ISSN: 2320 - 4230

Journal of Drug Discovery and Therapeutics

Available Online at www.jddt.in

CODEN: - JDDTBP (Source: - American Chemical Society)

Volume 11, Issue 2, March-April: 2023, 21-28

Review Article

Protective Effect of Herbal Medicinal Plant Extract against Carbon Tetrachloride Induced Liver Injury in Rat

Pawan Sharma*[1], Vinod Nakra[2], Vidhi Jain[3], Manmeet Singh Saluja[4]

[1]Research Scholars, Department of Pharmacy, SunRise University, Alwar, Rajasthan.
[2] Professor, Department of Pharmacy, SunRise University, Alwar, Rajasthan.
[3] Professor & Principal, Maa Vaisnavi education institute & Research Sansthan, Baran, Rajasthan.
[4] Professor, Department of Pharmacy, TIT- Pharmacy, Bhopal, Madhya Pradesh.

Article Info: Received: 18-01-2023 / Revised: 27-01-2023 / Accepted: 19-02-2023
DOI: https://doi.org/10.32553/jddt.v11i2.461
Corresponding author: Pawan Sharma
Conflict of interest: No conflict of interest.

Abstract:

Adiantum capillus (AC) Linn (Family: Adiantaceae) commonly known as “Hansraj” It is used in common cold, cough, pyrexia inflammation, acts as purgative. It improves digestion and renal function. This study was performed to investigate the hepato-defensive potential of Adiantum capillus in contradiction of carbon tetrachloride tempted hepatic damage in rats. The rodents were dosed with Acetonic extract (AEAC, 500mg/kg) extract of Adiantum capillus against Carbon Tetrachloride induced hepatic damage. Results shows thst Carbon Tetrachloride elevated the level of hepatic markers like SOPT, SGPT, bilirubin and ALP as well as altered the antioxidant enzymes. The level of hepatic biomarkers, as well as antioxidant enzyme, were recovered after the administration of at 500mg/kg, p.o., which was further confirmed by the histopathological. From above results it was concluded that The Acetonic extract (500 mg/kg, bw) extract of Adiantum capillus (ACE) recover the biochemical as well as antioxidant parameters, which were affected by Carbon Tetrachloride. The histopathological analysis supports the outcomes of antioxidants parameters.

Key Words: Adiantum capillus, Hepatoprotective activity, SGOT, Antioxidant, Carbon Tetrachloride, Histopathology.

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, Srilanka 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.**[5]** The extracts of the ***Adiantum capillus*** (ACE) leaves have anti-inflammatory, analgesic and anti-microbial activities.**[6]** Adiantum capillus (AC) contains 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]**

The major chemical constituents of ***Adiantum capillus*** are 5-7A-Isoprpenyl-4,5-dimethyloctahydro-1h-inden-4yl)-3-methyl-2-Penta, n hexadecanoicacidandgammasitosterolcis-vaccenicacid,5-7A-Isopropenyl-4,5-Dimethyloctahydro-inden-4-yl)-3-methyl-pent-2-EL9. Isolated new terpenoids that were 22, 29-epoxy-30- norhopan-13-ol from the ***Adiantum capillus*** leaves with strong antibacterial activity. 3- coumaroyl quinic acid, kaempferol-3-glucosides as a major phenolic compound.**[7]**

There is no work reported work on hepatoprotective activity of Acetonic extract of ***Adiantum capillus*** whole plant. Hence, the present study was carried out to evaluate the hepatoprotective activity of Acetonic extract ***Adiantum capillus*** against Carbon Tetrachloride induced liver toxicity in rats.

Procurement and Authentication of the Plant

The ***Adiantum capillus*** plant were gathered from the surrounding region of Alwar, Rajasthan, and then sent to Sunrise University, Alwar, Rajasthan, where they were certified by Dept. of Botany, Sunrise University, Alwar, Rajasthan.

Preparation of extracts of *Adiantum capillus*

In a Soxhlet apparatus, 500g of powdered ***Adiantum capillus*** plant were extracted with solvent in order to increasing polarity. The materials were concentrated by evaporation **[**8**]**.

Animals

Wistar albino rats (150-200 g) were procured from Central Drug Research Institute, Lucknow, India. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad libitum*. All the animals were acclimatized for a week before use. The experimental protocols were approved by Institutional Animal ethics Committee after scrutinization. Animals were received the drug by oral gavages tube. All the animals were care of under ethical consideration as per the CPCSEA guidelines with regular inspections of rats. The

laboratory conditions duly undertaken by registered veterinary practitioner **[9]**.

Chemicals

All the chemicals and solvents were of analytical grade. Silymarin was obtained as gift sample from Micro Lbs, Goa, India. Standard kits for SGOT, SGPT and ALP etc. were obtained from Span Diagnostics Ltd., India.

Preliminary phytochemical analysis

To determine which phytoconstituents were present in each extract, a preliminary phytochemical study was performed.

Toxicity studies

All of the extracts were subjected to an acute toxicity test in accordance with OECD 423 guidelines **[9]**. Research on acute toxicity was conducted on female albino rats. Before administering the extract orally at dosages of 100, 200, and 500mg/kg b.w., the animals were fasted for 24 hours with only water provided, and then monitored for toxic symptoms for up to 72 hours. The therapeutic oral dosage for all extracts was 500 mg/kg body weight.

Carbon tetrachloride induced hepatotoxicity

The rats, of both sexes, were split up into 6 groups of six each. (n = 6) [10, 11]

- **Group I (Control)**: administered water (5 mililitre/kilogram, p.o.) *o.d.* for nine days.
- **Group II (-ve control):** administered water (5 mililitre/kilogram, p.o.) *o.d.* for nine days, meanwhile on the seventh day, carbon tetrachloride (1 ml/kg in 50% v/v olive oil, s.c.) was given.
- **Group III (+ve control):** administered the normal medicine silymarin (25 mg/kg, p.o.) *o.d.* for nine days, meanwhile on the seventh day, carbon tetrachloride (1 ml/kg in 50% v/v olive oil, s.c.) was given.
- **Group IV (Test Sample)** administered The Acetonic extract (AEAC, 500mg/kg) of Adiantum capillus plant o.d. for nine days, meanwhile on the seventh day, carbon tetrachloride (1 ml/kg in 50% v/v olive oil, s.c.) was given.

On last day, blood was obtained from animals by puncturing retro orbital plexus. Blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and utilized for the estimation of various biochemical parameters including SGOT & SGPT [12], ALP [13], serum bilirubin [14] and serum protein [15] After collection of blood samples, the animals were sacrificed under deep ether anaesthesia. Morphological parameters like weight of animals, weight of liver have also been used to evaluate the protective effect of the drug. Hepatoprotective chemical causes loss in liver weight/100 gm body weight of rats [16, 17].

Histopathology studies

A portion of liver tissue of all the animal groups was excised and then washed with normal saline. The liver tissues were fixed in 10% buffered neutral formalin for 48 hrs and then with bovine solution for 6 hrs and were then processed for paraffin embedding. By using a microtome, sections of 5 mm thickness were taken and stained with hematoxylin and eosin. These sections were examined under light microscope using a magnification of 100X [18].

Statistical Significance

The results of the study were expressed as mean ± SEM, n=6. ANOVA [19] was used to analyze and compare the data, followed

by Dunnet's [20] test for multiple comparisons.

Results

Chemical testing identified the presence of the phytoconstituents in the different extracts. The findings demonstrate that Acetonic extract (AEAC, 500mg/kg) of Adiantum capillus plant contain the greatest amount of pharmacologically active compounds, such as glycosides, sponins, phytosterols, and flavonoids. As a result, these extracts were chosen for the pharmacological research. The findings are shown in Table 1. There was no mortality found amongst the graded dose groups of animals and they did not show any toxicity or behavioural changes at a dose level of 5000 mg/kg. This finding suggests that Acetonic extract (AEAC) of Adiantum capillus plant were safe or non-toxic to rats and hence doses of 500 mg/kg, p.o. were selected for the study. All groups of animals tested fell asleep after receiving an intramuscular injection of thiopentone sodium (40 mg/kg). When Carbon Tetrachloride was administered to rats, the beginning of sleep was significantly delayed (measured in seconds) and the total amount of time spent in sleeping was increased (measured in minutes). Pretreatment with Acetonic extract (AEAC, 500mg/kg) of Adiantum capillus plant and silymarin, substantially improved sleep onset but dramatically reduced sleep duration in rats compared to a Carbon Tetrachloride treatment group. The outcomes are shown in Table 2. An increase in liver weight and liver volume were seen in the Carbon Tetrachloride treated group, indicating that the livers of these individuals had grown in size. Liver weight was significantly restored in the groups given Acetonic extract (AEAC, 500mg/kg) of Adiantum capillus plant together with silymarin. The findings are shown in Table 3. There was a significant elevation in the levels of serum marker enzymes like SGOT, SGPT and ALP content of Carbon Tetrachloride treated groups, But pretreatment with Acetonic extract (AEAC, 500mg/kg) of Adiantum capillus plant and silymarin (25 mg/kg, p.o.) exhibited an ability to counteract the hepatotoxicity by decreasing serum marker enzymes. The findings are shown in Table 4. In Carbon Tetrachloride treated groups, there was a significant increase in total bilurubin and significant reduction in total protein content. Whereas, pretreatment with Acetonic extract (AEAC, 500mg/kg) of Adiantum capillus plant caused significant reduction in total bilirubin and significant increase in total protein. The findings are shown in Table 4.

Histopathological studies of liver also provided a supportive evidence for biochemical analysis. Histological changes such as steatosis (fatty changes in hepatocytes) and perivenular fibrosis were observed in Carbon Tetrachloride treated (toxic) control group. Both the extracts has prevented these histological changes. The results were showed in Figure 1.

Table 1: Preliminary Phytochemical studies of Extracts of Adiantum capillus

Constituents	*Acetonic Extract (AEAC)*
Carbohydrate	- ve
Glycosides	- ve
Oil and fats	+ ve
Proteins	+ ve
Saponins	- ve
Phenolic comp. and tannins	+ ve

Phytosterols	+ ve
Alkaloids	+ ve
Gums and mucilage	+ ve
Flavonoids	+ ve

Table 2: Effect of Acetonic extract (AEAC) of Adiantum capillus plant on functional parameters in Carbon Tetrachloride induced hepatotoxic rats.

Treatment/ Dose	Onset of sleep (Sec.)	Duration of sleep (Min.)
Normal	165.4 ± 3.08	110.41± 3.52
Carbon Tetrachloride	169.1 ± 3.42*	244.05 ± 9.12*
Silymarin	149.7 ± 3.09***	145.11 ± 2.09***
AEAC (500 mg/kg)	102.5 ± 3.31**	182.09 ± 7.30**

Values are mean ± SEM, n = 6. (One way ANOVA Followed by Dunnette multiple comparisons test). Statistically significance of ** P<0.01, *** P<0.001, when compared with Carbon Tetrachloride induced group and * P<0.05, when compared with normal group.

Table 3: Effect of Acetonic extract (AEAC, 500mg/kg) of Adiantum capillus plant on Physical Parameters in Carbon Tetrachloride induced hepatotoxic rats.

Treatment/ Dose	Liver Weight	Liver Volume
Normal	5.42 ± 0.05	5.49 ± 0.06
Carbon Tetrachloride	11.02 ± 2.06*	11.22 ± 2.41*
Silymarin	5.97 ± 1.13***	5.94 ± 1.75***
AEAC (500 mg/kg)	7.22 ± 1.30**	7.35 ± 1.42**

Values are mean ± SEM, n = 6. (One way ANOVA Followed by Dunnette multiple comparisons test). Statistically significance of ** P<0.01, *** P<0.001, when compared with Carbon Tetrachloride induced group and * P<0.05, when compared with normal group.

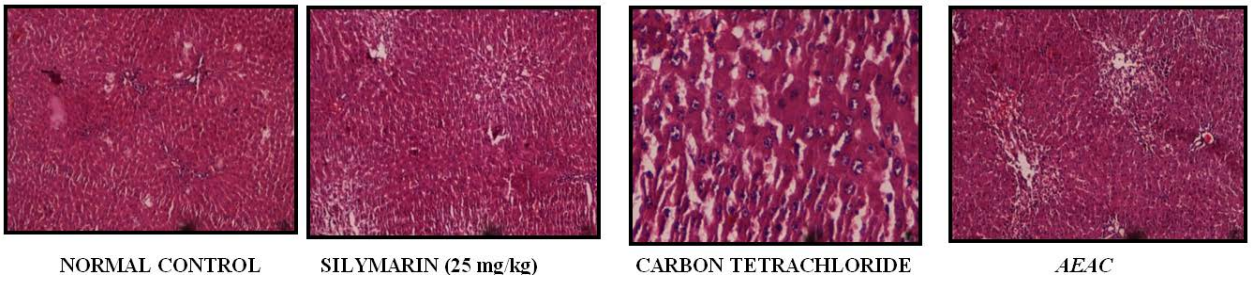

Figure 1: Effect of *Acetonic Extract (Aeac, 500mg/Kg) Of Adiantum Capillus Plant* On Histopathological Diagram Of Liver Tissue In Carbon Tetrachloride Induced Hepatotoxic Rats.

Discussion

There are many factors which are responsible for the liver damage or injuries such as chemicals and drugs. In the present study, Carbon Tetrachloride was used to induce hepatotoxicity, since it is clinically relevant. Elevated levels of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) are indications of hepatocellular injury **[21-27]**.

In Carbon Tetrachloride induced hepatotoxicity, Carbon Tetrachloride is metabolized in human cell (endoplasmic reticulum and mitochondria) with the formation of CCl3O-, the reactive oxidative free radical intermediate generated by cytochrome P450. The nascent oxygen O-

resulting from lipoperoxidation causes an increase in intracellular reactive Fe+2 ions, aldehyde, GSH depletion, and calcium restoration. In addition to direct covalent contact, oxidative CCl3 O- causes degeneration of Ca+2 sequestrations. Failure to sequester leads in increased intercellular Ca+2, aggregation by proteolytic enzymes, and a rise in Fe+2 ions, which precipitates aldehyde cytotoxicity through lipid peroxidation **[28]**.

Administration of CARBON TETRACHLORIDE to experimental animals produced statistically significant rise in the enzymes levels, namely SGOT, SGPT, ACP, ALP, etc indicating the chemical induced hepatocellular toxicity. Theinhibitory effect of the ***Acetonic extract (AEAC, 500mg/kg) of Adiantum capillus plant*** on hepatotoxicity were compared to that of positive control group. The significant protection in the biochemical parameters like SGOT, SGPT, ACP and ALP against Carbon Tetrachloride induced elevations in pretreatment of the animals with the ***Acetonic extract (AEAC, 500mg/kg) of Adiantum capillus plant***. Further there was increase in weight of the liver treated with the Carbon Tetrachloride were seen as compared to the normal. The treatment with the ***Acetonic extract (AEAC, 500mg/kg) of Adiantum capillus plant*** retains the liver weight near to the normal. Liver section of control rat showing a normal hepatic architecture wall brought about from the central vein. The liver samples of Carbon Tetrachloride treated rats showed gross necrosis of the centrilobular hepatocytes characterized by gross necrosis, degeneration, karyolysis and eosinophilic infiltration which are significantly prevented by treatment with the ***Acetonic extract (AEAC, 500mg/kg) of Adiantum capillus plant*** that showed the hepatoprotective activity. The histopathological pattern of the livers of the rats treated with Carbon Tetrachloride plus extracts showed minimal necrosis in centrilobular and regeneration of hepatocytes. A number of scientific reports indicated that certain flavonoids, triterpenoids and steroids have protective effect on liver due to its antioxidant properties. Administration of ***Acetonic extract (AEAC, 500mg/kg) of Adiantum capillus plant*** that showed significant hepatoprotective activity; while qualitative phytochemical investigations on the ***Acetonic extract (AEAC, 500mg/kg) of Adiantum capillus plant*** also showed test positive for flavonoids by chemical tests. Further, it has been reported that the flavonoid constituents of the plant possess antioxidant properties and was found to be useful in the treatment of liver damage .**[29]**

The administration of hepatoprotective drugs may induce the hepatocytes to resist the toxic effect of Carbon Tetrachloride. The results indicated that the ***Acetonic extract (AEAC, 500mg/kg) of Adiantum capillus plant*** has significant hepatoprotective activity. The obtained results indicated a high degree of protection against the hepatotoxic effect of CarbonTetrachloride.

References

1. Singh SS, Pandey SC, Shrivastava S, et al. Chemistry and medicinal properties of ***Adiantum capillus***. Indian J Pharmacol. 2003; 35: 83-9.
2. Kirtikar KR, Basu BD. Indian Medicinal Plants, Vol 1. 2nd ed. New Connaught Place, Dehra Dun; 1975.
3. Nayampalli SS, Ainapure SS, Samant BD, et al. A comparative study of diuretic effects of ***Adiantum capillus*** and hydrochloro-thiazide in rats and a preliminary phase I study in human volunteers. J Postgrad Med. 1988; 34: 233-6.
4. Aiyer KN, Kolammal M. Pharmacognosy of Ayurvedic Drugs,

Series 1. 1st ed. Trivendram: The Central Research Institute; 1963.

5. Raghunathan K, Mittra R. Pharmacognosy of Indigenous Drugs. New Delhi: Central Council for Research In Ayurveda & Siddh; 1982.

6. Nadkarni KM, Nadkarni AK. Indian Materia Medica, Vol 1. 3rd ed. Mumbai: M/S Popular Prakasan Pvt. Ltd; 1976.

7. Zhao TF, Wang X, Rimando AM, et al. Folkloric medicinal plants: Tinospora sagittata var. cravaniana and Mahonia bealei. Planta Medica. 1991; 57: 505.

8. Dhaliwal KS. Method and composition for treatment of diabetes. US Patent 5886029; 1999.

9. Kapil A, Sharma S. Immunopotentiating compounds from ***Adiantum capillus***. J Ethnopharmacol. 1997; 58: 89-95.

10. Mehrotra R, Katiyar CK, Gupta AP. Hepatoprotective compositions and composition for treatment of conditions related to hepatitis B and E infection. US Patent 749296; 2000.

11. Jana U, Chattopadhyay RN, Shw BP. Preliminary studies on anti-inflammaory activity of Zingiber officinale Rosc., Vitex negundo Linn. and ***Adiantum capillus*** (Willid) Miers in albino rats. Indian J Pharmacol. 1999; 31: 232-3.

12. Anonymous. Wealth of India: Raw materials, Vol X. New Delhi: CSIR; 1976.

13. Sethuraman MG, Lalitha KG, Raj Kapoor B. Hepatoprotective activity of Sarcostemma brevistigma against carbon tetrachloride-induced hepatic damage in rats. Current Science. 2003; 84: 1186-87.

14. Singh RP, Padmavathi B, Rao AR. Modulatory infl uence of Adhatoda vesica (Justica adhatoda) leaf extract on the enzyme of xenobiotic metabolism, antioxidant status and lipid peroxidation in mice. Mol Cell Biochem. 2000; 213: 99-109.

15. Harbone JB. Phytochemical methods - A guide to modern technique of plant analysis, 2nd edn, chapman and hall, Newyork; 1984. 85pp.

16. CPCSEA. (2003) Indian Journal pharmacology 35: 257-274.

17. Ward FM and Daly MJ. (1999) "Hepatic Disease. In: Clinical Pharmacy and Therapeutics (Walker R.and C.Edwards Eds.)". Churchill Livingstone, New York. pp. 195-212.

18. Yoganarasimhan SN. (1996) Medicinal plant of India.vol-1 Karnataka. Interline publishing pvt.ltd, Bangalore, pp 232.

19. Reitman S, Frankel S. (1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American Journal Clinical Pathology 28: 56–63.

20. Kind PRN, King EJ. (1954) Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. Journal Clinical Pathology 7(4): 322-326.

21. Amour FF D', Blood FR, Belden DA. (1965) The manual for laboratory work in Mammalian Physiology. The University of Chicago Press, Chicago, 126-128.

22. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. (1951) Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193: 265–275.

23. Avadhoot V, Rana V. (1991) Hepatoprotective effect of *Vitex negundo* against carbon tetrachloride-induced liver damage. Arch Pharmacy Research 14(1): 96-98.

24. Bhanwra V, Singh V, Khosla V. (2000) Effect of *Azadirachta indica* (Neem) leaf aqueous extract on paracetamol-induced liver damage in rats. Indian Journal of Physiolology and Pharmacology 44(1): 64-68.

25. Mankani KL, Krishna V, Manjunatha BK, Vidya SM, Singh SJ, Manohara YN, Raheman A, Avinash KR. (2005) A review of natural products with hepatoprotective Activity. Indian Journal of Pharmacology 37(3): 165-168.

26. Gennaro AR. (1995) Remington: The science and practice of pharmacy, vol. I, 19th ed, Mack Publishing Company, Easton (PA), pp.111.

27. Dunnet CW. (1964) New Tables for Multiple Comparisons with a Control. Biometrics 20: 482-491.

28. Yue M, Yu CH, Ren K, Chen W, Li Y. (2006) Transient elevation of hepatic enzymes in volunteers after intake of alcohol. Hepatobiliary and Pancreatic Diseases International 5(1): 52-55.

29. Zhou Z, Wang L, Song Z, Lambert JC, McClain CJ, Kang YJ. (2003) A critical involvement of oxidative stress in acute alcohol-induced hepatic TNF-alpha production. American Journal of Pathology 163: 1137-1146.