

ANTITUBERCULAR DRUG AND ANTIMALARIAL DRUG INDUCED HEPATOTOXICITY AND ITS PROTECTION BY SELAGINELLA CORYMBOSA LEAVES EXTRACT

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ABSTRACT

In the present study, the hepatoprotective activity of hydroalcoholic extract of Selaginella corymbosa leaves was investigated. Qualitative test confirmed the presence of flavonoids, sterols and saponins in the extract. The extract was studied for isoniazid and chloroquine induced hepatic injury using 500mg/kg oral dose in albino rats. The changes were assessed by serum enzyme profile, which includes glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), direct bilirubin and total bilirubin levels. Histo pathological study was performed to observe the cellular damage of liver. There was a significant reversal of biochemical and histopathological changes induced by isoniazid and chloroquine in rats by hydroalcoholic extract treatment. This study was therefore undertaken to demonstrate the hepatoprotective activity of hydroalcoholic extract of Selaginella corymbosa against isoniazid (INH) and chloroquine induced hepatotoxicity in rats.

Keywords: Selaginella corymbosa, Isoniazid, chloroquine, GPT, GOT.

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INTRODUCTION

Drug induced liver injury by indiscriminate use for various therapeutic agents, such as antimalarial drugs, antitubercular drugs etc., is an unresolved problem, which has emerged as the most frequent cause for postmarketing withdrawal of medications, despite a rigorous preclinical and clinical review process (Davidsons & Eastham 1966). As alcohol consumption is ever increasing in the society, cirrhosis of liver usually as a complication of alcoholism, is the third most frequent cause of death among those 25 to 64 years of age (Lieber 1988). In recent years, there has been an upsurge in the clinical use of indigenous drugs, like herbal plants, originally used in the traditional system of medicine. *Selaginella corymbosa* (family – Selaginellaceae) is a well known traditional medicine in India. But the systemic research of this extract by any possible mechanism, using different acute and chronic hepatotoxic models seems to be scarce. This study was therefore undertaken to demonstrate the hepatoprotective activity of hydroalcoholic extract of *Selaginella corymbosa* leaves against isoniazid (INH), chloroquine and alcohol induced hepatotoxicity in rats.

MATERIALS AND METHODS

Plant Material: The plant leaves *Selaginella corymbosa* was identified by S Rajan Botanist, Emerald, Coimbatore, India; **Extraction Procedure:** The fresh leaves were collected, shade dried at controlled temperature, pulverized using mechanical grinder and passed through a 40-mesh sieve. The powdered drug was extracted by successive percolation

procedure with hydroalcoholic solvent (30 : 70 rats) at room temperature. The solvent was removed at low temperature (40-50°C) to give a drug extract 8.2% yield w/w (with respect to the powdered material). The extract was stored in a refrigerator and a weighed quantity was suspended in 2% v/v Tween-80 solution for the experiment.

Phytochemical Studies:

The chemical make-up of hydroalcoholic extract of *Selaginella corymbosa* was investigated. From preliminary phytochemical analysis, it was found that the extract showed a positive response for the presence of flavonoids, steroids and saponins.

Chemicals:

Drug suspensions of isoniazid (INH) (sigma aldrich., banglore) and chloroquine phosphate (IPCA, Mumbai) were prepared in distilled water. Ethanol (99.8% v/v) (Ranbaxy, Fine Chemicals Ltd.) was diluted upto 40% v/v with distilled water. All other chemicals used were of analytical grade. The standard polyherbal drug, Liv-52 was purchased from Himalaya.

Animals:

Wistar rats of either sex, 180-200g, were used for the experiment. They were kept at standard animal housing conditions and a day-night (10 h : 14 h) cycle was maintained throughout the experimental period. They were allowed to acclimatize one week before the experimentation, in the department of Animal House and had free access to food and water.

Blood Collection and Biochemical Estimations:

Blood samples were collected by cardiac puncture of rats. It was allowed to clot for 30 min. Then, serum was separated by

centrifugation at 2500 rpm for 10 min and analysed for standard liver function tests, such as serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) levels as well as direct and total bilirubin levels. The ready to use kits of SGOT, SGPT (by DNPH method) and bilirubin (by modified DMSO method) were employed for the biochemical studies.

Animal Experiment:

Hydroalcoholic extract of *Selaginella corymbosa* was administered orally upto 1g/ kg to individual rat of a group containing six rats as a pilot study. There was no mortality observed due to above treatment. Hence, by considering this study as well as previous references (Mujamdar et al. 1998; Bhanwra et al. 2000), the arbitrary dose of 500 mg/kg body weight per oral was employed for the entire study. All the drugs were administered orally. The group containing six rats was administered 2% Tween-80 solution as vehicle for 30 days. This group was designated as a control group, which will be considered as the same for all the models. After 30 days, blood was collected and serum was estimated for biochemical parameters such as SGOT, SGPT and bilirubin levels. After removal of blood, animals were sacrificed and liver was isolated from each rat for histo pathological studies. Effect of the hydroalcoholic extract of *Selaginella corymbosa* against isoniazid induced hepatotoxicity. The method followed was as described by Ninbkar et al. (2000). Rats were divided into three group (n=6); such as intoxicated, test and standard. Isoniazid (50mg /kg) was administered orally to all the groups for continuous 30 days daily. The test and

standard groups received *Selaginella corymbosa*(500mg/kg) and a standard hepatoprotective drug Liv-52 (0.5ml/kg) concurrently with isoniazid intoxication respectively. While intoxicated group was administered vehicle along with the INH treatment, after 30 days, blood samples were collected and serum was analyzed for different biochemical tests. Effect of the hydroalcoholic extract of *Selaginella corymbosa* against chloroquine induced hepato toxicity. The method followed by Daas & Shah (2000) was employed for this study, chloroquine (970 mg/kg) was given as a single oral dose to induce toxicity to all the three groups as eighty day of experiment along with their respective therapies. Groups such as toxicant, test and standard received vehicle (2ml/kg), *Selaginella corymbosa* extract (500mg/kg) and Liv-52 (0.5ml /kg) respectively for eight days. After 24 h to the chloroquine administration, blood samples were collected and serum was analysed for different biochemical tests.

Effect of the Hydroalcoholic Extract of *Selaginella corymbosa* against Alcohol Induced Hepatotoxicity:

The method was modified from the studies of Gadeholt (1984) and Kapur et al. (1994). All the three groups were treated with chronic ethanol (40% v/v) ingestion orally for 21 days in the dose of 2ml/100g. In addition, intoxicated group was administered vehicle (2ml /kg), while test and standard groups received their respective drugs like *Selaginella corymbosa* (500mg /kg) and Liv-52 (0.5ml /kg) from the day fifteen onwards till day twenty one. Biochemical estimation were performed after blood collection.

Histopathological Studies:

Immediately after blood collection, animals were sacrificed. Liver was removed from each animal and stored in 10% formal saline for purpose of fixation followed by dehydration, clearing, embedding in paraffin wax and staining with haematoxyline / eosin stain.

Statistical Analysis: results are presented as mean \pm S.E.M. and statistical significance between control and intoxicated as well as intoxicated and treated groups was evaluated by the student's t-test. $P < 0.01$ was considered significant.

RESULTS AND DISCUSSION

The drugs having adverse effects for liver such as isoniazid (50mg/kg) and chloroquine (970mg /kg) as well as widely consumed, alcohol (Ethanol 40% v/v) in the dose of 2ml/100 g were used in the present study. There was a significant rise ($p < 0.001$) in the serum GOT, GPT, direct bilirubin and total bilirubin levels with treatment of above stated intoxicated groups. Histopathological studies also revealed several areas with necrotic foci and degeneration of hepatocytes in the isoniazid treated group. There was

CONCLUSION

In the present study, serum enzyme markers, like GOT and GPT levels in the *Selaginella corymbosa* extract treated groups demonstrated a significant reduction ($p < 0.01$) against isoniazid, chloroquine and ethanol intoxication. Similarly, a marked fall in direct and total serum bilirubin levels respectively ($p < 0.001$) against the above mentioned hepatotoxic drugs clearly revealed protective effect of hydro alcoholic extract of *Selaginella corymbosa* leaves.

marked congestion, necrosis and hydropic vacuolation in the chloroquine intoxicated animals. Lastly, in the ethanol (40% v/v) treated liver sections, mild steatosis with small fat droplets were observed. Isoniazid converts to its toxic metabolites, such as hydrazine, acetyl hydrazine, deacetyl hydrazine etc. by cytochrome P-450 dependent mixed function oxidase. Both isoniazid and chloroquine exhibit a decrease in glutathione stores, namely glutathione, glutathione catalase, glutathione-S-transferase etc. which exert oxidative stress on liver and further cause lipid peroxidation. Ahesson & Ahesson 1984; Sodhiet al 1997; Farombi et al. 2000). Ethanol also directly by cytochrome P-450 2E, or indirectly through its active metabolites like acetaldehyde generates free radicals, such as superoxide, hydroxyl or hydroxy ethyl radicals. They readily demonstrate protein covalent binding and lead to glutathione depletion through CD14 receptor and TNF- α mediated action in kupffer cells which further lead to lipid peroxidation (Poli 1993; Yin et al 2001).

Histopathological studies also indicates a remarkable recovery, as in the isoniazid induced hepatotoxicity, the liver sections from *Selaginella corymbosa* treated groups demonstrated normal prominent cords; whereas the chloroquine induced hepatotoxicity models demonstrated moderate recovery with few areas of mild to moderate necrosis with concurrent treatment of *Selaginella corymbosa*. Fatty changes and hydropic vacuolation were observed in minimal intensity in the alcohol induced hepatotoxicity, treated by *Selaginella*

corymbosa extract revealed a reasonable recovery than ethanol intoxicated animals. Literature reveals a marked decrease in hepatic lipid peroxidation with concurrent treatment

with extract o leaves (Arivazhagan et al. 2000). Thus, the anti-lipoperoxidative property of Selaginella corymbosamay be contributing towards its hepatoprotective activity.

TABLE 1: EFFECT OF SELAGINELLA CORYMBOSA EXTRACT ON BIOCHEMICAL PARAMETERS IN RATS WITH ISONIAZID INDUCED LIVER DAMAGE.

Biochemical Parameters	Control	INH Intoxication	INH+ Extract	INH + Liv-52
SGOT (U/ml)	83±5.29	162.8#±4.01	123.8**±4.35	95.6** ±4.16
SGPT (U/ml)	58.3±3.18	129.5#±4.92	97.36**±6.35	78.2**±4.04
Direct Bilirubin (mg/dl)	0.113±0.002	0.368#±0.027	0.261**±0.023	0.186**±0.006
Total Bilirubin (mg/dl)	0.218±0.007	0.8#±0.019	0.419**±0.012	0.386**±0.019

(n=6) Values are in Mean ± S.E.M. # p<0.001 when compared with control* p<0.01, **p<0.001 when compared with intoxicated group

TABLE 2: EFFECT OF SELAGINELLA CORYMBOSA EXTRACT ON BIOCHEMICAL PARAMETER IN RATS WITH CHLOROQUINE INDUCED LIVER DAMAGE.

Biochemical Parameters	Control	Chloroquine Intoxication	Chloroquine + Extract	Chloroquine + Liv-52
SGOT (U/ml)	83±5.29	239.8#±5.24	142**±4.13	116**±4.75
SGPT (U/ml)	58.3±3.18	179.8#±8.19	96.16*±5.14	82.36**±3.82
Direct Bilirubin (mg/dl)	0.113±0.002	0.0376#±0.01	0.262*±0.007	0.169**±0.013
Total Bilirubin (mg/dl)	0.218±0.007	0.7#±0.021	0.437**±0.009	0.37**±0.011

(n=6) Values are in Mean ± S.E.M. # p<0.001 when compared with control* p<0.01, **p<0.001 when compared with intoxicated group

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