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Hepatoprotective activity of flowers of *Cassia auriculata* R. Br. against paracetamol induced liver injury

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Abstract

The aqueous (100 mg/kg p.o.), methanolic (100 mg/kg p.o.) and petroleum ether (50 mg/kg p.o.) extracts of the flowers of *Cassia auriculata* linn, (Caesalpinaceae) were tested for their hepatoprotective activity against paracetamol induced hepatotoxicity in albino rats. Silymarin at a dose of 25 mg/kg i.p. was used as standard. The degree of protection was measured by using biochemical parameters like serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), alkaline phosphatase (ALP), direct bilirubin and total bilirubin. The histopathological studies were also conducted. The aqueous and methanolic extracts of the flowers showed a significant hepatoprotective activity comparable with those of Silymarin.

Key words: Hepatoprotective activity; *Cassia auriculata*; Paracetamol.

1. Introduction

Cassia auriculata Linn (Caesalpinaceae) popularly known, as Tarval in Hindi is a fast growing profusely branched, tall evergreen shrub, generally 1.2 - 3.0 m in height sometimes reaching a height of 6.0 m. [1] and is employed in indigenous and folk medicine for a variety of purposes, bark is used in rheumatism, ulcers, skin diseases, leprosy and eye diseases, it is recommended in diabetes, [2, 3] *C. auriculata* is the constituent of many herbal preparations used in liver disorders, Earlier studies have shown that *C. auriculata* leaf extracts have shown hepatoprotective activity against alcohol induced liver injury [4]. *C. auriculata* has shown anti diabetic activity [5] and antimicrobial activity [6]. *Cassia auriculata* Linn. root extract is found effective against cisplatin and gentamicin-induced renal injury [7] Polysaccharides, flavonoids, anthracene derivatives, dimeric procyanidins, myristyl alcohol, β-sitosterol β-D-glucoside, quercetin, rutin, di- 2-ethyl – hexylphthalate are present in *C. auriculata* [8].

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According to the United States Acute Liver Failure Study Group 1, Drug induced liver injury accounts for more than 50% of acute liver failure, including hepatotoxicity caused by overdose of acetaminophen (APAP, 39%) and idiosyncratic liver injury triggered by other drugs (13%) [9]. by multiple mechanisms of action. This paper deals with the evaluation of the hepatoprotective effect of different extract of flowers of *C. auriculata* on rat liver damage induced by paracetamol.

2. Materials and methods

2.1 Plant material

Flowers of the plant were collected from Satpuda valley of Nandurbar district, Maharashtra, India, in the month of August. The plant was authenticated at Department of Botany, S.S.V.P.S College of science, Dhule, Maharashtra, India. A voucher specimen has been kept in the department for further reference.

2.2 Chemicals

Silymarin, Paracetamol were purchased from Sigma Chemical. All other reagents were of analytical grade

2.3 Preparation of plant extracts

The plant material (8.0 kg) was dried under shade in a room. After 10 days of drying, the flowers were powdered in pulveriser and sieved with a 40 # sieve. *C. auriculata* flowers powder was packed in soxhlet apparatus and successively extracted with methanol (yield 14.28 %w/w) and petroleum ether (yield 12.23%w/w) The mark was finally subjected to cold maceration in distilled water to yield aqueous extract (yield 22.2. %w/w).

2.4 Experimental animals

Three months old Wistar albino rats of either sex weighing 180 - 240 g were obtained from animal house of the toxicology center, Pune, India. After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity and dark/light cycle. Before and during the experiment, rats were fed with standard diet (Gold Moher, Lipton India Ltd). The study was approved by institutional animal ethical committee of R. C. Patel College of Pharmacy, Shirpur, Maharashtra, India. CPCSEA registration No. 651/02/C/CPCSEA.

2.5 Paracetamol induced hepatotoxicity

All the animals were randomly divided into the six groups each group consists of 6 animals. The first group served as vehicle control and received normal saline. The second group served as paracetamol intoxicated control and received by gavage vehicle (normal saline) for seven days. Third group was given standard drug silymarin at the dose of 25 mg/kg body weight by intraperitoneal route, fourth group was given methanolic extract (100 mg/kg p.o.), and fifth group received aqueous extract (100 mg/kg p.o.) and sixth group received petroleum extract (50 mg/kg p.o.). The vehicle or test drugs were administered orally for 7 days. Paracetamol suspension (2% gum acacia) was administered in a dose of 2 gm/kg p.o. on 7th day. Forty-eight hours after paracetamol administration, blood was collected from all the animals through retro-orbital plexus. The 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 analyzed for various biochemical parameters.

2.6 Assessment of Biochemical parameters

SGOT and SGPT [10], ALP [11], direct bilirubin and total bilirubin [12] were analysed by using standard kits.

2.7 Histopathological studies

The rats were sacrificed under light ether anaesthesia by cervical dislocation and the liver of all the animals of all the groups were isolated, washed with water, dried gently with filter paper and preserved in 10% formaline. After 48 h the liver were dehydrated by passing successively in different mixtures of ethyl alcohol–water (50, 80, and 95%, and finally in absolute alcohol), cleared in xylene and embedded in paraffin. Sections (4–5 mm thick) were prepared and then stained with hematoxylin and eosin dye for microscopic observation. The histopathological studies were carried out at Maharishi, House of Histopathology and cytology, Ahmedabad, India.

2.8 Statistical analysis

Results of the biochemical estimations are reported as means ± standard error of mean (S.E.M.) and statistical analysis was made by one way ANOVA and post hoc Dunnet's *t* - test. At 95% confidence interval $P<0.05$ and $P<0.01$ were considered to be significant.

3. Results

The protective actions of *C. auriculata* pre-treatment on hepatotoxicity induced by paracetamol are summarised in Table 1.

3.1. Paracetamol induced liver injury

C. auriculata demonstrated protective effect in rats against paracetamol (PCM) induced hepatotoxocity. Oral administration of paracetamol at a dose of 2 gm/kg, caused a significant ($P<0.01$) rise in SGPT, SGOT, ALP, total Bilirubin and direct Bilirubin levels. Silymarin significantly ($P<0.01$) reduced these levels to normal. A significant ($P<0.01$) decrease was observed in the SGPT, SGOT, ALP, total Bilirubin and direct Bilirubin levels in the animals treated with different extracts of *C. auriculata*. The results revealed that methanolic and petroleum ether extract were more effective as compared with aqueous extract.

3.2. Histopathology of the paracetamol model

Liver section of normal control group showed normal histology showing central vein and normal hepatocytes and sinusoidal (Fig. 1). Paracetamol administration caused gross centrilobular necrosis. Focal areas of liver cell necrosis, lymphocytic infiltration were evident (Fig. 2). Pretreatment of rats with silymarin nearly reversed the toxicity caused by paracetamol (Figure 3). Rats pretreatment with different extract of *C. auriculata* shows mild protection in paracetamol induced liver damage. (Fig. 4 and 5, 6).

4. Discussion

Acetaminophen is a widely used analgesic and antipyretic drug. It causes severe centrilobular hepatic necrosis when ingested in large amounts in suicide attempts or accidentally by children. A single dose of 10 to 15 g, occasionally less, may produce clinical evidence of liver injury. Fatal fulminant liver disease is usually associated with ingestion of 25 g or more [13]. Paracetamol is metabolized to a minor electrophilic metabolite, N-acetyl-p-benzoquinoneimine (NAPQI), which during paracetamol overdose depletes glutathione and initiates covalent binding to cellular proteins. These events lead to the disruption of calcium homeostasis, mitochondrial dysfunction, and oxidative stress and which eventually culminate in cellular damage and death [9]. Protection against paracetamol induced toxicity has been used as a test for a potential hepatoprotective agent by several investigators [14, 15, 16, 17, 18]. Oral administration of paracetamol causes an elevation in SGOT, SGPT because of cellular leakage, due to cell necrosis, there is also an elevation in ALP, total and direct Bilirubin. Biochemical parameter were decreased significantly ($P< 0.01$) by the pre-treatment of the rats with silymarin. Pretreatment of rats with aqeous, methanolic and petroleum extract

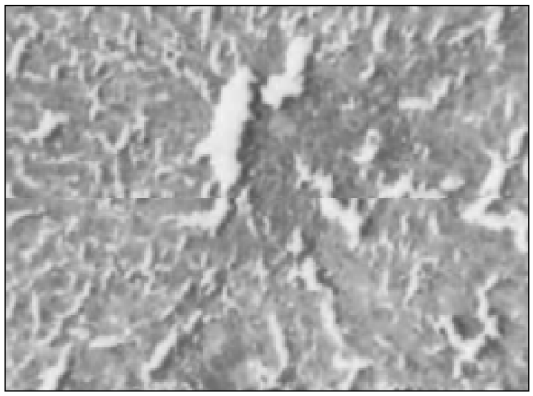

Fig. 1
Normal Liver
Normal histology showing central vein and normal hepatocytes and sinusoidal

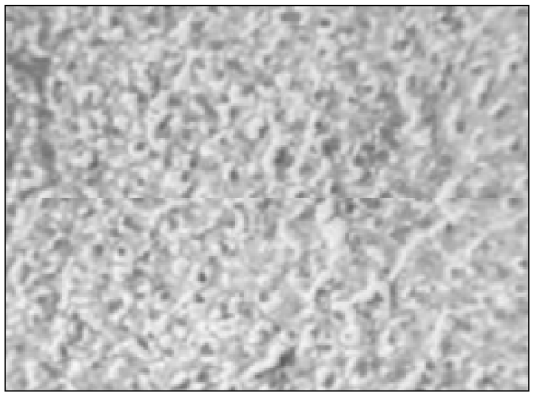

Fig. 2
Toxicant control (Paracetamol 2 gm/kg)
Focal areas of liver cell necrosis, Lymphocyric infiltration

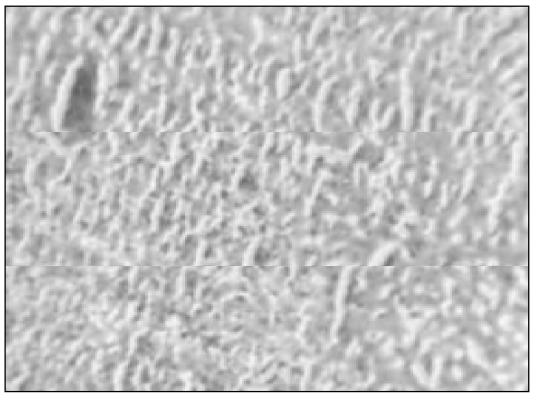

Fig. 3
Standard sylimarin (25 mg/kg) + Paracetamol (2 gm/kg) large areas of cell regeneration and lympholytic infiltration

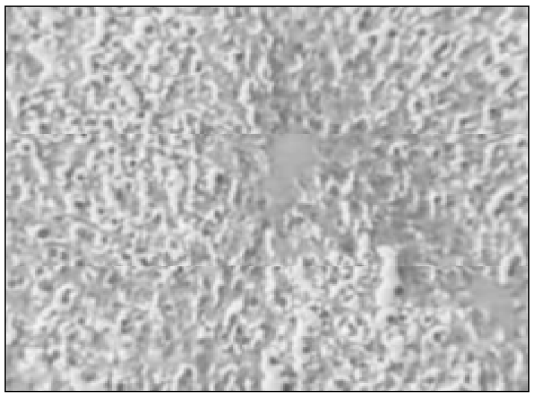

Fig. 4
Aqueous extract 100 mg/kg + Paracetamol (2 gm/kg) Areas of cell necrosi and lympholytic infiltration

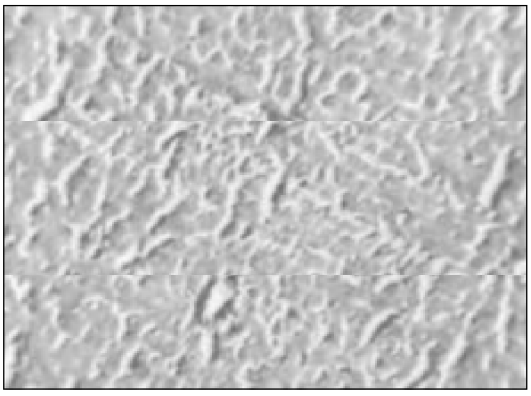

Fig. 5
Methanolic extract 100 mg/kg p.o. + paracetamol (2 gm/kg p.o.) Isolated areas of cell necrosis are seen, lympholytic infiltratration is also evident and also areas of cell regeneration are found

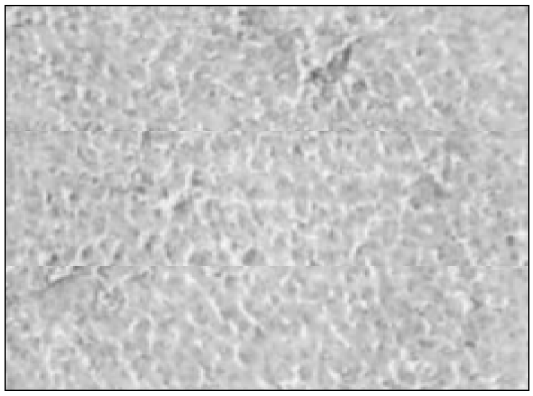

Fig. 6
Petroleum extract 100 mg/kg p.o. + paracetamol (2 gm/kg p.o.) areas of cell necrosis are seen but ocassional areas of cell regneration is also evident

Table 1. Effect of Aqueous, methanolic and petroleum ether extracts of flowers of *C. auriculata* and paracetamol on Serum Transaminases, Alkaline phosphatase, Total and Direct Bilirubin

Treatment	SGOT	SGPT	ALP	T. Bilirubin	D. Bilirubin
Normal control (No treatment)	144.83±6.18**	40.33±1.56**	164.5±3.43**	0.924±0.11**	0.31±0.01**
Toxicant Control Paracetamol (2 gm/kg)	322.16±11.81	96.33±4.24	416.33±8.2	4.019±0.09	0.61±0.019
Standard Sylimarin (25 mg/kg) + Paracetamol (2 gm/kg)	177.16±3.44**	56±1.86**	199±2.78**	1.61±0.19**	0.38±0.017**
Aqueous extract (100 mg/kg) + Paracetamol (2 gm/kg)	192.16±4.27*	63.33±3.3*	228.5±7.008*	3.27±0.039*	0.460±0.057*
Methanolic Extract (100 mg/kg) + Paracetamol (2 gm/kg)	188.5±3.5288	60.83±3.38	215.33±0.59*	2.747±0.06**	0.44±0.013*
Petroleum ether Extract (50 mg/kg) + Paracetamol (2 gm/kg)	187.3±2.10**	67.5+2.56*	205+7.27*	2.31±0.048*	0.864±0.02*

Values are expressed as mean±S.E.M.; (n=6), *P<0.05, when compared with the toxicant control groups (one-way ANOVA followed by Dunnetts test), **P<0.01, when compared with the toxicant control groups (one-way ANOVA followed by Dunnetts test), The levels of SGPT, SGOT in Serum are expressed as IU/L. The levels of ALP in Serum are expressed as K.A./L The levels of total and direct bilirubin in serum is expressed as mg/dl.

causes an significant (P<0.001) decrease in the levels of SGOT, SGPT, ALP, and total and direct Bilirubin, showing that these extracts have significant hepatoprotective activity.

Histopathological studies showed that paracetamol caused focal necrosis. Silymarin exhibited a marked protection against hepatic damage caused by paracetamol. Whereas *C. auriculata* extracts pre-treatment exhibits a mild protection, thus histopathological studies confirmed the results of biochemical findings. In summary, the present study demonstrates the hepatoprotective effect of different extracts of *C. auriculata* against paracetamol-induced hepatotoxicity.

5. Conclusion

The above observations shows that the different extracts of the flowers of *C. auriculata* contain some active principles which may be responsible for producing their characteristic effect on hepatotoxicity induced by paracetamol. Further studies involving isolation of different phytoconstituent from the plant and their pharmacological evaluation is needed to be carried out in order to develop an effective treatment against hepatotoxic effect of drugs.

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Maharishi, House of Histopathology and cytology, Ahmedabad - 380 014, India, for providing necessary experimental facilities for histopathology studies.

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