# Hepatoprotective action of *Lygodium flexuosum* (L.) Sw.: From Traditional Medicine to Molecular Biology

## Summary

*Lygodium flexuosum* (L.) Sw. is a climbing fern belongs to the family Lygodiaceae. The leaf paste, root and rhizome are used to cure jaundice in tribal medicine in India. The main objective of the present study was to evaluate the hepatoprotective activity of *L. flexuosum* and its toxicity, if any, in experimental models since there was no data available to verify the folk or tribal claims.

Preliminary studies started with the extraction of the plant powder in water, ethanol and n-hexane and pharmacological activity was studied in acetaminophen (2 gm/kg) intoxicated rats. All the three extracts showed efficacy at 62.5 mg/kg level. But at lower doses (12.5 mg/kg) n-hexane extract showed better efficacy and was, therefore, selected for further studies. In separate experiments, a single dose of carbon tetrachloride (150 μL/100 gm), D-galactosamine (800 mg/kg) and thioacetamide (100 mg/kg) were administered to induce liver damage in rats in preventive and curative models. The n-hexane extract (200 mg/kg and 100 mg/kg) was treated prior to or after the toxin treatment and the hepatotoxicity was prevented or reduced and was comparable with standard control, silymarin (50 mg/kg). Acute (5 gm/kg) and subacute toxicity (1 gm/kg) data showed that of the extracts (water, ethanol and n-hexane) at higher doses were devoid of any toxicity.

The protective effect of *L. flexuosum* n-hexane extract on carbon tetrachloride induced acute hepatotoxicity in rats was evaluated. Rats were killed 6, 24, and 48 hours after treatment with a single oral dose of carbon tetrachloride (150 μL/100 gm). Pre-treatment with n-hexane extract that was significantly prevented an increase in serum aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase activity and liver lipid peroxidation and prevented the depletion of hepatic glutathione. Rats treated with *L. flexuosum* had reduced

levels of the transcripts of proinflammatory cytokines like transforming growth factor-$\beta$1, tumor necrosis factor-$\alpha$ and interleukin-1$\beta$ in the liver of carbon tetrachloride intoxicated rats when compared to carbon tetrachloride control. The data showed that *L. flexuosum* significantly reduced carbon tetrachloride induced increase in proinflammatory cytokine levels.

The effect of *L. flexuosum* was studied in preventive and curative models of hepatic fibrosis and focusing on its effects on different parameters involved in liver fibrosis. Liver fibrosis was induced in male Wistar rats by exposure to carbon tetrachloride (150 $\mu$L/100 gm) for 10 weeks. In preventive groups rats were treated with the extract (200 mg/kg) along with carbon tetrachloride and in curative group extract (200 mg/kg) was administered for two weeks after 10 weeks of carbon tetrachloride induction. *L. flexuosum* significantly prevented or reversed liver damage in carbon tetrachloride intoxicated animals in both treatment groups. Rats treated with the extract had reduced levels of tumor necrosis factor-$\alpha$, interleukin-1$\beta$, transforming growth factor-$\beta$1, procollagen-I & III, and tissue inhibitor of metalloproteinase-1 transcripts and an increased level of matrix metalloproteinase-13. In preventive and curative treatments, procollagen III expression in *L. flexuosum* extract treated rats treated with carbon tetrachloride downregulated to a greater extent evidenced by Western blot analysis. Confocal images and immunohistochemical analysis of liver sections specifically stained for Collagen-III showed better protective effect by the extract in carbon tetrachloride treated rats.

The antiangiogenic activity of *L. flexuosum* on hepatocarcinogenesis induced by *N*-nitrosodiethylamine for 20 weeks was evaluated in preventive and curative models. The result showed that angiogenesis induced by *N*-nitrosodiethylamine was effectively inhibited by *L. flexuosum* extract at a dose of 200 mg/kg in both preventive and curative models. The data demonstrated that the mRNAs for vascular endothelial growth factor, angiopoietin-1 and 2 and its receptor Tie-2 were overexpressed in *N*-nitrosodiethylamine treated rats. Preventive

and curative treatment with *L. flexuosum* prevented or reversed the elevated levels of vascular endothelial growth factor, angiopoietin-1 & 2 and Tie-2 mRNA expression indicating decreased proliferation of tumor cells in *N*-nitrosodiethylamine treated rats. Immunohistochemical analysis showed the localization of overexpressed vascular endothelial growth factor around the periportal area in *N*-nitrosodiethylamine treated rats. The over expression of vascular endothelial growth factor was inhibited by the treatment with *L. flexuosum* in both treatment groups indicating its inhibitory role of the extract on neo-vasculature formation in rat liver.

Induction of apoptosis by *L. flexuosum* at different concentrations (100, 50, 25 and 10 μg/mL) and silymarin (50 μg/mL) in Hep 3B and PLC/PRF/5 human liver cancer cell lines were studied. Apoptosis is a potential mechanism by which *L. flexuosum* extract exerted its antiproliferative effects. Apoptosis was also demonstrated by poly (ADP-ribose) polymerase cleavage represented by the cleavage of 116 kDa into an 85 kDa peptide product, upregulation of pro-apoptotic gene *bax*, and downregulation of anti-apoptotic gene *bcl-2*. Annexin V-FITC binding in apoptotic cells clearly demonstrated the induction of apoptosis by *L. flexuosum* extract in liver cancer cells. Nuclear Factor-kappaB, a nuclear transcription factor, regulates the expression of various genes involved in inflammation and carcinogenesis. Tumor necrosis factor-α induced nuclear factor-kappaB-dependent reporter gene transcription was suppressed by the treatment with *L. flexuosum* as evidenced by Dual Luciferase reporter assay.

The findings suggest that *Lygodium flexuosum* functions as a potent antihepatotoxic, anti-inflammatory, anti-fibrotic and antiangiogenic agent, significantly reduces toxic hepatic injury induced by different chemicals. It also induces apoptosis in liver cancer cells.