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Hepatoprotective activity of *Cassia alata(Linn.)* leaves against paracetamol-induced hepatic injury in rats

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ABSTRACT

The present study was undertaken to investigate the hepatoprotective activity of alcoholic extract of *Cassia alata* (Linn.) leaves against paracetamol-induced hepatic damage. The activity was tested in wistar albino rats at dose level of 200 & 400 mg/kg, orally and compared with silymarin (100 mg/kg) as standard. Alteration in the levels of biochemical markers of hepatic damage like SGPT, SGOT, ALP, bilirubin, cholesterol, HDL and tissue GSH were tested in both treated and untreated groups. Paracetamol (2 g/kg) has enhanced the SGPT, SGOT, ALP, bilirubin and cholesterol levels and reduced the serum levels of HDL and tissue level of GSH. Treatment with alcoholic extract of *Cassia alata* leaves (200 mg/kg and 400 mg/kg) has brought back the altered levels of biochemical markers to the near normal levels in the dose dependent manner, indicating that *Cassia alata* (Linn.) has a significant Hepatoprotective effect.

Keywords*:* *Cassia alata*; Paracetamol; Hepatoprotective

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INTRODUCTION

The liver is a vital organ of paramount importance involved in the maintenance of metabolic functions and detoxification from the exogenous and endogenous challenges, like xenobiotics, drugs, viral infections and chronic alcoholism. If during all such exposures to the above mentioned challenges the natural protective mechanisms of the liver are overpowered, the result is hepatic injury.

Liver damage is always associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue GSH levels. In addition serum levels of many biochemical markers like SGOT, SGPT, triglycerides, cholesterol, bilirubin, alkaline phosphatase, are elevated [1,2].

In spite of phenomenal growth of modern medicine, there are no synthetic drugs available for the treatment of hepatic disorders. However there are several herbs/herbal formulations claimed have possess beneficial activity in treating hepatic disorders. Herbs play important role in the management of various liver disorders [3]. In one of our field survey we found that a wildly grown plant *Cassia alata* which was claimed to possess hepatoprotective property. It was found that this plant contains flavonoids [4], alkaloids [5], Antraquinone derivative,tannins, sterols, triterpenes. From literature it was found that leaves possess analgesic [6], antimicrobial [7], antitumour properties and antidiabetic activity [8]. A number of reports indicate that overdose of paracetamol can produce centrizonal hemorrhagic hepatic necrosis in humans and experimental animals. The present study was conducted to evaluate the hepatoprotective activity of the alcoholic extract of the *Cassia alata* leaves against paracetamol-induced hepatic injury in rats.

MATERIALS & METHODS

The plant *Cassia alata* Linn. was collected from Salem district, Tamil-Nadu. The Botanist Mr. Kumresh, M.Sc.M.Phil taxonomically identified the plant material. The plant material dried under shade and then powdered with a mechanical grinder. The dried powdered of leaves of *Cassia alata* Linn. were defatted with petroleum ether (60-80^0 C) in a Soxhlet apparatus. The defatted powder material thus obtained was further extracted with chloroform, acetone, ethanol and aqueous solvents. Based on the phytochemical studies alcohol extract was selected for the present study.

Drug formulation

Oral suspension containing 200 & 400 mg/ml of the alcoholic (70%) extract were prepared in 1% w/v carboxy methyl cellulose (CMC).

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Animals

Wistar albino rats (150-200g) of either sex were used in this investigation. They were maintained at standard housing conditions and fed with commercial diet (Hindustan lever ltd., Bangalore) and provided with water *ad libitum* during the experiment. Approval from the institutional animal ethical committee (Reg.no 1092/ac/07/CPCSEA) for the usage of animals in the experiments was obtained.

Hepatoprotective activity

The acute toxicity studies were carried out as per staircase method [9]. Fifty rats were divided into five groups of 10 each and were administered with aliquot doses of the extracts orally. Mortality was noticed and LD50 of the extracts was found to be 2000 mg/kg body weight. One-tenth of this dose was selected as the therapeutic dose for the evaluation [10].

Grouping of animals

The method of Chattopadhyay [11] was used in the study. The experiment design of the investigation was carried out in 5 groups with 6 animals each group in the following regimen

Group I (Control) : Received the vehicle 1% w/v CMC at a dose of 1 ml/kg p.o for one week.

Group II (Paracetamol) : Received the vehicle 1% w/v CMC at a dose of 1 ml/kg p.o for one week + paracetamol 2 g/kg s.c on fifth day of study week.

Group III (Standard) : Received Silymarin (Ranbaxy Lab. Dewas) at a dose of 100 mg/kg/day p.o for one week + paracetamol 2 g/kg s.c on fifth day of study week.

Group IV (Test-1) : Received alc. extract of cassia alata leaves in the dose of 200mg/kg p.o for one week + paracetamol 2 g/kg s.c on fifth day of study week.

Group V (Test-2) : Received alc. extract of cassia alata leaves in the dose of 400mg/kg p.o for one week + paracetamol 2 g/kg s.c on fifth day of study week.

Assessment of hepatoprotective activity

All the animals were killed on day 7 under light ether anaesthesia. The blood samples were collected separately by carotid bleeding into sterilized dry centrifuge tubes and allowed to

coagulate for 30 min at 37^0 C. The clear serum was separated at 2500 rpm for 10 min and biochemical investigations were carried out to assess liver function viz. SGPT [12], SGOT [13], ALP [14], biluribin, [15] cholesterol [16], and HDL [17] levels. The tissue GSH was evaluated according to Ayake et al. [18]. These results were tabulated in Table-1 & 2.

Statistical analysis

The results are expressed as mean±SEM, (N=6). Statistical significance was determined by one-way analysis of variance with $P<0.05$ considered significant. The analysis was performed by Prism software.

RESULTS AND DISCUSSION

Paracetamol has enhanced the levels of SGPT, SGOT, bilirubin (both total and direct bilirubin levels), alkaline phosphatase level (ALP), total cholesterol, whereas HDL and tissue GSH levels are decreased significantly. Treatment with silymarin and 200 mg/kg and 400 mg/kg of cassia alata leaves extract has significantly brought down the elevated levels of SGPT, SGOT, ALP, bilirubin, cholesterol and also significantly enhanced the decreased levels of tissue GSH and HDL. Results are reported in Tables 1 and 2.

Paracetamol is normally eliminated mainly as sulfate and glucoronide. Only 5% of the paracetamol is converted into N-acetyl-p-benzoquineimine. However, upon administration of toxic doses of paracetamol the sulfation and glucoronidation routes become saturated and hence, higher percentage of paracetamol molecules are oxidized to highly reactive N-acetyl-p-benzoquineimine (NAPQI) by cytochrome-450 enzymes. Semiquinone radicals, obtained by one electron reduction of NAPQI, can covalently binds to macromolecules of cellular membrane and increases the lipid peroxidation resulting in the tissue damage. Higher dose of paracetamol and NAPQI can alkylate and oxidize intracellular GSH and protein thiol group, which results in the depletion of liver GSH pool subsequently leads to increased lipid peroxidation and liver damage [19]. In our experiments it is observed that tissue GSH levels in the paracetamol group is decreased to the extent of around 65%. This clearly indicates that there is a significant hepatic damage due to paracetamol. This is further evident from the fact that there is elevationn in the levels of various biochemical markers of hepatic damage like SGPT, SGOT, bilirubin, and cholesterol. Treatment with silymarin and alc.ext of cassia alata has increased tissue GSH level and the elevated levels of above mentioned biochemical markers to the near healthy levels. The treatment has also demonstrated the reduced hepatic damage or improvement in the hepatic architecture (data not shown).

It may be concluded that the hepatoprotective effect of cassia lata leaves is due to the prevention of the depletion in the tissue GSH levels. The exact hepatoprotective mechanism of this herbal drug is not known. The phytochemical studies revealed the presence of flavonoids in test extract. Various flavonoids have been reported for their hepatoprotective activity [20] [21]. So, the hepatoprotective effect of leaves of cassia alata may be due to their flavonoids content.

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Table No.1: Effect of alcoholic extract of *Cassia alata* leaves on biochemical parameters in paracetamol-induced hepatic injury in rats.

Treatment	SGPT U/l	SGOT U/l	ALP U/l	Total bilirubin mg/dl	Direct bilirubin mg/dl	Cholesterol mg/dl	HDL mg/dl
Control (saline 1ml/kg)	68.57±0.42	131.76±0.43	135.57±1.05	0.92±0.01	0.25±0.01	103.42±2.71	47.14±1.60
Paracetamol (PCM, 2 g/kg s.c)	281.18±0.65	403.16±1.15	436.33±1.33	3.42±0.11	0.69±0.01	157.85±1.84	28.47±0.91
Silymarin (100 mg/kg)+PCM	74.57±0.61*	135.26±0.96*	166.35±0.70*	1.04±0.03*	0.26±0.01*	116.19±2.43*	45.12±1.59*
Alcoholic extract of *Cassia alata* (200 mg/kg)+PCM	161.28±0.94*	285.16±0.56*	286.43±1.27*	1.45±0.06*	0.38±0.01*	136.34±1.95*	33.41±1.62(NS)
Alcoholic extract of *Cassia alata* (400 mg/kg)+PCM	86.86±0.63*	152.35±0.60*	181.65±1.00*	1.07±0.04*	0.31±0.01*	118.69±1.73*	41.25±0.97*

N=6, *P<0.01 vs PCM group, NS non significant.

Table No.2

Effect of alcoholic extract of *Cassia alata* leaves on the liver tissue levels of GSH in the paracetamol-induced hepatic injury in rats.

Group	**Dose mg/kg (p.o.)**	**Tissue levels of GSH Absorbance 412 nm**	**% Increase**
Saline	1ml	0.932±0.06	-
Paracetamol (PCM)	2gm/kg(s.c)	0.316±0.03	-
Silymarin+PCM	100 mg/kg	0.564±0.09*	78.74
Alc.ext of *Cassia alata* +PCM	200 mg/kg	0.452±0.03*	43.17
Alc.ext of *Cassia alata* +PCM	400 mg/kg	0.544±0.04*	72.2

N=6, *P<0.01 vs PCM group

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CONCLUSION

In conclusion, our study demonstrates that the leaves extract of *Cassia alata* (Linn.) can be effective in treatment against liver injury.

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