

Hepatoprotective activity of the infusion of the dried leaves of *Cassia alata* Linn.

RAMASAMY ANANDAN^{1*}, BALASUNDARAM JAYAKAR¹ and RAJAPPAN MANAVALAN²

¹Vinayaka Mission's College of Pharmacy, Salem- 636 008 (India).

²Department of Pharmacy, Annamalai University, Chidambaram, Tamilnadu (India).

(Received: March 02, 2009; Accepted: April 12, 2009)

ABSTRACT

Hepatoprotective activity of the Infusion of the dried leaves of *Cassia alata* (ICA) was studied against Paracetamol induced hepatic injury in albino rats. Pretreatment of the Infusion (ICA) reduced the biochemical markers of hepatic injury like serum glutamate pyruvate transaminase (SGPT), serum oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), total bilirubin and gamma glutamate transpeptidase (GGTP). Histopathological observations also revealed that pretreatment with ICA protected the animals from paracetamol induced liver damage. The results indicate that the leaves of *C. alata* possess the Hepatoprotective activity. This property may be attributed to the flavonoids present in the leaves of *C. alata*.

Key words: *Cassia alata*, hepatoprotective, paracetamol, biochemical parameters, histopathological studies.

INTRODUCTION

Liver, the largest organ in the body, is being evolved to maintain the body's internal milieu and also protected itself from the challenges it faces during its functioning. Since it is involved in the biochemical conversions of various endogenous and exogenously administered substances, there is a possibility of generating various highly reactive species of free radicals. In spite of these free radicals generation, hepatotoxins like paracetamol overpower the protective mechanism of the liver and cause hepatic damage. Though the modern medicinal system has grown predominantly, the drug for treating hepatic disease is still a dream. Hence people are looking at traditional system of medicines for remedies to hepatic disorders.

Cassia alata Linn. (*Leguminosae*) is a shrub found throughout India, which is traditionally used by the tribes and native medicinal practitioners for the treatment of various ailments including asthma, ringworm, skin diseases, liver disorders and rheumatism¹. Literature review reveals that the plant possesses antiplasmodial, antimicrobial, anti-

inflammatory, larvicidal, antimutogenic, antifungal, analgesic and hypoglycemic activities²⁻⁴. The plant contains flavonoids, glycosides, tannins, phenolic compounds, sterols and terpenoids^{5,6}. However, there are no reports regarding the Hepatoprotective activity of the leaves of this plant. Preliminary phytochemical screening of the Infusion shows the presence of flavonoids. Flavonoids are reported to possess various properties including Hepatoprotective property⁷. The present study has been undertaken to screen for Hepatoprotective activity of the Infusion of the dried leaves of *Cassia alata* and to verify the claim using paracetamol induced hepatic injury model in rats.

MATERIAL AND METHODS

Plant materials

The leaves of *Cassia alata* were collected from Shervaroy's hills, Salem, Tamilnadu, India during the month of April 2006. The plant was identified and authenticated by the Botanist. A voucher specimen was kept in our laboratory for future reference (V/01/2006). The plant material was shade dried and pulverized.

Preparation of the Infusion

The Powdered plant material (50g) was macerated with 500ml of boiling water for 15 minutes. The entire mixture was allowed to cool, filtered (the marc was not pressed) and the filtrate was evaporated to dryness under reduced pressure to afford viscous residue (6.8 gm-13.6% w/w). Freshly prepared Infusion was used whenever it was necessary. The Infusion was suspended in 5% gum acacia and used for further experiments.

Animals

Swiss albino mice (20-25g) and male Wistar Rats (150-175 g) were procured from Venkateshwara Enterprises, Bangalore, Karnataka, India and used throughout the study. They were housed in microloan boxes in a controlled environment (temperature $25 \pm 2^\circ\text{C}$ and 12 h dark/light cycle) with standard laboratory diet and *ad libitum*. The study was conducted after obtaining Institutional Animal Ethical Committee's clearance (Protocol No.Pcol/02/2006 dated 28.01.2006).

Acute toxicity studies

Acute oral toxicity (AOT) of ICA was determined using Swiss albino mice. The animals were fasted for 3 h prior to the experiment and were administered with single dose of the Infusion dissolved in 5% gum acacia (doses ranges from 500-2000 mg/kg at various dose levels) and observed for mortality up to 48 h (short term toxicity). Based on the short-term toxicity, the dose of next animal was determined as per OECD guideline 425. All the animals were also observed for long-term toxicity (14 days). The LD_{50} of the test extract was calculated using 'AOT425' software provided by Environmental Protection Agency, USA.

Evaluation of Hepatoprotective activity

Five groups of animals (Male Wistar Rats) containing six each were used for the study. The animals from Group I served as the control and received the vehicle 5% w/v gum acacia at a dose of 1ml/kg/day, p.o. for 7 days. Group II animals were similarly treated as Group I. The standard drug Silymarin (Micro Labs, Silyban) was administered to Group III animals in the dose of 100mg/kg/day, p.o. for 7 days. Groups IV-V animals were treated with the ICA with the doses of 200-400mg/kg/day,

p.o. for 7 days respectively. The Paracetamol, Silymarin and the Infusion (ICA) were administered regularly to the respective groups of animals. On the 7th day Paracetamol suspension in dose of 750mg/kg p.o.^{8,9} was administered to all the rats except rats of group I, 30 min after the administration of Silymarin and ICA. After 36 h of paracetamol administration, all the animals were killed under chloroform anesthesia. The blood samples were collected separately into the sterilized dry centrifuge tubes and allowed to coagulate for 30min and serum was collected. The separated serum was analyzed to assess various biochemical markers like serum glutamic pyruvate transaminase (SGPT), serum glutamic oxaloacetate transaminase (SGOT)¹⁰, alkaline phosphatase (ALP)¹¹, total bilirubin¹² and gamma glutamate transpeptidase (GGTP)¹³.

Statistical analysis

The mean values \pm SEM were calculated for each parameter. For determining the significant inter group difference each parameter was analyzed separately and one-way ANOVA was carried out. The individual comparisons of the group mean values were done by using Dunnett's procedure.

Histopathology

After draining the blood, the abdomen of each animal was cut opened and the liver sample was excised, washed with normal saline and processed separately for Histopathological observation. The ratio of wet liver weight was calculated. The livers were examined grossly, were fixed in 10% buffered neutral formalin for 48 hours and then with bovine solution for 6 hour. Paraffin section were taken at 5 μm thickness processed in alcohol-xylene series and was stained with alum hematoxylin and eosin¹⁴. The sections were examined microscopically for the Histopathological changes.

RESULTS AND DISCUSSION

Hepatoprotective activity of ICA was studied. For the acute oral toxicity studies, the ICA treated animals were observed for mortality up to 48 h (short term toxicity) and for long-term toxicity (14 days). Based on the results the ICA did not produce any mortality up to 2000mg/kg body weight. The results of the biochemical parameter revealed the elevation of biochemical markers like SGPT,

SGOT, ALP, bilirubin and GGTP in toxicant treated group indicating that Paracetamol induced damage to the liver. Pretreatment with ICA (200 and 400 mg/kg p.o) significantly reduced ($P<0.01$) the elevated levels of all the above mentioned biochemical indicators. The enzyme levels were almost restored to the normal (Table 1).

It was observed that the size of the liver was enlarged in paracetamol intoxicated rats but it was normal in ICA treated groups. A significance ($P<0.01$) in liver weigh variation supports the findings (Table 2).

Histopathological examinations of the liver section of the rats treated with paracetamol showed

an intense centrilobular hepatocytes characterized by nuclear pyknosis and eosinophilic cytoplasm followed by large excessive hepatic lesion. Paracetamol toxicity is due to the formation of its toxic metabolite (an oxidative product) N-acetyl-p-benzoquinone-imine through the action of cytochrome P450. The covalent bonding of N-acetyl-p-benzoquinone-imine to sulphhydryl group of protein, resulting in lipid peroxidative glutathione level, thereby produces cell necrosis in the liver¹⁵. The rats treated with Silymarin and ICA showed a good sign of protection against the toxicant to considerable extent as it was evident from the formation of normal hepatic cords and absence of hepatic lesions.

Table 1: Effect of ICA of the dried leaves of Cassia alata on biochemical markers of Paracetamol induced hepatic injury in Rats

Design of treatment	SGPT U/I	SGOT U/I	ALP U/I	Total bilirubin mg/ml	GGTP U/I
Control (Vehicle 1ml/kg/day,p.o.)	78±1.10	118±2.12	138±1.72	0.75±0.03	126±0.97
Paracetamol (750mg/kg. p.o)	374±3.56a	356±2.95a	438±3.86a	2.42±0.17a	264±2.65a
Silymarin (100ml/kg/ a.cday,p.o.)	85±1.08b.c	121±1.46c	146±1.06a.c	0.8±0.03a,c	138±1.29
ICA(200mg/kg/ cday,p.o.)	92±0.89a,c	194±1.17a,c	238±2.14a,c	0.84±0.04a,c	198±1.56a,
ICA(400mg/kg/ a.cday,p.o.)	88±1.75a.c	163±1.39a.c	194±1.32a.c	0.82±0.02a,c	164±2.10

N=6; Values were expressed as mean ± SEM; a $P<0.01$; b $P<0.05$ Vs Control c $P<0.01$ Vs Paracetamol; Data were analyzed by one way ANOVA followed by Dennett's procedure.

The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effects or restoring the normal hepatic physiology, which has been distributed by hepatotoxins.

The Silymarin and the Infusion of the dried leaves of Cassia alata significantly decreased the Paracetamol induced elevated level of enzymes in the treatment groups, indicating the enhancement of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells by the extract. Decrease in the bilirubin after treatment with ICA

Table 2: Effect of ICA on average liver weight of treated animals

Design of treatmentweight	Liver weight / 100g of body
Control(Vehicle 1ml/kg/day,p.o)	2.51+0.42
Paracetamol(750mg/kg,p.o)	4.96+0.73a
Silymarin(100mg/kg/day,p.o.)	2.64+0.18b
ICA(200mg/kg/day,p.o.)	2.93+0.14b
ICA(400mg/kg/day,p.o.)	2.68+0.30b

N=6; Values were expressed as mean ± SEM a $P<0.01$ Vs Control, b $P<0.01$ Vs Paracetamol Data were analyzed by One way ANOVA followed by Dunnett's Procedure

indicated the effectiveness of the extract in the normal functional status of the liver. Histopathological analyses were good in agreement with the biochemical changes.

The chemical constituents of *Cassia alata* responsible for their hepatoprotective activity are not known. Preliminary phytochemical studies and literature review revealed the presence of flavonoids in ICA. Flavonoids are reported to possess antioxidant and hepatoprotective properties^{16,17}. The hepatoprotective activity of the leaves of *Cassia alata* may be assigned to flavonoids, However,

further studies are needed for confirmation.

In conclusion, the present study demonstrated that the Infusion of the dried leaves of *Cassia alata* possesses hepatoprotective activity. In addition, the hepatoprotective property may be attributed to the active principles of the plant namely, Flavonoids, tannins and other polyphenolic compounds. Further study is warranted to isolate, characterize and screen the active principles from the leaves of *Cassia alata* that possess hepatoprotective activity.

REFERENCES

1. Kirtikar, K.R., and Basu, B.D. Indian medicinal plants, International book publishers, Dehradun, **3**: 870-871 (1993).
2. Moriyama, H., Iizuka, T., Nagai, M., Miyataka, H., and Satoh, T. "Antiinflammatory activity of heat-treated *Cassia alata* leaf extract and its flavonoids glycoside." *Yakugaku Zasshi*, **123**(8): 607-11 (2003).
3. Somchit, M.N., Reezal, I., Nur, I.E., and Mutalib, A.R. "In vitro antimicrobial activity of ethanolic and water extracts of *Cassia alata*." *J. Ethnopharmacol*, **84**(1): 1-4 (2003).
4. Villasenor, I.M., Canlas, A.P., Pascua M.P., Sabando, M.N., and Soliven, L.A. "Bioactivity studies on *Cassia alata* Linn. Leaf extracts." *Phytother. Res, Suppl 1*: S93-6 (2002).
5. Moriyama, H., Iizuka, T., Nagai, M., and Murata, Y. "HPLC quantification of keampferol-3-O-gentiobioside in *Cassia alata*." *Fitoterapia*, **74**(5): 425-30 (2003).
6. Yadav, S.K., and Kalidhar, S.B. "Alquinone: An Anthraquinone from *Cassia alata*." *Planta Med*, **60**(6): 601 (1994).
7. Allan, L and Miller, N.D. "Antioxidant flavonoids: Structure, function and clinical usage." *Alternative Med. Rev*, **1**: 111 (1996).
8. Hiroshi, A., Toshiharu H, Masahiro H., and Shoji A., *J. Pharma Pharmacol*, **39**: 1047 (1987).
9. Kapur, V., Pillai, K.K., Hussian, S.Z and balani, D.K *Indian J. Pharmacol.*, **26**: 35 (1994).
10. Reitman, S and Frankel, S. "A colorimetric method for determination of serum glutamate oxaloacetate and glutamic pyruvate transaminase." *Amer. J. Clin. Path.*, **28**: 56-58 (1957).
11. Kind, P.R and king, E.J. "Estimation of plasma phosphatase by determination of hydrolysed phenol with antipyrin." *J. Clin. Path.*, **7**: 322-326 (1954).
12. Mallay, H.T and Evelyn, E.A. "Estimation of serum bilirubin levels with the photoelectric colorimeter." *J. Biol. Chem.*, **119**: 481-484 (1937).
13. Szaszi, G. "A kinetic photometric method for serum gamma glutamyl transpeptidase." *Clin. Chem.*, **15**: 124-126 (1969).
14. Galigher, A.E and Kozloff, E.N. Essential practical Microtechnique, 2nd edition, Lea and Febiger, *Philadelphia*, 77 (1971).
15. Kapur, V., Pillai, K.K., Hussian, S.Z and balani, D.K *Indian J. Pharmacol.*, **26**: 35 (1994).
16. Allan, L and Miller, N.D. "Antioxidant flavonoids: Structure, function and clinical usage." *Alternative Med. Rev*, **1**: 111 (1996).
17. Wegener, T and Fintelmann, V. "Flavonoids and Bioactivity." *Wein Med. Wochem Schr.*, **149**: 241-247 (1999).