

## HEPATOPROTECTIVE ACTIVITY OF *ACHYRANTHES ASPERA* LINN AGAINST PARACETAMOL INDUCED TOXICITY

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### ABSTRACT

Methanol extract and its methanol fraction from aerial parts of *Achyranthes aspera* exhibited significant hepatoprotective effect against paracetamol induced toxicity in rats. Glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase, total bilirubin, total cholesterol, total protein and albumin in serum indicated the hepato-protective effect of the extracts. Histopathological examination of liver sections confirmed that pre-treatment with methanol extract and methanol fraction prevented hepatic damage induced by paracetamol. The results were comparable with the standard hepatoprotective drug silymarin. The extract and its fraction showed no signs of toxicity up to a dose level of 2000 mg/kg. It is suggested that, the presence of flavonoids in methanol extract and methanol fraction may be responsible for hepatoprotective properties. An attempt was also made to quantify the total flavonoid content of the extracts.

**Keywords:** Paracetamol, Hepatoprotective, Methanol extract, Silymarin.

### INTRODUCTION

*Achyranthes aspera* (Amaranthaceae) commonly known as prickly chaff in English, chirchira in Hindi, is a perennial herb distributed throughout the tropical and sub tropical regions, of India. The presence of alkaloids, saponins, flavonoids<sup>1</sup> has been reported in the plant. The plant is described as abortifacient, diuretic, emmenagogue, anti hypertensive, spasmodic<sup>1</sup>, anti inflammatory<sup>2</sup>. The Wealth of India<sup>1</sup> reported the use of decoction of the leaves in liver disorders. In an attempt to explore scientifically its usage in liver disorders, in present study the bioactive methanol extract has been further fractionated to enrich hepatoprotective activity and to identify the constituents responsible for activity. The hepatoprotective activity of methanol extract and its methanol fraction at various dose levels was assessed using paracetamol as a hepatotoxin. Silymarin was used as positive control.

### MATERIALS AND METHODS

#### Plant material

Entire plants of *Achyranthes aspera* were collected from college of premises and their identity was confirmed at The Botanical Survey of India, Southern circle, Coimbatore, India. The voucher specimen (COG/CESCOP/01/SVSK/2009) has been deposited in the Herbarium of the Institute.

#### Preparation of extracts

Powdered (500 g) aerial parts of the plant was defatted with petroleum ether (60-80° C), and then completely extracted with methanol using soxhlet apparatus. The methanol extract concentrated in vacuum yielded solid mass (12.05%, W/W). About 50 g of methanol extract was adsorbed on silica gel (60-120 mesh) and fractionated using chloroform and methanol. The recovered fractions were finally evaporated to give yields of 1.33% and 24.6% respectively. Preliminary thin layer chromatographic studies<sup>3</sup> of methanol extract revealed the presence of flavonoids, phenolics, terpenoids and steroids; the chloroform fraction (CFME) showed the presence of flavonoids and one positive spot for each of terpenoids and steroids; while the methanol fraction (MFME) showed flavonoids, phenolics, terpenoids and steroids. The ME was tested for hepatoprotective activity at dose levels of 100, 200 and 300 mg/kg po, where as MFME was tested at dose levels of 50, 150 and 250 mg/kg po. Chloroform fraction being with minor concentration of flavonoids, terpenoids and steroids and less in quantity was not selected for activity. The results were compared with a standard hepatoprotective drug silymarin (100 mg/kg). All the test substances were suspended in vehicle (5% acacia mucilage).

#### Animals

Wistar albino rats of either sex weighing 175-225 g, maintained under standard husbandry conditions were used. Animals were

allowed to take standard laboratory feed and water *ad libitum*. The experiments were performed after the experimental protocol was approved by the institutional animal ethics committee.

#### Estimation of phenolic Content

The total phenolic content of the methanol extract of *A. aspera* was estimated by the method of Folin ciocalteu<sup>4</sup>. Stock solution (20mg/10ml) of the extract was prepared in methanol. From the stock solution 1ml of the extract was taken into a 25 ml volumetric flask. To this added 10 ml of water and 1.5 ml of Folin ciocalteu reagent. The mixture was kept aside for 5 min and then 4 ml of 20% sodium carbonate solution was added and volume was made up to 25 ml with double distilled water. The mixture was kept aside for 30 min and absorbance of blue colour developed was recorded at 765 nm. For the preparation of calibration curve the solutions of standard gallic acid was prepared in concentration range of 50-250 µg/ml.

#### Estimation of total flavonoid content

The total flavonoid content of the methanol extract of *A. aspera* was estimated by reported methods. The aluminium chloride colorimetric method was used for estimating Flavones, flavonols and isoflavones as reported by Chang et al<sup>5</sup>. Quercetin was used to make the calibration curve. From the stock solution of standard 0.1, 0.2, 0.3, 0.4 and 0.5 ml were taken which gave 10, 20, 30, 40 and 50 µg concentrations respectively. The diluted standard solutions (0.5 ml) were separately mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. The absorbance of the reaction mixture was measured at 415 nm, after incubation at room temperature for 30 min. The amount of 10% aluminium chloride was substituted by the same amount of distilled water in blank. Similarly 0.5 ml of methanol extract of *A. aspera* (2 mg/ml) was reacted with aluminium chloride for determination of total flavonoid content.

The method reported by Chang et al. was used for estimation of flavanones. Naringenin was used as a reference standard to prepare calibration curve. 20 mg of naringenin was dissolved in methanol and then diluted to give concentrations of 250,500, 1000,1500 and 2000 µg/ml. one millilitre of each of the diluted standard solutions was separately mixed with 2 ml of 1% 2, 4-dinitro phenyl hydrazine reagent and 2 ml of methanol at 50°C for 50 min. After cooling to room temperature the reaction mixture was mixed with 5 ml of 1% potassium hydroxide in 70% methanol and incubated at room temperature for 2 min. Then 1 ml of the mixture was taken, mixed with 5 ml of methanol and centrifuged at 1000 rpm/min to remove the precipitate formed. The supernatant was collected and adjusted to 25 ml. the absorbance of the supernatant was measured at 495 nm. Similarly 5 ml each of methanol extract of *A. aspera* (5 mg/ml) were similarly treated with 2, 4-dinitro phenyl hydrazine reagent for determination of flavonoid content. The sum of the values obtained from these two methods was considered as total flavonoid content.

## Hepatoprotective activity in vivo

### Toxicity studies

Acute toxicity studies were performed for ME and MFME according to the acute toxic classic method<sup>6</sup> described by OECD. Female albino rats were used for acute toxicity study. The animals were kept fasting for overnight providing only water. The rats were divided into two groups of 3 animals each. The groups of rats were administered orally with appropriate extracts of *A. aspera* at a dose of 300 mg/kg. The animals were observed continuously after dosing during first 30 min, periodically for first 24 hr with special attention given during first 4 hr and daily thereafter, for a total of 14 days. As there was no mortality seen at this dose level, the procedure was repeated with further dose (2000 mg/kg) using fresh animals.

### Hepatotoxins and test substances

Test substances including silymarin were suspended in vehicle i.e.5 % acacia mucilage for administration. Paracetamol at a dose level of 3 g/kg in 5 % acacia mucilage was administered orally.

### Paracetamol-induced hepatotoxicity<sup>7</sup>

Rats were divided into 7 groups of 6 each, control, paracetamol, silymarin and test groups. The rats of control and paracetamol group received four doses of 5% acacia mucilage (1 ml/kg, p.o.) at 24 hr intervals (0 h, 24 h, 48 h and 72 h). The rats of paracetamol group received a single dose of paracetamol (3 g/kg p.o.) 30 min after the administration of the third dose of vehicle. The animals in silymarin and test groups received three doses of respective test substances (silymarin 100 mg/kg; ME and MFME 50, 150 and 250 mg/kg p.o.) at 0 h, 24 h, and 48 h. Paracetamol (3 g/kg p.o.) was administered 30 min after the third dose of respective test substances. After 48 h of paracetamol intoxication blood was collected and serum was separated for estimation of biochemical parameters.

### Assessment of liver function

Glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) were estimated by a UV-Kinetic method based on the reference method of International Federation of Clinical Chemistry<sup>8</sup>. Alkaline phosphatase (ALKP) was estimated by the method described by Mac comb and browers<sup>9</sup>, total bilirubin (TBL) by Jendrassik and Groff method<sup>10</sup>, total cholesterol (CHL) by CHOD-PAP Method of Richmond<sup>11</sup>, total protein (TPTN) was estimated by Biuret method<sup>12</sup>; while albumin (ALB) was estimated by bromo cresol green method<sup>13</sup>. All the estimations were carried out using standard kits on semi auto analyser.

### Histopathological studies

Animals were sacrificed to remove the liver. The liver was fixed in Bouin's solution for 12 h, and then embedded in paraffin using conventional methods<sup>14</sup>, cut into 5 µm thick sections and stained using haematoxylin-eosin dye. The sections were then observed for histopathological changes.

### Statistical Analysis

The mean values±SEM were calculated for each parameter. Percentage restoration against the hepatotoxin by the test samples

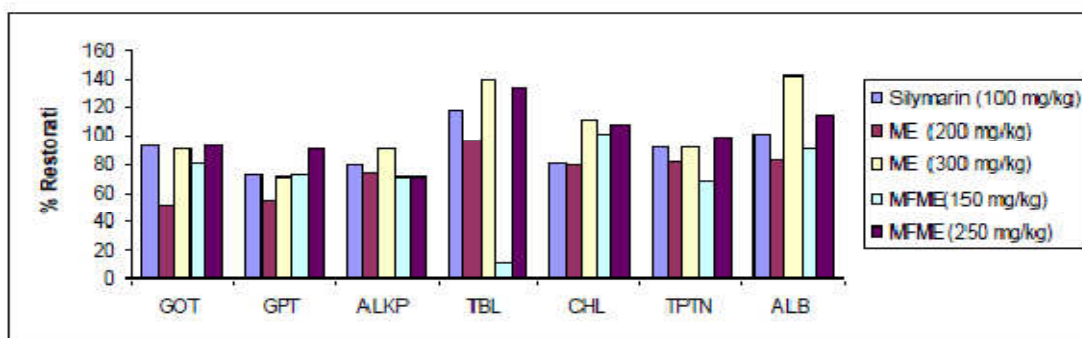
was calculated by considering the difference between the hepatotoxin treated group and the control group as 100% restoration. For the determination of significant inter group difference each parameter was analyzed separately and one way analysis of variance (ANOVA)<sup>15</sup> (Gennaro, 1995) was carried out. After that individual comparisons of group mean values were done using Dunnet's test<sup>16</sup>.

## RESULTS

The phenolic content of methanol extract of *A. aspera* was found to be 11.5% w/w representing the presence of various phenolic compounds like poly phenols, flavonoids, phenolic acids etc. From the calibration curve of the quercetin, the concentrations of the flavonols and flavones in the methanol extract of *A. aspera* was found to be 4.88% w/w. From the calibration curve of naringenin, the amount of flavanones in the methanol extract of *A. aspera* was found to be 6.66% w/w. The sum of the values obtained from these two methods was considered as total flavonoid content and are found to be 8.34% w/w.

The ME and MFME did not cause any mortality upto 2000 mg/kg and were considered as safe. Paracetamol intoxication (3 g/kg p.o.) induced a marked increase in the serum levels of GOT (135.08 ± 23.36 to 335.68 ± 29.33), GPT (56.77 ± 4.23 to 210.91 ± 16.68), ALKP (286.55 ± 23.17 to 741.30 ± 97.72), TBL (0.84 ± 0.33 to 2.27 ± 0.48), and CHL (79.32 ± 10.0 to 303.80 ± 98.6); and decrease in the levels of TPTN (8.17 ± 0.41 to 3.36 ± 1.06) and ALB (2.28 ± 0.08 to 1.03 ± 0.39) when compared to normal rats indicating acute centrilobular necrosis. The groups of rats which received ME at dose levels of 200 and 300 mg/kg p.o. and MFME at dose levels of 150 and 250 mg/kg p.o. showed a significant decrease (p<0.05) in almost all the elevated levels of biochemical parameters and significant (p<0.05) increase in depleted TPTN and ALB levels similar to that observed in the case of rats of silymarin treated group. There was no significant (p<0.05) difference in the activity, exhibited by ME at dose levels of 200 and 300 mg/kg; MFME at dose levels of 150 and 250 mg/kg. The activity exhibited by ME was statically similar to the activity exhibited by MFME. The results obtained are shown in Table 1 and Figure 1. Percentage restorations of various parameters by ME and MFME against paracetamol-induced hepatotoxicity are represented in Graph 1. The maximum percentage restoration, in the levels of ALKP, TBL, CHL and ALB was observed with ME at dose level of 300 mg/kg, while MFME at a dose level of 250 mg/kg afforded highest percentage restoration in the levels of GOT, GPT and TPTN levels.

Histological examination of liver sections of rats of control group, revealed normal cellular architecture (Figure 1a) while those intoxicated with paracetamol (3 g/kg p.o.) showed disarrangement and degeneration of normal hepatic cells with intense centrilobular necrosis and bridged necrosis, characterised by bands of necrosis linking one central vein to another, sinusoidal haemorrhages and dilatation (Figure 1b). Treatment with ME (200 and 300 mg/kg) and MFME (150 and 250 mg/kg) followed by paracetamol intoxication resulted the absence of necrosis, sinusoidal dilatation and lesser degree of disarrangement and degeneration of hepatocytes (Figure 1d-g) indicating marked protective activity similar to that observed in silymarin treated rat liver sections (Figure 1c).



Graph 1: Percentage restoration of various parameters by ME and MFME against paracetamol-induced hepatotoxicity

**Table 1: Effect of ME and MFME of *A. aspera* on Paracetamol-induced hepatotoxicity in rats**

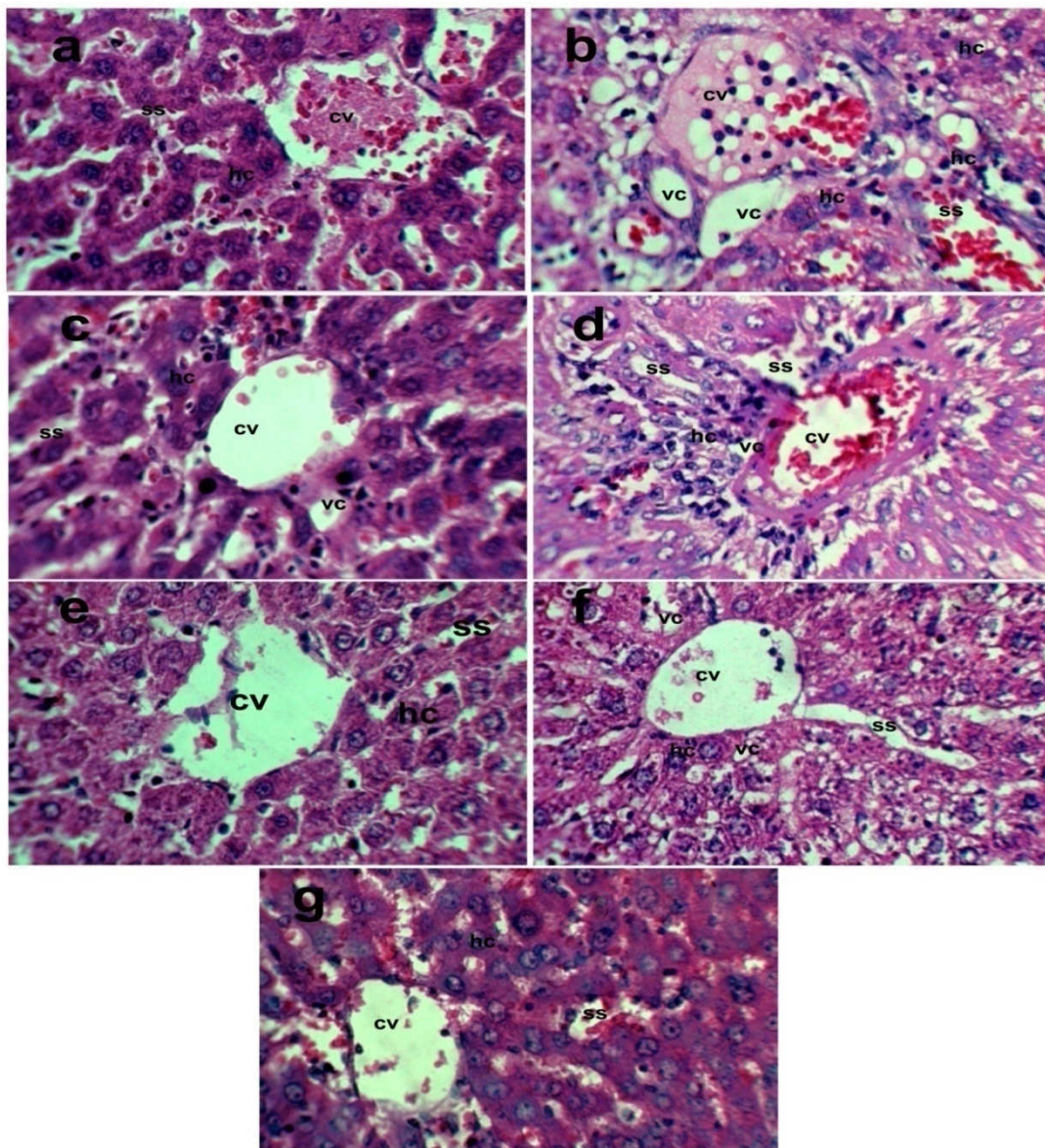
Group	GOT (IU/L)	GPT (IU/L)	ALKP (IU/L)	TBL (mg/dl)	CHL (mg/dl)	TPTN (g/dl)	ALB (g/dl)
Control	135.08 ± 23.36	56.77 ± 4.23	286.55 ± 23.17	0.84 ± 0.33	79.32 ± 10.0	8.17 ± 0.41	2.28 ± 0.08
Paracetamol	335.68 ± 29.33	210.91 ± 16.68	741.30 ± 97.72	2.27 ± 0.48	303.80 ± 98.6	3.36 ± 1.06	1.03 ± 0.39
Silymarin	147.90 ± 15.29*	99.95 ± 26.76*	378.53 ± 82.94*	0.58 ± 0.26*	121.97 ± 48.83*	7.79 ± 1.14**	2.30 ± 0.18**
ME 1	230.23 ± 23.57*	127.42 ± 20.21*	404.55 ± 88.7*	0.88 ± 0.32	124.93 ± 35.92*	7.27 ± 0.40**	2.07 ± 0.37**
ME 2	154.23 ± 21.65*	101.62 ± 22.65*	328.62 ± 76.57*	0.26 ± 0.47*	55.21 ± 7.59*	7.80 ± 0.66**	2.79 ± 0.12**
MFME1	173.65 ± 7.75*	99.86 ± 25.63*	419.50 ± 79.97*	0.83 ± 0.35*	75.89 ± 20.01*	6.71 ± 1.04**	2.16 ± 0.21**
MFME2	149.44 ± 12.61*	71.36 ± 19.56*	418.70 ± 78.34*	0.35 ± 0.18*	62.97 ± 7.88*	8.16 ± 0.27**	2.46 ± 0.12**
F Calculated	12.48	5.907	3.539	3.441	3.731	4.62	5.07
DV	75.92	77.22	294.14	1.351	167.5	2.95	0.91

Data represents the mean ± SEM of six animals. F theoretical = 2.42 (p<0.05).

ME 1 and ME 2: Methanol extracts 200 and 300 mg/kg; MFME 1, and MFME 2: Methanol fraction of methanol extract 150 and 250 mg/kg.;

DV: Dunnett value.

\* Significant reduction compared to Paracetamol (p<0.05). \*\* Significant increase compared to Paracetamol (p<0.05).



**Fig. 1: Photomicrographs representing effect of ME and MFME against Paracetamol induced hepatotoxicity in rats.**

a: Normal rat liver section; b: Liver section of the rat intoxicated with Paracetamol; c: Liver section of the rat treated with silymarin and intoxicated with Paracetamol; d: Liver section of the rat treated with ME 200 mg/kg and intoxicated with Paracetamol; e: Liver sections of the rat treated with ME 300 mg/kg and intoxicated with Paracetamol; f: Liver section of the rat treated with MFME 150 mg/kg and intoxicated with Paracetamol; g: Liver section of the rat treated with MFME 250 mg/kg and intoxicated with Paracetamol. Eosin-Haematoxylin stain. 400X. cv: central vein, vc: vacuole, ss: sinusoidal spaces, hc: hepatocytes.

## DISCUSSION

Paracetamol produces hepatic necrosis when ingested in large doses. It is metabolised in the liver primarily to glucuronide and sulphate conjugates. Paracetamol toxicity is due to formation of toxic metabolites when a part of it is metabolised by cytochrome P<sub>450</sub>. Induction of cytochrome P<sub>450</sub> or depletion of glutathione is a prerequisite for paracetamol induced hepatotoxicity. Therefore the hepatoprotective activity of the ME, and MFME of aerial parts of *A. aspera* against paracetamol induced hepatotoxicity may be due to inhibition of cytochrome P<sub>450</sub>; stimulation of hepatic regeneration or activation of the functions of reticuloendothelial systems.

Thus the hepatoprotective activity of these extract and fraction may be due to their ability to affect the cytochrome P<sub>450</sub> mediated functions or stabilisation of endoplasmic reticulum resulting in hepatic regeneration.

In literature many authors reported the hepatoprotective activity of flavonoid compounds. Hepatoprotective activity of flavonoids of *Rosmarinus tomentosus* was reported by Galisteo et al<sup>17</sup>. The hepatoprotective effect of quercetin was reported by Janbaz et al<sup>18</sup>. Protective effect of rutin was reported by Janbaz et al<sup>19</sup>. Silymarin obtained from *Silybum marianum* is a good hepatoprotective agent<sup>20</sup>. In accordance with these results, it may be hypothesized that flavonoids with their anti-oxidant properties, which are present ME, and MFME, of *A. aspera* are responsible for the hepatoprotective activity.

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