HEPATOTOXICITY AND NEPHROTOXICITY ACTIVITY OF ETHANOL EXTRACT OF ABIES WEBBIANA LINDL. LEAVES

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ABSTRACT

The present study was conducted to evaluate the effect of alcoholic extract A. webbiana leaves Lindl on liver and kidney. Wistar rats were divided into three groups (n=3) for hepatotoxicity and nephrotoxicity. Alcoholic leaves extract of A. webbiana was evaluated for protective effects against CCl4 induced hepatotoxicity and cisplatin induced nephrotoxicity. Hepatotoxicity studied by comparing parameters to control group, CCl4 treated group and induced CCl4 followed by alcoholic extract group such as SGOT, SGPT, SALP, bilirubin and also compare histopathological examination of liver tissue. Nephrotoxicity studied by comparing parameters to control group, cisplatin treated group and induced cisplatin followed by alcoholic extract group serum BUN, serum creatinine, serum protein and % change in body weight and histological examination of kidney tissues. Effect of ethanol extract of Abies webbiana at dose of 400 mg/kg was studied by comparing the liver and kidney parameters with carbon tetra chloride and cisplatin induced as control respectively. Ethanol extracts of Abies webbiana at dose 400 mg/kg was found to be hepato and nephro protective effect and histopathological examination also support these activity. It is concluded that alcoholic extract possess hepatoprotective and nephroprotective activity significantly.

KEYWORDS: Abies webbiana, Hepatotoxicity, Nephrotoxicity, Cisplatin and CCl4

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INTRODUCTION

*Abies webbiana* commonly known as Talispatra in Bengali and Hindi, Talispatram in Sanskrit and Indian Silver Fir in English, is a large, tall, evergreen tree occurring in the Himalayan region from Kashmir to Assam in India. It comes under the Family: Pinaceae. The leaves of this plant have different uses in Ayurveda, the traditional system of Indian medicine and have been described for using against swasa, kasa, amadosha, hikka, chhardi and mukharoga. A. *webbiana* leaves have been reported as antibacterial and antifungal, mast cell stabilizing, anxiolytic, anti-tumor, anti-inflammatory, anti-tussive, female antifertility, febrifuge, anti-spasmodic properties, central nervous system (CNS) depressant actions and are effective against hyperglycemia, conception, rheumatism and high temperature. In phytochemical screening certain chemical constituents, mainly monoterpenes (from essential oil), flavonoids, biflavonoid glycosides, phytosterols, amino acids, saponins, tannins, alkaloids, lipids, interpenoids, steroids and glycosides were found and a new alkoidal namely 1-(4'-methoxyphenyl)-aziridine, a nitrogenous compound and a new biflavonoid, Abiesin have been isolated. There have been no reports on the in vivo hepatotoxicity and nephrotoxicity activities of *A. webbiana*. Therefore, the present study has been carried out to evaluate the hepatoprotective and the nephroprotective activity of *A. webbiana* extract in carbon tetrachloride and cisplatin induced hepato and nephrotoxicity in rats.

MATERIALS AND METHODS

(i) Collection of plant material

The leaves of *Abies webbiana* Lindl. were collected from the forest of Tungnath (Garhwal, Uttarakhand). Plant material was authenticated by Head, Department of Pharmacognosy and Ethnopharmacology, NBRI, Lucknow. The voucher specimen was preserved for the future reference. The leaves were separated from the branches and dried at the temp 40ºC for one hour before pulverization of mechanically grinder. The powder was passed through 40 mesh sieve, and preserved for future purpose in tightly sealed container.

(ii) Preparation of alcoholic extract

About 500 gm of the powdered shade dried leaves of *Abies webbiana* Lindl. was extracted with 70% v/v of alcohol, by continuous heat extraction in a soxhlet extractor. The extract was concentrated to a small volume and evaporated on a water bath to dryness. 10% w/v extract was prepared using distilled water containing 2% v/v Tween 80 (as a suspending agent).

(iii) Animals

Albino rats (Wistar) weighing 150-200g of either sex were used in the present study. The animals were acclimatized for one week under laboratory conditions in SGIT, Ghaziabad (U.P.). They were housed in polypropylene cages and maintained at 22°C ± 2°C under 12 hrs dark / light cycle. They were fed with standard rat feed and water ad libitum. The experimental protocol was approved by the Institutional animals ethical committee (IAEC, Registration No SGIT/2014/04) prior to the beginning of the work.

(iv) Acute toxicity study

Different doses (5, 50, 300, 1000 and 4000 mg/kg, p.o.) of alcoholic extract of *Abies webbiana* Lindl. and isolated alkaloids and flavonoids (0.5, 5, 300, 500 mg/kg, p.o.) to the animals were used for acute toxicity in accordance to Organization for Economic Cooperation Development (OECD, 2002) guideline 423. Three rats, each sequentially dosed at intervals of 48 hrs, were used for the test. Once daily cage side observations included changes in skin, fur, eyes, mucous membrane (nasal), autonomic (salivation, lacrimation, perspiration, piloerection, urinary incontinence and defecation) and central nervous system (drowsiness, gait, tremors and convulsions) changes. Mortality, if any, was determined over a period of 2 weeks. For the assessment of activity, one dose level was chosen in such a way that, high dose was approximately one-tenth of the maximum dose during acute toxicity studies, which was 400 mg/kg, p.o.

(v) Method for Evaluation of Hepatotoxicity activity

In the dose response experiment, Wistar albino rats were taken randomly assigned into 3 groups of 6 individuals each.

Group-I Animals (-ve control) were administered 1ml distill water p.o., for 5 days

Group-II Animals (+ve control) were administered (0.1ml/kg) CCl₄ (i.p.) for 5 days

Group-III Animals were administered (CCl₄ + 400 mg/kg) alcoholic extract p.o. for 5 days

Animals were sacrificed on the 6th day under mild ether anesthesia. Blood samples were collected and centrifuged (after adding heparin) immediately to get clear serum for evaluating the serum biochemical parameters. The liver samples were dissected out, blotted off blood, wash with saline and were used to estimate the liver weight and liver volume and also stored it in 10% formalin and processed for histopathology to evaluate the details of hepatic architecture in each group microscopically. Serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxaloacetate transaminase (SGOT), Serum alkaline phosphates (SALP), Serum total bilirubin and Serum direct bilirubin were estimated by commercially available kits.

(vi) Method for Evaluation of Nephrotoxicity activity

In the dose response experiment, albino rats were taken randomly assigned into 3 groups of 6 individuals each.

Group- I Animals (-ve control) were administered 1ml distill water p.o., for 7 days

Group- II Animals (+ve control) were administered (6 mg/kg, i.p.) Cisplatin, for 7 days

Group-III Animals were administered (Cisplatin + 400 mg/kg) alcoholic extract p.o. for 7 days

The body weight of all the animals was taken on every day. The animals were sacrificed on day 7 under mild

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ether anesthesia. Kidney tissues and blood samples were collected. Kidneys were weighed; the samples of kidney tissue were stored in 10% formalin and processed for histopathology. The blood samples were used to measure serum creatinine and blood urea nitrogen (BUN).

(vii) Histological study
Small pieces of liver and kidney fixed in 10% buffered formalin were processed for embedding in paraffin. Sections of 4–6 µm thickness were stained with hematoxylin and eosin and examined for histopathological changes of liver and kidney.

(viii) Statistical Analysis
The values were expressed as Mean ± SEM. Statistical analysis was performed by tukey multiple comparison test. One way analysis of variance (ANOVA) by Tukey multiple comparison test, was carried out & p<0.05 was considered as significant. P<0.01 more significant and ***P<0.001 highly significant. Groups were compared with control and Negative control group.

RESULTS
There was no toxicity was found in alcoholic extract in acute toxicity study. Assessment for activity, one dose level were chosen in such a way that, high dose was approximately one-tenth of the maximum dose during acute toxicity studies, which was 400 mg/kg, p.o.).Hepatotoxicity study was performed and level of SGOT, SGPT, SALP and total bilirubin (Total and Direct) shown in (Table No 1). It was found that the biochemical measurement of CCl₄ (Group II) were significant increased as compared to control (Group I). After induced CCl₄ followed by alcoholic extract (Group III) which are significant decreased the increased level of biochemical parameter by CCl₄ as compare to negative control (Group II) which are treated by CCl₄.

Nephrotoxicity study was assessed and level of serum BUN, serum creatinine and serum protein and % change of body weight shown in (Table No 2). The levels of serum BUN, serum creatinine and serum protein and % change of body weight were increased significantly in cisplatin treated animals (Group II) when compared to normal control animals (Group I). The extent of elevation was reduced significantly in animals which received by alcoholic extract (Group III).

Table 1
Effect of alcoholic extract of A. webbiana on liver biomarkers

<table>
<thead>
<tr>
<th>Group</th>
<th>Level of Biochemical Parameters</th>
<th>%change in body weight</th>
<th>Serum creatinine (mg/dl)</th>
<th>Serum BUN(mg/dl)</th>
<th>Serum protein(g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>1.275±0.06</td>
<td>0.781±0.01</td>
<td>16.92±0.04</td>
<td>3.89±0.09</td>
</tr>
<tr>
<td>CCl₄</td>
<td></td>
<td>13.55±0.95***</td>
<td>2.079±0.01***</td>
<td>26.87±0.06***</td>
<td>9.49±1.25***</td>
</tr>
<tr>
<td>ALC. Extract (400 mg/kg)+ CCl₄</td>
<td></td>
<td>2.11±0.41***</td>
<td>1.41±0.23***</td>
<td>3.5±0.12***</td>
<td>6.47±0.25***</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (N=6) one way ANOVA followed by Tukey’s multiple comparison column test. Where * represent significant at P<0.05, ** more significant at P<0.01 and *** highly significant compare with CCl₄ group and “” represent significant compare with Control group.

Table 2
Effect of alcoholic extract of A. webbiana on kidney biomarkers

<table>
<thead>
<tr>
<th>Group</th>
<th>LEVEL OF BIOCHEMICAL PARAMETERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>%change in body weight</td>
<td>Serum creatinine (mg/dl)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Cisplatin</td>
<td></td>
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<td>ALC. Extract (400 mg/kg)+ Cisplatin</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM (N=6) one way ANOVA followed by Tukey’s multiple comparison column test. Where * represent significant at P<0.05, ** more significant at P<0.01 and *** highly significant compare with Cisplatin group and “” represent significant compare with Control group.

Figure 1
Histopathological study of rat’s liver CCl₄ induced hepatotoxicity

Figure 2
Histopathological study of the rat’s Kidney Cisplatin induced nephrotoxicity


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DISCUSSION

In the present study it was found that the leaves extract of *A. webbina* can modulate hepatotoxicity induced CCl₄ and nephrotoxicity induced by cisplatin. Assessment of liver function can be made by estimating the activity of enzyme (SGOT, SGPT and SALP) which are originally present in higher concentration in the cytoplasm and liver plasma membrane. It is damaged these enzymes released into the blood stream. Pretreatment with *A. webbiana* leaves extract showed the ability lower the increased serum enzymes caused by CCl₄. Determination of serum bilirubin (total and direct) represents an index for the assessment of hepatic function and any abnormal increase in the levels of bilirubin serum indicate disturbance of hepatocellular function. Ethanol extracts of *A. webbiana* leaves reduced the increased level of bilirubin suggest the possibility of extract stabilization bilirubin dysfunction of liver. Histopathological observation revealed confluent necrosis, showing fatty changes and hydropic degeneration and after treatment with extract of *A. webbiana* leaves was found to reduced focal hemorrhage, inflammation and partial protection of hepatocytes focal necrotic area. It is concluded that ethanol extract of *A. webbiana* leaves has hepatoprotective against CCl₄. Cisplatin induces oxidative stress causing damage to intracellular organelles and alters this functions which lead to inhibition of protein synthesis glutathione depletion lipids per oxidation and mitochondrial damage. Induction of nephrotoxicity by cisplatin is assessed to be a rapid process involving reaction with protein in renal tubules. As renal damage occurs within an hour after administration of cisplatin and significantly elevated the levels of BUN and serum creatinine in rats. Ethanol extracts of *A. webbiana* leaves reduced the increased level of these parameters. Organ weight can be the most sensitive indicator of an experimental compound as significant differences between treated and untreated (control) may occur in the absence of morphological changes. In this study ethanol extract of the drug significantly reduced the weight of kidney in comparison with cisplatin treated group of animal. Histopathological examination showed widespread tubular necrosis and dilatation in Cisplatin-treated rats which states cisplatin induced nephrotoxicity. Ethanol extract of *A. webbiana* decreased the cisplatin-induced tubular necrosis and most of the changes were caused by cisplatin treatment. Flavonoids are a group of polyphenolic compounds which are present widely in plant kingdom both in the free state as glycosides and posses wide biological activities. Flavonoid has reported that protective against environmental toxic agents. Phytochemical analysis revealed that various chemical constituents like; monoterpenes (from essential oil), flavonoids, biflavonoid glycosides, phytoestrogens, amino acids, saponins, tannins, alkaloids, lipids, triterpenoids, steroids, diterpene glycosides and alkaloids are present in the leaf of *Abies webbiana*. In the present investigation, it was observed that pretreatment with alcoholic extract (400 mg/kg p.o.) has significantly reduced the elevated biochemical markers of liver and kidney. On the basis of biochemical and histopathological observation can say that the alcoholic extract has shown significant protective effect on liver and kidney.

CONCLUSION

Based on the present study, it can be concluded that alcoholic extract (400 mg/kg p.o.) of *Abies webbiana* leaves have potent hepatoprotective and nephroprotective activity.

SCOPE FOR THE FURTHER STUDY

Present work is preliminary study of the ethanol extracts used for nephrotoxicity and hepatotoxicity. It can help the isolation and formulation of extract or isolated active constituents like flavonoid and alkaloid.

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CONFLICT OF INTEREST

All authors have no conflict of interest.
REFERENCES