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Research Article

PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF *ACALYPHA COMMUNIS* MULL. ARG. FOR THEIR HEPATOPROTECTIVE ACTIVITY

RAJASEKARAN S[1]*, ANANDAN R[2]

[1]Department of Pharmacology, Institute of Pharmaceutical Sciences and Research Center, Bhagwant University, Ajmer, Rajasthan, India.
[2]Department of Pharmacology, Erode College of Pharmacy, Erode, Tamil Nadu, India. Email: raj4ma@gmail.com

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ABSTRACT

Objective: *Acalypha communis* is a synonym of *Ricinocarpus communis* (Müll.Arg.) belonging to the family of Euphorbiaceae. *A. communis* possess a wide variety of activities like antimicrobial. Hence, the present study was intended to evaluate ethanolic leaf extract of *A. communis* for hepatoprotective activities.

Methods: The hepatoprotective activity was studied by paracetamol (2 g/kg) and D-galactosamine (400 mg/kg) induced models. Acute toxicity study and preliminary phytochemical screening were also studied to evaluate the toxicity.

Results: No toxicity profile was observed in rats after oral administration of the ethanolic leaf extract at the dose of 5 g/kg body weight. The different dose of 200 mg/kg and 400 mg/kg administered with the extract of *A. communis*. There was significant ($p<0.001$) reduction in biochemical parameters with respect to control. Phytochemical screening of the plant extract revealed the presence of tannins, alkaloids, flavonoids and saponins, and terpenoids.

Conclusion: It can be concluded that the hepatoprotective activity elucidated by *A. communis* could be mainly due to the presences of high-value class of compound like the phenolic group as the major content in the plants.

Keywords: *Acalypha communis*, Paracetamol, D-galactosamine, Biochemical parameters and histopathological studies.

INTRODUCTION

India has a rich culture of medicinal herbs and spices, which includes more than 2000 species and has a vast geographical area with high potential abilities for the Ayurvedic, Unani, and Siddha traditional medicines, but only very few have been studied chemically and pharmacologically for their potential medicinal value [1,2]. Hence, natural products from medicinal plants need to be investigated by scientific methods for their hepatoprotective activity. The plant *Acalypha communis* is a synonym of *Ricinocarpus communis* (Müll.Arg.) belonging to the family of Euphorbiaceae. It includes herbs, shrubs, and small trees, Shrubs or suffrutex frequently with resinous bright droplets on leaves and inflorescences; indumentum of simple or glandular hairs. Inflorescences spicate, usually unisexual [3].

METHODS

Collection, identification, and authentification of the plants

The leaves of *A. communis* Müll.Arg., were collected from the Malappuram District, Kerala, India, during the month of October 2013. The plant materials were identified and authenticated by Dr. Pradeep Botanist Calicut University, Kozhikode. Voucher specimens were kept in our laboratory for future reference.

Preparation of extracts

The granulated, dried leaves of *A. communis* (500 g) were packed in a Soxhlet apparatus and subjected to continuous hot percolation for 8 hrs using 450 ml of ethanol (95% v/v) as a solvent. The extract was concentrated to dryness under reduced pressure and controlled temperature and dried in a desiccator (yield 75 g, 15% w/w). The extract was suspended in 5% gum acacia and used for further experiments.

Preliminary phytochemical screening

The extract was screened qualitatively for the presence of various groups of phytoconstituents using different chemical tests [4].

Procurement of experimental animals

Animals were selected as per the OECD guidelines. Healthy young and nulliporous, non-pregnant Sprague Dawleys female rats weighing from 160 to 180 mg of 8-12 weeks old were selected, because literature survey of lethal dose 50% test shows that usually there is little difference in sensitivity between sexes, but generally females were found slightly more sensitive, were procured from listed suppliers of Sri Venkateswara Enterprises, Bengaluru, India. The animals were fed with standard pellet diet (Hindustan Lever Ltd. Bengaluru) and water ad libitum. All the animals were housed in polypropylene cages. The animals were kept under the alternate cycle of 12 hrs of darkness and light. The animals were acclimatized to the laboratory conditions for 1 week before starting the experiment. The animals fasted for at least 12 hrs before the onset of each activity. The experimental protocols were approved by Institutional Animal Ethics Committee (IAEC No.- P.Col/02/1868/26/09/2013/IAEC/JSPC) after scrutinization. The animals received the drug treatments by oral routs.

OBSERVATIONS

Animals were observed individually for 48 hrs after dosing at the first 30 minutes, periodically and during the first 24 hrs, with special attention given during the first 4 hrs and daily thereafter, for a total of 14 days. Additional observations were also made if the animals continue to display signs of toxicity. Observations included were changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity, and behavior pattern. Observations were also made and checked for tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma. Results were tabulated in Table 1.

Experimental procedure [5]

The rats were divided into the five groups each containing six rats.
Group I: Control rats, which fed normal diet and water.
Group II: Rats treated with paracetamol (2 g/kg) for 28 days.

Group III: Rats treated with silymarin (100 mg/kg) + paracetamol (2 g/kg) orally once daily for 28 days.
Group IV: Rats treated with AC (200 mg/kg, i.p.) + paracetamol (2 g/kg) once daily for 28 days.
Group V: Rats treated with AC (400 mg/kg, i.p.) + paracetamol (2 g/kg) once daily for 28 days.

Experimental procedure [5]

The rats were divided into the five groups each containing six rats.
Group I: Control rats, which fed normal diet and water.
Group II: Rats treated with D-galactosamine (400 mg/kg) for 28 days.
Group III: Rats treated with silymarin (100 mg/kg) + D-galactosamine (400 mg/kg) orally once daily for 28 days.
Group IV: Rats treated with AC (200 mg/kg, i.p.) + D-galactosamine (400 mg/kg) once daily for 28 days.
Group V: Rats treated with AC (400 mg/kg, i.p.) + D-galactosamine (400 mg/kg) once daily for 28 days.

Statistical analysis

Values were represented as mean ± standard error of mean of three parallel data's.

Table 1: Acute toxicity study of ethanolic extracts of leaves of *A. communis* based on OECD guidelines 423

S. No	Number of animals	Dose in mg/kg	Report
1	3	5	No death
2	3	50	No death
3	3	300	No death
4	3	2000	No death
5	3	5000	No death

A. communis: Acalypha communis

RESULTS

Preliminary phytochemical screening

The preliminary phytochemical analysis of fractions of *A. communis* shows the presence of steroids, alkaloids, flavonoids, glycosides, saponins, tannin, and carbohydrate.

DISCUSSION

The present study reveals the hepatoprotective activity of *A. communis* against paracetamol and D-galactosamine-induced hepatic damage in rats. Hepatotoxic drugs such as d-galactosamine and acetaminophen reduces liver functional capacity, which leads to an accumulation of waste products such as ammonia in the blood [5]. The results show that *A. communis* is effective in low and medium doses (200 mg/kg, p.o and 400 mg/kg, p.o) paracetamol is a commonly used as analgesic

Table 2: Results of gross behavioral studies in rats on administration of *A. communis*

Observation	Effects								
Gross activity	Upto 3 hrs	3 ½ hrs	4 hrs	4 ½ hrs	5 hrs	5 ½ hrs	6 hrs	12 hrs	24 hrs
Respiration	+	+	+	+	+	+	+	+	+
Writhing	–	–	–	–	–	–	–	–	–
Tremor	–	–	–	–	–	–	–	–	–
Convulsions	–	–	–	–	–	–	–	–	–
Hind limb paralysis	–	–	–	–	–	–	–	–	–
Sense of touch and sound	+	+	+	+	+	+	+	+	+
Salivation	+	+	+	+	+	+	+	+	+
Diarrhea	–	–	–	–	–	–	–	–	–
Mortality	–	–	–	–	–	–	–	–	–

A. communis: Acalypha communis,

Table 3: Results of the effects of biochemical markers of paracetamol induced hepatic injury in rats

S. No.	Group/drug	Dose (mg/kg)	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total bilirubin (mg/dl)	Total protein (mg/dl)
1	Group I - normal control (NaCl 0.9% w/v)	5 ml/kg	51.23±2.31	60.12±1.24	27.17±1.72	1.14±0.13	8.74±0.84
2	Group II - paracetamol	(2 g/kg)*	193.60±1.74*	173.10±1.36*	58.75±1.32*	12.18±0.74*	4.89±1.31*
3	Group III - silymarin+paracetamol	100 mg/kg+(2 g/kg)**	59.41±1.32**	56.78±2.32**	29.34±1.54**	1.27±0.22**	8.03±0.41**
4	Group IV - AC+paracetamol	200 mg/kg+(2 g/kg)**	73.67±1.23**	67.31±1.14**	38.31±1.81**	2.68±0.35**	6.85±0.23**
5	Group V - AC+paracetamol	400 mg/kg+(2 g/kg)**	61.23±2.13**	58.47±1.87**	30.14±1.22**	1.61±0.16**	7.91±0.13**

n=6; values were expressed mean±SEM; Group II was compared to Group I. Groups III to V were compared to Group II. *p<0.01 versus paracetamol group: Significant; **p<0.001 versus paracetamol group: Highly significant data were analyzed by one-way ANOVA followed by Dunnett's t-test. SEM: Standard error of mean. SGOT: Serum glutamic oxaloactetic transaminase, SGPT: Serum glutamic pyruvic transaminase, ALP: Alkaline phosphatase

Table 4: Results of the effects of biochemical markers of D-galactosamine-induced hepatic injury in rats

S. No.	Group/drug	Dose (mg/kg)	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total bilirubin (mg/dl)	Total protein (mg/dl)
1	Group I - normal control (NaCl 0.9% w/v)	5 ml/kg	64.23±1.31	77.52±1.04	19.17±1.35	1.39±1.42	6.98±0.25
2	Group II - D-galactosamine	(400 mg/kg)*	167.70±1.07*	181.02±1.24*	42.83±2.01*	8.31±0.36*	3.66±1.46*
3	Group III - silymarin+D-galactosamine	100 mg/kg+(400 mg/kg)**	67.74±1.93**	80.21±2.06**	18.64±1.27**	1.43±1.52**	7.09±0.68**
4	Group IV - AC+D-galactosamine	200 mg/kg+(400 mg/kg)**	79.31±1.03**	91.21±1.62**	27.94±1.42**	2.13±1.41**	5.91±1.29**
5	Group V – AC+D-galactosamine	400 mg/kg+(400 mg/kg)**	69.23±1.42**	83.71±2.47**	20.75±1.61**	1.83±0.12**	7.21±1.02**

n=6; values were expressed mean±SEM; Group II was compared to Group I. Groups III to V were compared to Group II. *p<0.01 versus D-galactosamine group: Significant; **p<0.001 versus D-galactosamine group: Highly significant data were analyzed by one-way ANOVA followed by Dunnett's t-test. SEM: Standard error of mean.
SGOT: Serum glutamic oxaloactetic transaminase, SGPT: Serum glutamic pyruvic transaminase, ALP: Alkaline phosphatase

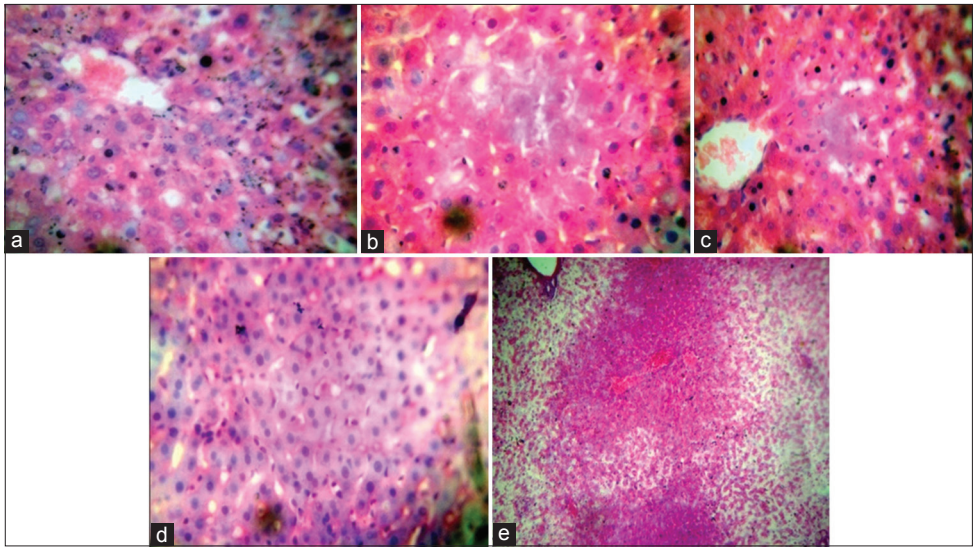

Fig. 1: Histopathological studies of liver (paracetamol induced), (a) Group I, (b) Group II, (c) Group III, (d) Group IV, (e) Group V

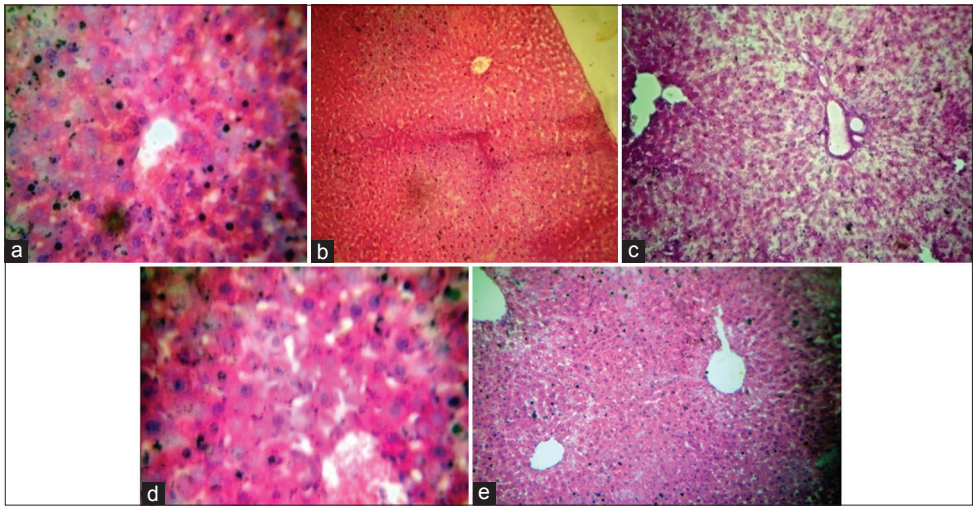

Fig. 2: Histopathological studies of liver (D-galactosamine-induced), (a) Group I, (b) Group II, (c) Group III, (d) Group IV, (e) Group V

and antipyretic drug and is safe in therapeutic doses but produces fatal hepatic necrosis with toxic doses [6]. The toxic effect of paracetamol is due to oxidative damage induced by its metabolite, N-acetyl-p-benzoquinoneimine, produced by the action of cytochrome P-450 in the liver. This metabolite reacts with reduced glutathione (GSH) to yield non-toxic 3-GS-yl-PCM. Depletion of GSH causes the remaining quinone and other natural endogenous oxygen species to bind to cellular macromolecules leading to cell death [7]. D-galactosamine is a well-established hepatotoxicant, which is widely used model which closely resembles human viral hepatitis in its morphologic and functional characteristics therefore considered very useful for evaluation of hepatoprotection [8,9]. D-galactosamine hepatotoxicity is considered as an experimental model of acute hepatitis although it does not affect other organs [10]. D-galactosamine is known to selectively block the transcription and indirectly hepatic protein synthesis and as a consequence of endotoxin toxicity, it causes fulminant hepatitis. The toxicity of D-galactosamine results from inhibition of RNA and protein synthesis in the liver. The metabolism of D-galactosamine may deplete several uracil nucleotides including uridine diphosphate (UDP)-glucose, UDP-galactose and uridine triphosphate deficiency, which trapped in the formation of uridine-diphosho galactosamine. Accumulation of UDP-sugar nucleotide may contribute to the change in the rough endoplasmic reticulum and to the disturbance of protein metabolism. Intense galactosamine of the membrane structures is thought to be responsible for loss in the activity of ionic pumps. The impairment in the calcium pumps, with consequent increase in the intracellular calcium is considered to be responsible for cell death [11-14].

This study shows that hepatic injury induced by paracetamol and D-galactosamine caused a significant rise in marker enzymes serum glutamic oxaloactetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), and total bilirubin. The serum enzymes such as SGOT, SGPT, ALP, and total bilirubin of treated animals were significantly reduced ($p<0.01$) by 28 days pretreatment of ethanolic extract of leaves of *A. communis* at two dose levels 200 and 400 mg/kg p.o, when compared with paracetamol and D-galactosamine-treated control (Group II). From the result, it is clear that the drugs show dose-dependent activity. Histopathological observation also revealed that pretreatment with AC protected the animals from paracetamol and D-galactosamine-induced liver damage. The results indicate that the leaves of *A. communis* possess the hepatoprotective activity.

CONCLUSION

From the present work, we conclude that species of *A. communis* are highly potential in biological activity. The preliminary screening of the samples revealed the presences of high-value class of compound like the phenolic group as the major content in the plants.

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