EVALUATION OF HEPATOPROTOCTIVE EFFECT OF ADIANTUM INCISUM FORSK LEAF EXTRACT AGAINST CCL4 INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT
Oxidative damage is involved in the pathogenesis of various hepatic injuries. In the present study, the protectivity of methanolic extract of leaves of Adiantum incisum forsk (MEAI), as an antioxidant to protect against CCL4-induced oxidative stress and hepatotoxicity in Albino Wistar rats was investigated. Intraperitoneal injection of CCL4 produced a marked elevation in the serum levels of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP) and total bilirubin, and decrease in the total protein. Histopathological analysis of the liver of CCL4-induced rats revealed marked liver cell necrosis with inflammatory collections that were conformed to increase in the levels of SOD, GPx, LPO and CAT. Daily oral administration of methanolic extract of Adiantum incisum forsk at 100 and 200 mg/kg doses for 10 days produced a dose-dependent reduction in the serum levels of liver enzymes. Treatment with A. incisum forsk normalized various biochemical parameters of oxidative stress and was compared with standard Silymarin. Therefore, the results of this study show that A. incisum forsk can be proposed to protect the liver against CCL4-induced liver damage in rats, and the hepatoprotective effect might be correlated with its antioxidant and free radical scavenger effects.

KEYWORDS: Adiantum incisum, hepatoprotective, CCL4, silymarin, antioxidant.

INTRODUCTION
Free radicals are critically involved in various pathological conditions such as cancer, cardiovascular disorder, arthritis, inflammation and liver diseases. Liver disease is an acute or chronic damage to the liver, usually caused by infection, injury, exposure to drugs or toxic compounds, an autoimmune process, or by a genetic defect. Chemicals such as carbon tetrachloride catabolised radicals induced lipid peroxidation, damage the membranes of liver cells and organelles, causes the swelling and necrosis of hepatocytes and result to the release of cytosolic enzymes such as AST, ALT and ALP into the circulating blood. These enzymes are the indications of chemical induced liver damage.

Medicinal value of pteridophytes is known to man for more than 2000 years. Adiantum incisum Forsk belongs to the family Adiantaceae, is a pteridophyte grown in slopes of hills in Punjab, tamilnadu, rajasthan. Fronds (leaves) of A.incisum having a bud in its apical region, which serves the purpose of vegetative propagation that is why, this fern is called as ‘Walking fern’. A lot of Adiantum species have been used in traditional Chinese medicine to cure human and animal diseases including relief of internal heat or fever, enhancement of urination, removal of urinary calculus, and sundry other curative claims. Adiantum incisum forsk is used for hemicranias, cough, fever, skin diseases, internal burning of body, jaundice and liver problems. Hence, the present investigation deals the hepatoprotective effect of the Methanolic extract of Adiantum incisum Forsk (MEAI) against CCL4 induced hepatotoxicity in rats. The study includes serum biochemical parameters, antioxidant parameters of tissue homogenate and histopathological examination of liver of treated rats.

MATERIALS AND METHODS
Collection and Authentication of plant
Adiantum incisum Forsk is grown in the slopes of tamilnadu. For the present work the plant was collected from Shevroy hills of Tamil Nadu. The plant was positively identified by Dr.A. Balasubramaniam, Siddha Research Consultant, ABS Botanical garden, Karipatti, Salem district, Tamilnadu. and authenticated the plant as Adiantum incisum Forsk of family Adiantaceae from available literature.

Preparation of the leaves extract
The powdered leaves of Adiantum incisum Forsk was defatted with pet-ether for 12 hrs and successfully extracted with methanol using soxhlet apparatus for 2 days. The obtained extract was concentrated by distillation stored in a desiccator and used for subsequent experiments.

Animals
Healthy male Wistar albino rats of 2 to 3 months of age and approximately weighing between 150-250g were used in the present study. Rats were housed in a polypropylene cages and allowed free access to feed and tap water under strictly controlled pathogen free conditions with room temperature 25±2°C. All the animals were followed the internationally accepted ethical guidelines for the care of laboratory animals. The experimental protocol has been approved by institutional animal ethics committee, JKKMMRF College of Pharmacy, B.Komarapalayam, Namakkal. (Regd. No. JKKMMRFCP 1158/PO/ac/07/PCSEA).

Experimental protocol
The rats were divided into five groups, comprising of six animals in each group.

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Effect of the effects of Adiantum incisum Forsk 100mg/Kg.

GROUP – II: Received methanolic extract of leaves of Adiantum incisum Forsk 200mg/Kg.

GROUP – III: Received Silymarin, the standard drug (25mg/kg).

GROUP – IV: Received methanolic extract of leaves of Adiantum incisum Forsk 100mg/Kg.

GROUP – V: Received methanolic extract of leaves of Adiantum incisum Forsk 200mg/Kg.

0.7 ml/kg of CCl₄ was injected intra-peritoneally (i.p.) to all groups except normal control to induce hepatotoxicity on 3, 6 and 10th days of experiment. Duration of the study was 10 days. All the animals were sacrificed on the 11th day for the estimation of biochemical parameters.

**Estimation of serum biochemical parameters**

On 11th day blood was collected from animals under anaesthesia by cardiac puncture. Blood samples collected centrifuged at 3500 rpm for 15 mins at room temperature for separation of serum. The clear, non-haemolysed sera was separated using clean dry disposable plastic syringe and stored at -20°C for measurements of SGOT, SGPT, Total protein, total bilirubin and ALP.

**Estimation of antioxidant parameters**

The liver was perfused with 0.86% cold saline to completely remove all the red blood cells. Then it was suspended in 10% (w/v) ice-cold 0.1 M phosphate buffer (pH 7.4) cut into small pieces, and the required quantity was weighed and homogenized using a homogenizer. The homogenate was centrifuged at 3000 rpm for 20 min to remove the cell debris. The supernatant was used for the estimation of Catalase, Superoxide dismutase, Gluthathione peroxidase and Lipid peroxidase.

**Histopathology of liver**

The liver was fixed in 10% formalin and embedded in paraffin wax. Sections were made using rotary microtone and haematoxylin-eosin was used as stain. Histological observations were made light microscope.

**Statistical analysis**

The data were statistically analyzed using one-way ANOVA followed by Tukey's method using Graph pad prism statistical software and all values are expressed as Mean ± SEM. Values are considered as statistically significant at *p*<0.05, **p**<0.01, ***p***<0.001.

**RESULTS**

**Effect of MEAI on serum parameters in CCl₄ induced hepatic damage in rats**

The effects of extract at two dose levels (100 and 200 mg/kg, p.o.) on serum marker enzymes and total bilirubin in CCl₄-induced hepatic injury are shown in Table 1(Fig: 1 & Fig: 2). Hepatic injury induced by CCl₄ caused significant rise in marker enzymes AST, ALT, ALP and serum bilirubin. Administration of EENA leaf at three different dose levels attenuated the increased levels of the serum enzymes, produced by CCl₄, and caused a subsequent recovery towards normalization almost like that of Silymarin treatment.

**Effect of MEAI on antioxidant parameters in CCl₄ induced hepatic damage in rats**

The effects of extract at two dose levels (100 and 200 mg/kg, p.o.) on liver antioxidant enzymes in CCl₄-induced hepatic injury are shown in Table 2. Hepatic injury induced by CCl₄ caused significant increases in liver antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase and lipid peroxidase. Administration of MEAI at different dose levels shows significant dose-dependent decreases, when compared with diseased control animals. Results were shown in table. 2 and Fig: 3.

**Histopathology of the liver**

In histopathological studies, the liver sections of rats treated with vehicle showed normal hepatic architecture. CCl₄-induced liver of rats shows loss of hepatic architecture with intense peripheral central vein necrosis, fatty changes, crowding of central vein. In rats treated with silymarin, a normal hepatic architecture with moderate mild degree of necrosis. Pre-administration of MEAI at the dose of 100 and 200 mg/kg and silymarin for 10 days reduces the hepatic injury score of fatty degeneration and necrosis, clearly indicating the protection offered by silymarin and leaf extract. Histological examination showed a protective effect of A. incisum on CCl₄-induced hepatotoxicity. (Fig:4)

**DISCUSSION AND CONCLUSION**

Carbon tetrachloride is commonly used for inducing liver damage. It causes peroxidative degradation in adipose tissue and it is metabolised to trichloromethyl radical and trichloromethyl peroxy radical which are involved in pathogenesis of liver. CCl₄ produces oxidative damages and leakages of SGOT, SGPT, ALP in serum and increase in TB levels and decrease in total protein. Therefore, the reduction in levels of SGPT and SGOT by the A incisum leaves extract is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCl₄. This effect shows that serum levels of transaminases return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes. It also suppressed the increased level of cholesterol.

Antioxidant activity has been hypothesized that one of the principal causes of CCl₄-induced liver injury is formation of lipid peroxides by free radical derivatives of CCl₄ (CCl₃ free radical). Thus, the antioxidant activity or the inhibition of the generation of free radicals is important in the protection against CCl₄-induced hepatopathogy. The body has an effective defence mechanism to prevent and neutralize the free radical-induced damage. This is accomplished by a set of antioxidant enzymes such as SOD, CAT and GPX. The increased level of SOD, catalase and GPX observed point out the hepatic damage in the rats administered with CCl₄. But the groups treated orally with methanolic extract of A. incisum extract showed significant decrease in the level of these enzymes, which indicates the free radical scavenging property of A. incisum.

The hepatoprotective effect of A. incisum extract was confirmed by histological examination of the liver tissue of control and treated animals. The histological architecture of CCl₄ treated liver section showed fatty degeneration of hepatocytes. However administration of A. incisum extract almost normalized these defects in the histological architecture of the liver, almost to the level of the Silymarin treated groups showing its potent hepatoprotective effects. The administration of methanolic extract of A. incisum extract revealed significant protection in hepatocyte regeneration against the toxic effect of carbon tetrachloride. Hence, the histological examination of extract treated group showing hepatoprotective effects and it is supported to biochemical studies. The qualitative phytochemical analysis on the methanolic extract of Adiantum incisum Forsk shows the presence of flavonoids.
The dominant constituents of *A. incisum* are the flavonoids and triterpenoids. Flavonoid constituent of plant possess antioxidant and hepatoprotective properties15-17. Based on the results obtained from the present study, it can be concluded methanolic extract of *Adiantum incisum* Forsk (MEAI) is found to be more potent and effective hepatoprotective activity. More detailed studies are however necessary to identify the active principle(s) and its exact mechanism of action.

REFERENCES

**TABLE-1** Effect of *A.incisum* extract on serum parameters in CCl4 induced hepatic damage in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>ALP (U/L)</th>
<th>TOTAL BILIRUBIN (mg/dl)</th>
<th>TOTAL PROTEIN (mg/dl)</th>
<th>CHOLESTEROL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP I</td>
<td>71.50 ± 0.90</td>
<td>141.5 ± 3.24</td>
<td>143.6 ± 1.53</td>
<td>0.97 ± 0.01</td>
<td>10.7 ± 0.03</td>
<td>106.3 ± 0.43</td>
</tr>
<tr>
<td>GROUP II</td>
<td>289.4 ± 3.85***</td>
<td>414.2 ± 1.08***</td>
<td>441.5 ± 0.64***</td>
<td>3.6 ± 0.14**</td>
<td>7.2 ± 0.12***</td>
<td>162.7 ± 0.37***</td>
</tr>
<tr>
<td>GROUP III</td>
<td>84.2 ± 1.51***</td>
<td>157.6 ± 1.15***</td>
<td>167.6 ± 3.13**</td>
<td>1.08 ± 0.01***</td>
<td>9.5 ± 0.12**</td>
<td>122.4 ± 0.69**</td>
</tr>
<tr>
<td>GROUP IV</td>
<td>173.1 ± 1.22**</td>
<td>291.0 ± 1.13***</td>
<td>292.6 ± 1.27***</td>
<td>1.52 ± 0.03***</td>
<td>8.4 ± 0.09**</td>
<td>154.0 ± 5.42*</td>
</tr>
<tr>
<td>GROUP V</td>
<td>95.1 ± 2.36***</td>
<td>162.9 ± 0.83**</td>
<td>192.5 ± 0.53**</td>
<td>1.16 ± 0.02**</td>
<td>8.1 ± 0.08***</td>
<td>136.9 ± 2.86***</td>
</tr>
</tbody>
</table>

Values are given as mean ± Standard error mean (S.E.M) for five groups of six animals each. Values are statistically significant at *p*<0.05, *p*<0.01, ***p*<0.001. Group II compared with group I and Groups III, IV & V were compared with group II.

**TABLE-2** Effect of *A.incisum* extract on antioxidant parameters in CCl4 induced hepatic damage in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Catalase (U/mg protein)</th>
<th>Superoxide dismutase (U/mg protein)</th>
<th>Glutathione peroxidase (U/mg protein)</th>
<th>Lipid peroxidase (nmol/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP I</td>
<td>307.14 ± 0.89</td>
<td>86.10 ± 2.50</td>
<td>167.22 ± 1.26</td>
<td>4.02 ± 0.42</td>
</tr>
<tr>
<td>GROUP II</td>
<td>318.80 ± 3.65</td>
<td>96.21 ± 2.19***</td>
<td>68.17 ± 0.81***</td>
<td>9.17 ± 0.14***</td>
</tr>
<tr>
<td>GROUP III</td>
<td>295.95 ± 2.51**</td>
<td>79.33 ± 2.33***</td>
<td>148.22 ± 1.85***</td>
<td>3.63 ± 0.04***</td>
</tr>
<tr>
<td>GROUP IV</td>
<td>264.16 ± 4.04***</td>
<td>70.34 ± 0.42**</td>
<td>119.32 ± 4.16***</td>
<td>4.35 ± 0.13***</td>
</tr>
<tr>
<td>GROUP V</td>
<td>271.27 ± 1.47***</td>
<td>73.62 ± 0.78***</td>
<td>131.56 ± 1.89 **</td>
<td>4.11 ± 0.05***</td>
</tr>
</tbody>
</table>

Values are given as mean ± Standard error mean (S.E.M) for five groups of six animals each. Values are statistically significant at *p*<0.05, *p*<0.01, ***p*<0.001. Group II compared with group I and Groups III, IV & V were compared with group II.
Figure: 1. Effect of MEAI on SGOT (U/I), SGPT (U/I), ALP (U/I), Cholesterol (mg/dl) levels in CCl₄ induced hepatotoxicity in rats.

Figure: 2. Effect of MEAI on Total protein (mg/dl) and total bilirubin (mg/dl) in CCl₄ induced hepatotoxicity in rats.

Figure: 3. Effect of MEAI on antioxidant parameters in CCl₄ induced hepatotoxicity in rats.
Figure: 4. Effect of MEAI on Histopathology of liver

Photomicrograph of liver shows a normal hepatic cellular arrangements in Group I (a) whereas, in group II (b) showing loss of hepatic architecture with intense peripheral central vein necrosis, fatty changes, crowding of central vein. In rats treated with silymarin (c), a normal hepatic architecture with moderate mild degree of necrosis. Group IV and group V (d & e) reduces the hepatic injury score of fatty degeneration and necrosis, clearly indicating the protection offered by MEAI.

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