Evaluation of the Hepatoprotective Potential of Adiantum capillus Against Carbon Tetrachloride-Induced Hepatopathy in Rodents

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INTRODUCTION

The liver is the largest gland in the body and weight about 1.4 gm. The liver is detoxified and metabolize different drugs and xenobiotics by cytochrome P450 (CYP) enzymes and add polar functional groups onto a drug molecule. Cytochrome belongs to the isozymes family which are responsible for the reduction and hydrolysis of organic molecules.1 Liver diseases, which are still a world health problem, may be divided into acute or chronic hepatitis (inflammatory liver diseases) and cirrhosis (degenerative disorder due to fibrosis in the liver). Treatments of liver diseases are disputed because conventional or synthetic drugs for the treatment of these diseases are no up to mark and causes side effects.2 Liver diseases are shown the highest mortality among all the digestive diseases. Adiantum capillus-veneris Linn (Family: Adiantaceae) commonly known as “Hansraj, avenca and maidenhair”3,4 cultivated throughout India, Sri Lanka and many tropical countries. Adiantum capillus (AC) is used in cold and cough bronchitis, inflammation, fever, liver and skin diseases. It is also used as a stimulant, emollient and purgative to improve appetite, digestion, stimulate renal function febrifuge, galactagogue and alopecia.3 The major chemical constituents of Adiantum capillus (ACE) leaves contain flavonoids, alkaloids, tannins, saponins, glycosides, steroids and terpenoids. Adiantum leaves extract contains a high level of flavonoids that are a good source of antioxidants.3 Isolated new terpenoids that were 22, 29-epoxy-30-norhopan-13-ol from the Adiantum capillus leaves with strong antibacterial activity.3 3- coumaroyl quinic acid, kaempferol-3-glucosides as a major phenolic compound.7
There is no work reported on hepatoprotective activity of *Adiantum capillus*. Hence, the present study was carried out to evaluate the hepatoprotective activity of *Adiantum capillus* against CCl₄ induced liver toxicity in rats.

**MATERIALS AND METHODS**

Collection and identification of Plant material
Plants were freshly collected and authenticated at National Botanical Research Institute, Lucknow and a voucher specimen (NBRI-SOP-201) for the collected sample has been submitted for future reference.

Preparation of extract
The whole plant of *Adiantum capillus* (AC) was freshly collected and subjected to shade-dried the dried whole plants were milled to a coarse powder. Successive extracts of *Adiantum capillus veneris* Linn whole plant powder was prepared using soxhlet apparatus petroleum ether (50-80°C), chloroform and 50% ethanol. The extracts were filtered, pooled and concentrated through a rotary evaporator.

Phytochemical test
A phytochemical screening test of 50% ethanolic extract of *Adiantum capillus* was performed for alkaloids, carbohydrates, flavonoids, glycosides, triterpenoids, resins, saponins, steroids and tannins as per reported method.⁸ ⁹

Experimental animals
Wistar rats weighing 140±20 gm were used for the study. They were kept in well-ventilated cages at room temperature (24±2°C) and relative humidity respectively. Animals were given with pellet diet (Bharat Ansh Scientific Industries, Lucknow) and the food was stopped 18–24 h before the experiment, water was allowed *ad libitum*. The Institutional Animal Ethics Committee of the Mahatma Gandhi Institute of Pharmacy, Lucknow (1957/PO/Re/S/17CPCSEA), approved the protocol.

Acute toxicity studies
The oral toxicity study was performed as per OECD-423 guideline. The pilot study was done by using three rats per treatment. 50% ethanolic extract of *Adiantum capillus* (ACE) extract was given at up to 2 g/kg.¹⁰

Hepatoprotective Activity
Wistar rats (140 ± 20 g) were categorized into six groups, each group having six rats. Group I served as control, given a single dose of carboxymethyl cellulose (1 ml of 1%, w/v, p.o.). Group II given Carbon tetrachloride (1 ml/kg body weight, i.p., 1:1 v/v mixture of CCl₄ and olive oil) alone, while group III given orally 100 mg/kg silymarin and group IV, V and VI given 100, 200 and 300 mg/kg body weight of 50% ethanolic extract of *A. capillus* (ACE) in (1%, w/v, CMC) respectively along with Carbon tetrachloride as in group II. The 50% ethanolic extract of *Adiantum capillus* (ACE) was given daily while Carbon tetrachloride was given every 72 h for 14 days. Animals were sacrificed 48 h after the last dose of the drug. Blood samples and tissue (liver) were collected.¹¹ The serum was separated for biochemical estimation.

Histopathological studies
For histopathologic analysis, samples of the liver are obtained from all groups and store in 10% phosphate-buffered formalin dehydrated in graded (50%-100%) alcohol and embedded in paraffin, sectioned at (5 µm), and stained with routine hematoxylin and eosin (H&E) stain for photomicroscopic assessment.

Antioxidant activity
The liver was clean with ice-cold saline to remove as blood residue. The liver was homogenized in ice-cold 0.9% sodium chloride solution with a Potter-Elvehjem glass homogenizer. The homogenate was separated at 840 rpm for 15 min and the supernatant was again separated at 1200 rpm for 15 min and the got fraction was used for Lipid peroxidation (LPO), Catalase (CAT), Superoxide Dismutase (SOD), and Reduced Glutathione (GSH) activity estimation.

Lipid Peroxidation (LPO) assay
Homogenate was dissolved with 0.2 ml of an 8.1% (w/v) sodium dodecyl sulfate solution, 1.50 ml of a 20% acetic acid solution and 1.50 ml of a 0.8% (w/v) solution of TBA and make up the volume, 4.0 ml with distilled water. Every vial was tightly packed and heated in a boiling water bath for 60 min, cooled with water. An equal volume of incubated samples and 10% trichloroacetic acid were placed into a centrifuge tube, mixed well and centrifuged at 1000 rpm for 10 min. The absorbance of the supernatant fraction was measured at 532 nm. TBA was replaced with distilled water served as control.¹²

Reduced Glutathione (GSH) assay
The concentration of GSH found the basis on the development of a yellow colour when 5,5’-dithiobis (2-nitrobenzoic acid) was added to compounds having sulphhydryl groups. The mixture having 4% sulfosalicylic acid and tissue samples homogenized in 4 volume of ice-cold 0.1 ml phosphate buffer (pH 7.4). The procedures used for determining the Glutathione and determine the non-protein sulphhydryl concentration inclusive of Glutathione. Enzyme activity was expressed as milligram per hundred gram.¹³
Assay of Superoxide Dismutase (SOD)
Superoxide dismutase scavenges the superoxide radical (O$_2^-$) and thus provides a defence against free radical damage.0.5ml of liver homogenate was diluted with water, 0.25 ml ethanol and 0.15ml of chloroform. The composition was centrifuged at 2000 rpm and mixed with 0.5 ml of the supernatant. Superoxide dismutase performance was measured as U/l change in optical density per minute at 50% reduced to adrenochrome transition.14

Assay of Catalase
In animals, catalase is present in all organs, especially being concentrated in the liver and erythrocytes. During β-oxidation of fatty acids by flavoprotein dehydrogenase, hydrogen peroxide was generated, which was accepted upon by catalase present in peroxisomes. To 0.1ml of liver homogenate, 1.0ml of each phosphate buffer and hydrogen peroxide were added and mixed. The reaction was initiated by the addition of a 2.0 ml dichromate acetic acid reagent and heated on the water bath. The green colour was developed, absorbance was taken at 570 nm.15

Statistical Analysis
The results were shown as Mean ± SEM. Statistical data was measured by Graph pad prism software, a one-way ANOVA statistical program. The obtained results were considered significant at P<0.05.

RESULT

Phytochemical investigation
The phytochemical investigation showed the presence of various phytoconstituents like carbohydrates, alkaloids, phenols, flavonoids, saponins, tannins and triterpenoids in extracts.

Acute toxicity studies:
Ethanic extract of A. capillus (ACE) did not show any behavioural change and mortality up to the 2000mg/kg dose level. Therefore, one-tenth (200mg/kg) of the 2000mg/kg, just half of 1/10th (100mg/kg) and just double of 1/10th half (300mg/kg) were selected as the dose of A. capillus extracts.

Hepatoprotective activity

Effect of ACE on serum hepatic biomarkers parameters
The consequence of various doses of 50% ethanolic extracts of A. capillus (ACE) was studied on serum marker enzymes like SGOT, SGPT, total bilirubin (TBB), direct bilirubin (DBB) and ALP. The hepatic bruise due to toxic dose of CCl$_4$ significantly (P>0.001) rise the level of enzyme SGOT by 264.98%, SGPT by 380.96%, TBB by 200%, DBB by 664.70% and ALP by 132.66% compared to the control group.

The dose of extract, ACE 100 (Group-IV), ACE 200 (Group V) declined the elevated level of SGOT 41.66% (P< 0.05), 71.14% (P< 0.001), SGPT 35.96% (P< 0.05), 55.97% (P< 0.01), TBB 53.33% (P< 0.05), 96.66% (P< 0.01) DBB 25.66% (P< 0.05), 55.30% (P< 0.01) and ALP 49.25% (P< 0.05), 74.24% (P< 0.01) as compared to group II. The dose of extract, ACE 300 (Group VI), declined the maximum level of SGOT 97.65% (P< 0.001), SGPT 97.72% (P< 0.001), TBB 110.4% (P< 0.001) DBB 77.61% (P< 0.001) and ALP 97.55% (P< 0.001) compared to group II. Similarly, silymarin decrease the level of SGOT 96.64% (P< 0.001), SGPT 95.23% (P< 0.001), TBB 115.40% (P< 0.001) DBB 80.08% (P< 0.001) and ALP 97.32% (P< 0.001) compared to group II. The results are tabulated in Table 1.

Effect of ACE on GSH, LPO, CAT and SOD level
In our study, the CCl$_4$ treated group showed a significantly elevated MDA level of 247.5 (P < 0.001) compared to group I. However, rats treated with different doses of ACE 100 (Group-IV), ACE 200 (Group V) and ACE 300 (Group VI) significantly declined the elevated level of MDA as 47.47% (P< 0.05), 73.73% (P< 0.05) and 93.99% (P< 0.01) as compared to group II.

ACE treated groups had significantly elevated the level of GSH, CAT and SOD content whereas CCl$_4$ intoxicated group had shown significant decrease in these parameters compared to control group. The dose of extract, ACE 100 (Group-IV), ACE 200 (Group V) and ACE 300 (Group VI) elevated the declined level of GSH 28.57% (P< 0.06), 50.87% (P< 0.05) 70.01 (P< 0.01), CAT 40.21% (P< 0.05), 56.91 (P< 0.05), 86.89% (P< 0.01) , and SOD 36.25% (P< 0.05) , 65.56% (P< 0.05), 90.42% (P< 0.01) as compared to group II. Silymarin (Group III) elevated the level of GSH 82.45%, (P< 0.001), CAT 89.99%, (P< 0.01), SOD 96.18% (P< 0.001) as well as decrease the level of MDA 94.94% (P< 0.01) as compared to group II. The results are tabulated in Table 2. In different doses the level of ACE, 300 mg/kg shown the maximum protection.

Histopathological Studies
The histological observations help the results obtained from serum enzyme assays Histopathology of the liver section is well described in Figure 1.

DISCUSSION

In the present investigation, ACE hepatoprotective activity using CCl$_4$ was evaluated in the rat. CCl$_4$ is a toxic compound, which metabolized by the action of cytochrome P450 that leads to the formation of unstable and complex
The present study revealed a -

metabolites of CCl₄ which may cause hepatotoxicity. CCl₄ is stimulated in the presence of cytochrome P₄₅₀ (CYP 2E1), and (CYP2B, CYP3A) both are converted CCl₄ to its metabolites such as tri-chloro-methyl (CCl₃') free radicals convert into trichloromethyl peroxo radical (CCl₃OO') in the presence of oxygen. CCl₄ free radical covalently binds to the macromolecules and CCl₄O₂ involves lipid peroxidation to dissolve the polyunsaturated fatty acid and change it into a small fragment called MDA or 4-hydroxynonenal. The free radicals alter the integrity of the cell membrane due to the oxidation of polyunsaturated fatty acid in cellular membranes.¹⁶

Liver function is determined by estimating the activities of SGOT, SGPT, TBB, DBB and ALP that are original, present in higher concentration in the liver cell. During liver disease, these enzymes leak into the bloodstream in conformity with the extent of liver damage.¹⁷ The present study revealed a significant rise in the activities of SGOT, SGPT, TBB, DB and ALP due to CCl₄ intoxication hepatic cell injury. The administration of different doses of ACE (100, 200 and 300 mg/kg) and silymarin treated group exhibited the lower level of SGOT, SGPT, TBB, DB and ALP compare to CCl₄ treated group. The stabilization of SGOT, SGPT, BB, DB and ALP levels by ACE is a clear manifestation of the improvement of the functional status of the liver cells. The histopathological examination disclosed that the hepatic cells, central vein, and portal triad are almost normal in the ACE (300 mg/kg, p.o.) group in contrast to the group, which received CCl₄.

Lipid peroxidation (LPO) is cell death due to the autocatalytic process. This process may cause peroxidative tissue damage in inflammation. MDA is one of the products in the lipid peroxidation process.¹⁸ The data obtained in our present study (Table 2) clearly shows an increase in the serum rat treated with CCl₄ suggesting enhanced liver peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent the formation of excessive free radicals. Treatment with ACE at 300 mg/kg significantly 93.99% (P < 0.01) inhibited the formation of MDA levels in the CCl₄ treated group and this is seen to be comparable with the standard drug silymarin.

In hepatic injury, superoxide radicals generate at the site of damage and modulate SOD and CAT, resulting in the loss of activity and accumulation of superoxide radicle, which damages the liver.¹⁹ The reduced levels of parameters of SOD and CAT, in CCl₄ administered rat but treated with ACE (100, 200, 300) mg/kg group of ACE showed the significant increase in the level of these enzymes, which observed the antioxidant property of ACE oxygen-free radicals.²⁰

GSH is capable of preventing damage to important cellular components caused by ROS such as free radicals, peroxides, lipid peroxides, and heavy metals.²¹ The intoxicants with CCl₄ causes a reduction in the synthesis and functioning of GSH. The increase in hepatic GSH level in rat treated with 100, 200 and 300 mg/kg of ACE due to de novo GSH synthesis or GSH regeneration.

The phytochemical studies carried out on ACE reveal the presence of carbohydrates, alkaloids, phenols, flavonoids, saponins, tannins and triterpenoids. are the major chemical constituents. These antioxidant phytochemicals might contribute to the hepatoprotective and antioxidant activities of the whole plant of A. capillus.

**CONCLUSION**

This study showed that the 50% ethanolic extract of A. capillus has hepatoprotective effects that were proven by biochemical and histopathological analysis. The ACE has shown dose-dependent activity among which at the dose level of 300 mg/kg p.o shows greater activity which comparable with a toxic and standard group.

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**Conflict of interest**

The authors declare that they have no conflicts of interest with the contents of this article.

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Table 1: Effect of 50% Ethanolic extract of Adiantum capillus (ACE) on biochemical parameters against CCl4-induced liver toxicity in rat

| Group | Treatment & Dose (mg/kg) | SGOT (U/L) | SGPT (U/L) | Total bilirubin (mg/dL) | Direct bilirubin (mg/dL) | ALP (U/L) |
|-------|--------------------------|------------|------------|------------------------|--------------------------|-----------|
| I     | Control                  | 106.27 ± 18.21 | 48.50 ± 8.21 | 1.2 ± 0.11             | 0.17 ± 0.01              | 68.14 ± 7.88 |
| II    | CCl4                     | 387.87 ± 35.21** | 233.27 ± 23.86*** | 3.6 ± 0.39***          | 1.30 ± 0.03***           | 158.54 ± 13.56*** |
| III   | Silymarin (100 mg/kg)    | 115.78 ± 11.76*** | 57.35 ± 6.62*** | 0.90 ± 0.13***         | 0.3862±0.209***          | 70.56 ± 7.86*** |
| IV    | ACE (100 mg/kg)          | 270.55 ± 22.24*  | 170 ±17.32*   | 2.32 ± 0.14*           | 1.01 ± 0.094*            | 114.21 ± 11.22* |
| V     | ACE (200 mg/kg)          | 186.55 ± 18.80*** | 103.45 ± 13.23** | 1.28 ± 0.12**          | 0.6752±0.027**           | 91.426 ± 9.61** |
| VI    | ACE (300 mg/kg)          | 112.81 ± 16.53*** | 58.24 ±6.80*** | 0.952 ± 0.13***        | 0.4232±0.027***          | 70.352±7.34*** |

Value expressed as ± S.E.M., n = 6. Significance level: *P< 0.05, **P< 0.01 and ***P< 0.001. CCl4 group compared to control while other group i.e treated group compared to CCl4 100 mg/kg, 200 mg/kg and 300 mg/kg represent the dose of 50% ethanolic extract of Adiantum capillus

Table 2: Effect of 50% Ethanolic extract of Adiantum capillus (ACE) on liver LPO (MDA nmole/min//mg of protein), GSH parameters (nmole/min//mg of protein), SOD (unit/mg of protein) and CAT (unit/mg of protein against CCl4-induced liver toxicity in rat

| Group | Treatment (mg/kg) | GSH (Mean±SEM) | LPO (Mean±SEM) | CAT (Mean±SEM) | SOD (Mean±SEM) |
|-------|-------------------|----------------|----------------|----------------|----------------|
| I     | Control           | 0.96 ± 0.08    | 0.40±0.09      | 58.38±6.62     | 26.52±2.92     |
| II    | CCl4              | 0.39 ± 0.05*** | 1.39 ±0.18***  | 35.23 ± 2.40*** | 8.96±1.82***   |
| III   | Silymarin (100 mg/kg) | 0.86 ±0.07*** | 0.45 ±0.08**   | 56.23 ± 2.61*** | 25.83 ± 2.42*** |
| IV    | ACE (100 mg/kg)   | 0.53 ± 0.06*   | 0.97±0.14*     | 44.62 ± 2.72*  | 15.32 ± 1.30*  |
| V     | ACE (200 mg/kg)   | 0.68 ± 0.09*   | 0.66±0.17*     | 48.52 ± 3.41*  | 20.52±2.83*    |
| VI    | ACE (300 mg/kg)   | 0.76 ± 0.08**  | 0.46±0.08**    | 55.45 ±4.60**  | 24.82±2.42**   |

Value expressed as ± S.E.M., n = 6. Significance level: *P< 0.05, **P< 0.01 and ***P< 0.001. CCl4 group compared to control while other group i.e treated group compared to CCl4 100 mg/kg, 200 mg/kg and 300 mg/kg represent the dose of 50% ethanolic extract of Adiantum capillus
Figure 1: Histopathology of liver tissues. A. Normal liver section show prominent central vein, normal hepatocytes & sinusoids; B. Toxic liver section shows massive fatty changes along with congestion in central vein; C. Liver sections of rats treated CCl₄ and ACE 100 mg/kg showing inflammatory collection around central vein & focal necrosis with sinusoidal dilation; D. Liver section treated with CCl₄ and ACE 200 mg/kg showing regeneration of hepatocytes around central vein toward near normal liver architecture; E. Liver section treated with CCl₄ and ACE 300 mg/kg showing normal liver architecture.