, the plant material was subjected to correct identification, organoleptic evaluation, pharmacognostic evaluation, volatile matter, quantitative evaluation (ash values, extractive values), phytochemical evaluation, TLC and HPTLC profiling, GC-MS analysis, toxicity testing, and biological evaluation. Qualitative and quantitative phytochemical profiling of the plant revealed the presence of various secondary metabolites namely alkaloids, flavonoids, terpenes, coumarins, sterols, saponins and tannins. Acute toxicity studies in animals are essential for any therapeutical substance intended for humans as the results of these studies may be used in choosing doses for repeat-dose studies, providing preliminary identification of target organs of toxicity and occasionally, revealing delayed toxicity. Hence the plant was assessed for its toxicity as per OECD guidelines before proceeding with *in vivo* studies and the results showed that the plant extract did not show any toxicity and mortality upto a dose of 5g/Kg bw. in experimental animals which assured the safe usage of the plant for further experimentations. For pharmacological assessment ELEHB in doses of 100, 200 and 300 mg/kg bw. was administered to male wistar albino rats and its effect
on various biochemical parameters was assessed in the CCl₄ induced experimental animals. The hepatic injury induced by CCl₄ resulted in an increase in serum AST, ALT, ALP and LDH levels due to the leakage of cellular enzymes into circulation and notable decrease in elevation of serum enzymes was observed following treatment with increasing concentrations of ELEHB. Fluctuating levels of membrane bound ATPases on CCl₄ induction confirming the dysfunctioning of the hepatocytes which was well reversed to normalcy on dose dependent administration of ELEHB. The antioxidant enzymes SOD, CAT, GSH, GPx and GST constitute a mutually supportive team of defense against ROS which were all observed to be decreased with elevated levels of LPO after CCl₄ induction. A significant increase in antioxidant enzyme levels accompanied with a fall in LPO levels on treatment with ELEHB suggested that it had a direct free radical scavenging property. The estimated levels of the serum biochemical markers namely A/G ratio, hepatic glycogen, hydroxyl proline, serum bilirubin, DNA, liver weight and lipid profile (LDL, HDL and PL) showed notable alterations indicating the loss of functional and structural integrity of hepatocytes on CCl₄ intoxication which was restored to normal levels following treatment with ELEHB in a dose dependent fashion. Histopathological examination of liver sections of the rat intoxicated with CCL₄ showed disarrangement and degeneration of normal hepatic cells with intense centrilobular necrosis with sinusoidal haemorrhages and dilatation. However the liver sections of the experimental animals treated with ELEHB at dose dependent concentrations showed less vacuole formation, reduced sinusoidal dilatation, less disarrangement and degeneration of hepatocytes indicating better regenerative activity of ELEHB which was well comparable with the standard drug silymarin. The observations basically supported the results obtained from the biochemical assays and would serve as a full diagnostic support for the preclinical and clinical studies. The observations of in vivo investigation in the present study was well supported by the in silico analysis with the chosen target NS5BRdRp docked with compounds retrieved from GC-MS analysis of ELEHB.

In conclusion, the present investigation demonstrated that ELEHB has hepatoprotective effect against CCl₄ induced hepatotoxicity. However, it is necessary to isolate and purify the active principles involved in the pharmacological potency of this plant and determine its mechanism of action.

Keywords: Hiptage benghalensis, CCL₄, hepatoprotective, Malpighiaceae