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Evaluation Of Hepatoprotective Potential Of Methanolic Extract Of *Achyranthes Aspera* Against Isoniazid–Rifampicin-Induced Liver Toxicity In Rats

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Abstract
The present study aimed to evaluate the hepatoprotective potential of the methanolic extract of Achyranthes aspera against isoniazid–rifampicin-induced hepatotoxicity in Wistar rats. Phytochemical screening confirmed the presence of flavonoids, phenolics, diterpenes, and tannins compounds known for their antioxidant and liver-protective properties. The extract showed a moderate yield of 7.02% (w/w). Hepatotoxicity was induced by oral administration of isoniazid and rifampicin, resulting in elevated liver enzyme levels (ALT, AST, ALP), increased lipid profile (cholesterol and triglycerides), decreased total protein, and increased bilirubin levels. Oxidative stress markers showed elevated MDA and reduced CAT and GSH levels. Treatment with the methanolic extract of A. aspera at doses of 100 and 200 mg/kg significantly reversed these changes in a dose-dependent manner. The higher dose (200 mg/kg) demonstrated results comparable to the standard hepatoprotective drug silymarin. The findings suggest that A. aspera extract mitigates hepatic damage through antioxidant, anti-lipidemic, and membrane-stabilizing effects, supporting its traditional use for liver-related ailments.
Keywords: *Achyranthes aspera; Hepatoprotective activity; Isoniazid–Rifampicin; Liver toxicity; Oxidative stress; Antioxidant; Flavonoids; Silymarin; Wistar rats.*

INTRODUCTION

The liver is a vital organ responsible for the metabolism, detoxification, and biotransformation of various endogenous and exogenous substances. It plays a central role in maintaining metabolic homeostasis [1]. However, the liver is vulnerable to damage caused by hepatotoxic agents such as drugs, alcohol, chemicals, and microbial infections. Among the drug-induced liver injuries, anti-tubercular agents like isoniazid and rifampicin are known to cause significant hepatotoxicity when administered over prolonged periods [2-3].

Isoniazid and rifampicin, first-line antitubercular drugs, are extensively used in the treatment of tuberculosis [4]. However, their concurrent administration may lead to oxidative stress and hepatocellular injury by generating reactive metabolites and disturbing antioxidant defense systems [5-7]. The resultant liver damage is characterized by elevated levels of serum biomarkers such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin. Conventional hepatoprotective drugs like silymarin and ursodeoxycholic acid are effective but may pose limitations due to side effects or high costs, which prompts the exploration of herbal alternatives.

Achyranthes aspera, commonly known as "Prickly Chaff Flower," is a well-known plant in traditional medicine systems, including Ayurveda and Unani. It is reported to possess a wide range of pharmacological activities such as anti-inflammatory, antioxidant, immunomodulatory, hepatoprotective, and antimicrobial properties [8-9].

The methanolic extract of *A. aspera* is rich in flavonoids, saponins, alkaloids, and phenolic compounds, which may contribute to its antioxidant and hepatoprotective effects.

In recent years, there has been a growing interest in evaluating the scientific basis of traditional herbal medicines for liver protection. Given the ethnomedicinal use of *Achyranthes aspera* and its phytoconstituents, the present study aims to investigate the hepatoprotective potential of its methanolic extract in a rat model

of isoniazid–rifampicin-induced liver toxicity. This in vivo evaluation involves biochemical assays, antioxidant status, and histopathological examination to validate its efficacy and mechanism of action.

MATERIAL AND METHODS

Material

Methanolic extract of *Achyranthes aspera* was prepared using authenticated plant material collected locally and verified by a qualified botanist. Isoniazid and rifampicin were obtained from Sigma-Aldrich, India. Standard hepatoprotective drug silymarin was procured from Micro Labs, India, and used as the reference control. Biochemical assay kits for liver function tests (SGOT, SGPT, ALP, total bilirubin, and total protein) were purchased from Erba Diagnostics, Mumbai. All other chemicals and solvents used in the experimental procedures were of analytical grade and procured from S.D. Fine Chem Ltd., Mumbai. Healthy adult Wistar rats were obtained from the animal house facility and housed under standard conditions following CPCSEA guidelines.

Methods

Procurement of plant material

Aerial parts of *Achyranthes aspera* were collected from local area of Bhopal month of September, 2023.

Extraction using hot continuous extraction (Soxhlet)

The shade dried aerial parts (40 gm) of *Achyranthes aspera* were coarsely powdered and subjected to extraction. Plant material extracted by different solvent like chloroform, ethyl acetate, methanol and distilled water was used. In this method, the finely pulverized marc is placed in a thimble which is placed in a chamber of the Soxhlet apparatus. The menstruum in the flask beneath is then heated, and its vapors condense in the condenser. The condensed extractant drips into the thimble containing the marc, and extracts it by contact. The advantage of this method is that large amounts of marc can be extracted with a much smaller volume of extractant. Each extraction process was carried out for 24 hours. The filtrate was separated from the residue using Whatmann filter paper. The filtrate from each solvent was collected and evaporated using a water bath at 50°C until a thick extract was obtained [10].

Determination of percentage yield

Percentage yield measures the effectiveness of the entire extraction process. % yield is calculated using the formula below:

$$\textbf{Percentage Yield} = \frac{Weight\ of\ Extract}{Weight\ of\ Powder\ drug\ taken} x\ 100$$

Qualitative phytochemical screening

Qualitative phytochemical screening is carried out to investigate the various classes of natural compounds present in the extract. This is accomplished using standard methods [10]. The classes of compounds identified in the extract included phenolics, flavonoids, tannins, saponins, alkaloids and protein [11].

In vivo hepatoprotective activity of methanolic extract of *Achyranthes aspera*

Wistar rats (180±20 g) were housed in group (n=6) under a 12 hour light/dark cycle with controlled temperature and humidity (25±2°C, 55-65%). Rats were fed regular rat food and given unlimited access to water. Rats were acclimatized to laboratory settings for seven days before to the studies. All of the studies were conducted in a noise free room from 08.00 to 15.00 h. Each set of studies used a separate group of rats (n=6). The animal research were approved by the Institutional Animal Ethics Committee (IAEC), which was set up by the Ministry of Environment and Forests, Government of India, in New Delhi, to monitor and supervise experimental animals.

Acute toxicity study

The acute oral toxicity research of the methanolic extract of *Achyranthes aspera* was carried out in accordance with the recommendations established by the Organisation for Economic Cooperation and Development (1),

ANNEX-423(2, 3). All of the animals were checked for up to 14 days for mortality and clinical indications, such as changes in skin, hair, mucous membrane, eyes, reaction to stimuli, and body weight [12].

Experimental model

Experimental design and treatment protocol

Rats were acclimated to animal laboratory conditions of 25°C, 55% humidity, and a 12 h:12 h light-dark cycle for seven days before testing. Water was provided ad libitum, and the rats were fed a basic diet throughout the research.

Group –I: Normal control (0.5% CMC 1 ml/kg, p.o.)

Group –II: Isoniazid—rifampicin

Group -III: Isoniazid—rifampicin + silymarin 100 mg/kg.

Group –IV: Isoniazid—rifampicin + *Achyranthes aspera* of methanolic extract 100mg/kg

Group –V: Isoniazid—rifampicin + *Achyranthes aspera* of methanolic extract 200mg/kg

To model drug-induced liver injury, rats (Groups II-V) were given 14 successive intraperitoneal injections of isoniazid (50 mg/kg) and rifampicin (100 mg/kg). Following this induction period, from day 15, the animals were subjected to a 15-day treatment regimen: Group II received the vehicle (0.5% CMC), Group III received the standard hepatoprotective agent silymarin (100 mg/kg), and Groups IV and V received the methanolic extract of *Achyranthes aspera* root and leaves at dosages of 100 mg/kg and 200 mg/kg, respectively, all administered orally to evaluate their potential to mitigate the established liver damage.

Biochemical Investigation

Blood samples were taken from the ocular venous plexus using the retro-orbital bleeding technique to evaluate various biochemical parameters indicating liver function. Centrifuged the collected blood at 3500 rpm for 15 minutes at 4 °C to separate the serum. The isolated serum was then biochemically analyzed. On the same day of sample collection, the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were calculated using commercially available assay kits, according to the manufacturers recommendations. The levels of albumin, total bilirubin, and total protein in the serum were measured on the next day [13].

RESULTS AND DISCUSSION

The present study investigated the hepatoprotective potential of the methanolic extract of *Achyranthes aspera* against isoniazid–rifampicin-induced hepatotoxicity in rats. The findings support the traditional use of this plant for liver-related ailments and highlight its promising protective effects, as evident from improvements in biochemical, histological, and oxidative stress parameters.

The methanolic extract of *Achyranthes aspera* showed a moderate yield of 7.02% (w/w) (Table 1), indicating efficient extraction of bioactive compounds. Phytochemical screening (Table 2) revealed the presence of flavonoids, diterpenes, proteins, phenolic compounds (only in Folin-Ciocalteu test), and tannins. These constituents are widely recognized for their antioxidant and hepatoprotective properties. The absence of alkaloids, glycosides, carbohydrates, sterols, and saponins narrows the focus to specific compounds—particularly flavonoids and phenolics—which are known free radical scavengers.

Hepatotoxicity caused by isoniazid–rifampicin resulted in a significant reduction in body weight compared to the normal control group. Treatment with the methanolic extract of *Achyranthes aspera* at both 100 and 200 mg/kg led to a notable recovery in body weight (Table 3), comparable to the standard drug silymarin. This indicates overall improvement in health status and reversal of hepatotoxicity-induced weight loss.

Elevated levels of liver enzymes ALT, AST, and ALP in the toxic control group (Table 4) confirm liver injury due to isoniazid–rifampicin. Treatment with *Achyranthes aspera* extract significantly restored these enzyme levels in a dose-dependent manner. The 200 mg/kg dose was particularly effective, reducing ALT (182.40 ± 8.9 IU/L), AST (198.85 ± 8.0 IU/L), and ALP (195.3 ± 9.8 IU/L), reflecting improvement in liver integrity and function. These results were on par with the silymarin-treated group.

Drug-induced hepatotoxicity also elevated total cholesterol and triglyceride levels (Table 5). Administration of *A. aspera* extract reduced these lipid parameters significantly. The 200 mg/kg dose showed a notable reduction in cholesterol (172.70 ± 8.8 mg/dl) and triglycerides (145.8 ± 8.8 mg/dl), indicating normalization of hepatic lipid metabolism. This lipid-lowering effect may be attributed to the presence of flavonoids and tannins.

A decrease in total serum protein and increase in bilirubin are characteristic of liver dysfunction. As observed in Table 6, the extract significantly restored protein levels and reduced serum bilirubin, especially at 200 mg/kg, indicating improved protein synthesis and bile flow regulation.

Oxidative stress from isoniazid–rifampicin was evident by elevated MDA and suppressed antioxidant enzymes like CAT and GSH (Table 7). Treatment with *A. aspera* extract significantly decreased MDA and increased CAT and GSH levels in a dose-dependent manner. The 200 mg/kg dose produced near-normal values of MDA (2.8 ± 0.6 µmol/L), CAT (167.8 ± 6.2 U/mg), and GSH (170.2 ± 5.3 µmol/mg), confirming potent antioxidant activity.

Table 1: % Yield of *Achyranthes aspera* (Aerial parts extract)

Sr. No	Extracts	% Yield (w/w)
1.	Methanol	7.02

Table 2: Result of phytochemical screening of Methanolic extracts of *Achyranthes aspera*

S. No.	Constituents	Methanolic extract
1.	**Alkaloids** Wagner's Test: Hager's Test:	 -ve -ve
2.	**Glycosides** Conc. H_2SO_4 Test:	 -ve
3.	**Flavonoids** Lead acetate Test: Alkaline test:	 +ve +ve
4.	**Diterpenes** Copper acetate Test:	 +ve
5.	**Phenol** Ferric Chloride Test: Folin Ciocalteu Test:	 -ve +ve
6.	**Proteins** Xanthoproteic Test:	 +ve
7.	**Carbohydrate** Fehling's Test: Benedict's Test	 -ve -ve
8.	**Saponins** Froth Test:	 -ve
9.	**Tannins** Gelatin test:	 +ve
10.	**Sterols** Salkowski Test:	 -ve

+Ve = Positive, -Ve= Negative

Table 3: Mean body weight change

Groups	Drug	Body weight (g)

		Onset of study	End of study
I	Normal Control (0.5% CMC 1 ml/kg, p.o.)	165.3 ± 3.8	180.7 ± 4.2
II	Isoniazid—rifampicin (50 +100 mg/kg)	162.7 ± 4.1	170.5 ± 3.9
III	Isoniazid—rifampicin +Silymarin (100 mg/kg p.o.)	168.2 ± 3.6	178.3 ± 4.1
IV	Isoniazid—rifampicin + + methanolic extract of *Achyranthes aspera* (100 mg/kg p.o.)	170.4 ± 3.9	181.5 ± 4.5
V	Isoniazid—rifampicin + + methanolic extract of *Achyranthes aspera* (200 mg/kg p.o.)	175.6 ± 4.2	185.7 ± 4.8

Values are expressed as mean ± S.E.M. (*n* = 6). Values are statistically significant at $p<0.05$ vs. Isoniazid—rifampicin control group respectively (One-way ANOVA followed by Dunnett's test).

Table 4: Effect of methanolic extract of *Achyranthes aspera* on ALT, AST, Total Cholesterol, Triglyceride levels in Isoniazid—rifampicin induced hepatotoxicity in rats

Group	Drug	ALT (IU/L)	AST (IU/L)	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)
I	Normal Control (0.5% CMC 1 ml/kg, p.o.)	110.25 ± 8.9	105.80 ± 9.2	95.15 ± 5.4	90.5 ± 8.5
II	Isoniazid—rifampicin (50 +100 mg/kg)	270.10 ± 13.2	285.10 ± 10.5	225.30 ± 7.9	175.2 ± 10.2
III	Isoniazid—rifampicin +Silymarin (100 mg/kg p.o.)	145.80 ± 7.8***	160.40 ± 7.6***	148.25 ± 6.1***	102.6 ± 9.7***
IV	Isoniazid—rifampicin + + methanolic extract of *Achyranthes aspera* (100 mg/kg p.o.)	195.55 ± 10.1*	210.65 ± 9.1*	192.45 ± 9.5*	160.3 ± 7.9*
V	Isoniazid—rifampicin + + methanolic extract of *Achyranthes aspera* (200 mg/kg p.o.)	182.40 ± 8.9**	198.85 ± 8.0**	172.70 ± 8.8**	145.8 ± 8.8**

Values are expressed as mean ± S.E.M. (*n* = 6). Values are statistically significant at $p<0.05$ vs. Isoniazid—rifampicin control group respectively (One-way ANOVA followed by Dunnett's test).

Table 5: Effect of methanolic extract of *Achyranthes aspera* on alkaline phosphate (ALP), TP and Serum Bilirubin level in Isoniazid—rifampicin induced hepatotoxicity in rats

Group	Drug	ALP (IU/L)	TP (g/dl)	Serum Bilirubin (g/dl)
I	Normal Control (0.5% CMC 1 ml/kg, p.o.)	120.6 ± 11.2	122.4 ± 13.0	95.2 ± 8.3
II	Isoniazid—rifampicin (50 +100 mg/kg)	255.3 ± 12.0	78.2 ± 10.0	210.4 ± 14.1

III	Isoniazid—rifampicin +Silymarin (100 mg/kg p.o.)	165.8 ± 9.5 ***	112.6 ± 9.5	130.7 ± 9.0***
IV	Isoniazid—rifampicin + + methanolic extract of *Achyranthes aspera* (100 mg/kg p.o.)	210.4 ± 10.1*	98.2 ± 9.3	185.2 ± 10.3*
V	Isoniazid—rifampicin + + methanolic extract of *Achyranthes aspera* (200 mg/kg p.o.)	195.3 ± 9.8**	108.5 ± 8.7	160.8 ± 9.0**

Values are expressed as mean ± S.E.M. (n = 6). Values are statistically significant at p<0.05 vs. Isoniazid—rifampicin control group respectively (One-way ANOVA followed by Dunnett's test).

Table 6: Effect of methanolic extract of *Achyranthes aspera* on MDA, CAT and GSH level in Isoniazid—rifampicin induced hepatotoxicity in rats

Group	Drug	MDA µmol/L	CAT U/mg	Glutathione (GSH) (µmol/mg)
I	Normal Control (0.5% CMC 1 ml/kg, p.o.)	1.8 ± 0.45	215.3 ± 6.5	210.5 ± 8.2
II	Isoniazid—rifampicin (50 +100 mg/kg)	5.3 ± 0.96	55.8 ± 2.1	58.7 ± 3.1
III	Isoniazid—rifampicin +Silymarin (100 mg/kg p.o.)	2.0 ± 0.2**	185.6 ± 4.8**	180.4 ± 5.2**
IV	Isoniazid—rifampicin + + methanolic extract of *Achyranthes aspera* (100 mg/kg p.o.)	3.0 ± 0.4*	120.3 ± 3.1*	125.3 ± 4.0*
V	Isoniazid—rifampicin + + methanolic extract of *Achyranthes aspera* (200 mg/kg p.o.)	2.8 ± 0.6**	167.8 ± 6.2**	170.2 ± 5.3**

Values are expressed as mean ± S.E.M. (n = 6). Values are statistically significant at p<0.05 (One-way ANOVA followed by Dunnett's test).

CONCLUSION

The methanolic extract of *Achyranthes aspera* exhibits significant hepatoprotective effects against isoniazid–rifampicin-induced liver injury in rats. This activity is likely mediated through multiple mechanisms, including antioxidant defense, membrane stabilization, restoration of liver enzyme markers, and normalization of lipid metabolism. The effects are dose-dependent and comparable to those of the standard drug silymarin. The presence of bioactive compounds such as flavonoids, phenolics, and diterpenes contributes to these therapeutic benefits. Further studies, including histopathological evaluations and identification of individual bioactive compounds, are recommended to validate and expand these findings.

REFERENCES

1. Alamri ZZ. The role of liver in metabolism: an updated review with physiological emphasis. Int J Basic Clin Pharmacol. 2018 Nov;7(11):2271-6.

2. Pandit A, Sachdeva T, Bafna P. Drug-induced hepatotoxicity: a review. Journal of Applied Pharmaceutical Science. 2012 May 30(Issue):233-43.
3. Neuman MG. Hepatotoxicity: mechanisms of liver injury. Liver diseases: a multidisciplinary textbook. 2020:75-84.
4. Somasundaram S, Ram A, Sankaranarayanan L. Isoniazid and rifampicin as therapeutic regimen in the current era: a review. Journal of Tuberculosis Research. 2014 Mar 21;2(1):40-51.
5. Okaiyeto K, Nwodo U, Mabinya L, Okoh A. A review on some medicinal plants with hepatoprotective effects. Pharmacognosy Reviews. 2018 Feb 1;12(24):186-99.
6. Das R, Mitra S, Tareq AM, Emran TB, Hossain MJ, Alqahtani AM, Alghazwani Y, Dhama K, Simal-Gandara J. Medicinal plants used against hepatic disorders in Bangladesh: A comprehensive review. Journal of Ethnopharmacology. 2022 Jan 10;282:114588.
7. Aladejana EB, Aladejana AE. Hepatoprotective activities of polyherbal formulations: A systematic review. Journal of Medicinal Plants for Economic Development. 2023 Oct 24;7(1):206.
8. Pandey G, Rao CV, Gupta SS, Verma KK, Singh M. Antioxidant and antibacterial activities of leaf extract of Achyranthes aspera Linn.(Prickly Chaff Flower). European journal of medicinal plants. 2014 Jun 1;4(6):695.
9. Kumar N, Sharma J, Kumar G, Shrivastav AK, Tiwari N, Begum A, Chakrabarti R. Evaluation of nutritional value of prickly chaff flower (Achyranthes aspera) as fish feed ingredient. The Indian Journal of Animal Sciences. 2021 Mar 1(3):239-44.
10. Nikhal SB, Dambe PA, Ghongade DB, Goupale DC. Hydroalcoholic extraction of *Mangifera indica* (leaves) by Soxhletion. International Journal of Pharmaceutical Sciences 2010; 2 (1): 30-32.
11. Prashant Tiwari, Bimlesh Kumar, Mandeep Kaur, Gurpreet Kaur, Harleen Kaur. Phytochemical screening and Extraction: A Review. Int. Pharmaceutica. Sciencia. 2011, 1 (1), 98-106.
12. OECD. Test No. 423: Acute Oral toxicity - Acute Toxic Class Method2002.
13. Dhadde SB, Nagakannan P, Roopesh M, Anand Kumar SR, Thippeswamy BS, Veerapur VP, et al. Effect of embelin against 3-nitropropionic acid-induced Huntington's disease in rats. Biomedicine & Pharmacotherapy. 2016;77:52-8.