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Research Article

HEPATOPROTECTIVE ACTIVITY OF VARIOUS EXTRACTS OF *Cayratia carnosa* (Wall. Ex Wight) Gagnep. IN PARACETAMOL INDUCED HEPATOTOXICITY IN ALBINO RATS

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ABSTRACT

Objective: In the present study, the antihepatotoxic activity of the various extracts of the whole plant of *Cayratia carnosa* was evaluated using paracetamol induced hepatotoxicity in albino rats.

Method: The degree of protection against liver toxicity was determined by measuring the serum biochemical parameters viz. SGPT (serum glutamic pyruvate transaminase), SGOT (serum glutamine oxalo acetate transaminase), ALP (serum alkaline phosphatase) and Bilirubin (Total, Direct and Indirect). In addition, histopathological examination of the liver was also studied. Silymarin at the dose of 25 mg/kg, p.o. was used as reference standard drug and it exhibited significant hepato-protection.

Result: Ethanolic extract at 400 mg/kg, which was comparable to that of standard drug silymarin while the aqueous extract 200 mg/kg treated animals exhibited the least hepato-protective activity.

Conclusion: The results obtained in this study provide a scientific support to the traditional folkloric use of *Cayratia carnosa* as a hepato-protective agent.

Keywords: *Cayratia carnosa*, Hepato-protective, Serum marker enzymes, Histopathology

INTRODUCTION

Liver is the most important organ concerned with metabolic activities[1]. It plays a major role in detoxification and excretion of many endogenous and exogenous compounds. Any injury to it or impairment of its functions may lead to implications on one's health[2,3]. Living in the world of inadequately controlled environmental pollution and use of potent drugs has made the liver to get exposed to a variety of xenobiotics and therapeutic agents. Thus the disorders associated with this organ are numerous and varied[4]. Heavy metal toxicity has been shown to affect almost every organ system of the body with continued exposure[5]. Management of liver diseases is still a challenge to modern medicine. Modern medicine has little to offer for alleviation of hepatic ailments.

Most of the hepato-protective agents now available are expensive and hence a genuine need is felt to devise some cost effective drugs based on plant principles in this regard[6]. Numerous medicinal plants and various formulations are used for liver disorders in ethno medical practices as well as in traditional systems of medicine in India[7]. Many plants possess hepato-protective activity against carbon tetrachloride, ethanol, paracetamol, anti tubercular drugs and galactosamine induced liver damage in albino rats and hence a similar study mentioned is presented in this study.

Cayratia carnosa (Wall. Ex Wight) Gagnep. (Vitaceae) is a small fleshy tendril climber: tendrils short, slender and branched; leaves trifoliate, usually pubescent, leaflets dentate; flower buds globose; fruits fleshy berries; seeds pyriform or triangular, rounded and rugose on the back. The plant grows wildly throughout the Western Ghats, in India. The whole plant is used for tumors, fever, neuralgia and splenomegaly. It purifies the blood and is given for liver diseases, cardiac disorders, ulcers, wounds, dropsy and hemorrhoids. During the present study, an herbal drug *Cayratia carnosa* is chosen for the evaluation of hepato-protective activity which could not be attempted by any researcher earlier.

MATERIALS AND METHODS

Plant materials

The whole plant of *Cayratia carnosa* was collected for the study in the month of August, the flowering season period, from the wild source in the plains area of Ulloor of Thiruvananthapuram city, Kerala. *Cayratia carnosa* was authenticated by Dr. P. Brindha, Botanist, Department of Botany, Captain Srinivasa Murti Drug Research Institute for Ayurveda, Arumbakkam, Chennai, Tamilnadu, India.

Preparation and selection of the extracts

The whole plant of *Cayratia carnosa* after collection was washed in running tap water to remove the soil and adhering materials, shade dried, coarsely powdered and packed in the soxhlet extractor. The packed material was extracted successively with the following solvents: Petroleum ether, benzene, chloroform, acetone, ethanol and water. Total aqueous extract was also prepared separately by cold maceration method[8]. These extracts were filtered, distilled and concentrated. The concentrated extracts were dried under *vacuum* in desiccators containing anhydrous calcium chloride. Since the ethanol, aqueous and total aqueous extracts contains the maximum number of phytoconstituents, these extracts alone were selected for the pharmacological activity.

Animals

Male albino rats of Wistar strain was used for hepato-protective activity. These animals were taken from the inbreed colony maintained in the animal house of our Institution. Male albino rats weighing 220 to 250g were used for the study. These animals were fed with pelleted diet manufactured by Amrut laboratory animal feed company, Sangli, Maharashtra and drinking water *ad libitum*. They were kept in 12hrs/12hrs light/ dark cycle for acclimatization towards the laboratory conditions before starting the experimental procedure. Ethical clearances for performing the experiments on animals were obtained from SB College of Pharmacy, Instituitional Animal Ethical Committee with reference no: IAEC/SBCP/2004-2005/F.7 (f) /252a dated 23/06/2004.

Paracetamol Induced Hepato Toxicity in Albino Rats

Hepato-protective activity was evaluated against paracetamol induced hepatic damage. Paracetamol toxicity is due to the formation of toxic metabolites (N-acetyl-p-benzoquinoneimine) through the action of cytochrome P_{450}. Induction of cytochrome P_{450} – depletion of hepatic glutathione is a prerequisite for paracetamol induced hepatotoxicity[9]. Liver tissue, rich in both transaminases (SGOT and SGPT) contains more SGPT and SGOT while both transaminases are elevated in sera of patients with acute hepatic

diseases. SGPT which is only slightly elevated by cardiac necrosis is a more specific indicator of liver damage[10].

Paracetamol is a well-known antipyretic and analgesic which produces hepatic necrosis at higher doses. Indiscriminate ingestion can lead to accidental poisoning and potentially lethal hepato-toxicity. Its mode of action in liver is by covalent binding of its toxic metabolite n-acetyl p-benzoquinoneimine to tissue macromolecules, resulting in cell necrosis[11].

Damage to the structural integrity of liver is reflected by an increase in the levels of serum transaminases, because they are cytoplasmic in location and are released into the circulation after cellular damage[12,13]. Protection against paracetamol induced toxicity has been used as a test for a potential hepatoprotective agent by several investigators[14,15].

Hepato-Protective Activity of *Cayratia carnosa*

Male albino rats of Wistar strain were selected and divided into nine groups of 6 animals each. They should be treated for seven days as follows:

Group-I = Normal control (Normal saline 10 ml/kg)

Group-II = Paracetamol 2.5 kg/kg.

Group-III = Silymarin (25 mg/kg)

Group-IV = Ethanolic extract 200 mg/kg

Group-V = Aqueous extract 200 mg/kg

Group-VI = Total Aqueous extract 200 mg/kg

Group-VII = Ethanolic extract 400 mg/kg

Group-VIII = Aqueous extract 400 mg/kg

Group-IX = Total Aqueous extract 400 mg/kg

On the seventh day paracetamol suspension (2.5 g/kg) in 0.5% C.M.C. was given by oral route to all the animals except animals in Group 1. This dose is known to cause liver damage in albino rats[16]. After 36 hours, all the animals were sacrificed under ether anesthesia; blood was collected by cardiac puncture in sterile centrifuge tubes and allowed to clot. Serum was separated by centrifuging at 2500 rpm for 10 min and used for the estimation of SGOT, SGPT[17], alkaline phosphatase[18] and serum bilirubin[19] levels.

Histopathological examination

After the animals were sacrificed, the abdomen was cut open and the liver was removed. The ratio of wet liver weight *per* 100 g of body weight was calculated and recorded[20]. The liver excised was washed with normal saline. Initially the materials were fixed in 10% buffered neutral formalin and then with bovine solution. They were processed for paraffin embedding following the microtome technique[21]. The sections were processed in alcohol, xylene series and were stained with alum haematoxylin and eosin. The stained sections were examined microscopically for histopathological changes.

Statistical Analysis

All the values are expressed as Mean ± SEM. The data obtained from hepato-protective study was subjected to one-way ANOVA followed by Dunnett's test for statistical significance. $P<0.05$ is considered to be statistically significant.

RESULTS AND DISCUSSION

Effect of *Cayratia carnosa* on paracetamol induced hepato-toxicity in albino rats

The results of the hepato-protective activity are presented in **Table-1**. Paracetamol causes significant increase in the activity of serum transaminases, alkaline phosphatase and bilirubin levels. Results indicate that various test extracts of *Cayratia carnosa* provides significant protection against the paracetamol induced toxic effect on liver. In paracetamol induced toxic hepatitis, toxicity begins with the changes in endoplasmic reticulum, which results in the loss of metabolic enzymes located in intra cellular structures[22]. The blood samples of the paracetamol alone treated animals showed drastic increase in the levels of liver weight (5.05 g), SGOT (63.5 units/ml), SGPT (47 units/ml), alkaline phosphatase (29 units/ml) and total bilirubin (1.5 mg/dl) as compared to control group showing liver weight (2.7 g), SGOT (25 units/ml), SGPT (21 units/ml), alkaline phosphatase (7.5 units/ml) and total bilirubin (0.35 mg/dl). Elevation of serum enzyme and bilirubin levels is considered as an index of liver damage[23,24,25] and liver necrosis. Histopathological sections of the paracetamol treated animals showed focal necrosis and portal inflammation as compared to the intact arrangement of cells of the control animal.

Administration of silymarin and various extracts of *Cayratia carnosa* showed hepatoprotective effect against the toxic effects of paracetamol as shown in the **Table-1**. Among these extracts significant hepato-protection was noticed in the Group-7 animals treated with ethanolic extract at 400 mg/kg, which was comparable to that of standard drug silymarin while the aqueous extract 200 mg/kg treated animals exhibited the least hepato-protective activity. Histopathological profile of all the test extracts treated animals showed recovery against the paracetamol induced necrosis in their normal compact arrangement of hepatic cells as compared to control.

Table 1: Hepatoprotective activity of various extracts of *Cayratia carnosa* on paracetamol induced hepatotoxicity in albino rats

Gr. No	Drug Treatment	Dose (mg/kg)	Liver weight g/100g body wt	SGOT units/ml (8-20)	SGPT units/ml (5-40)	Alkaline phosphatase KA.Units/ml (5-13)	Total Bilirubin mg/dl (0.2-0.55)	Direct Bilirubin mg/dl	Indirect Bilirubin mg/dl
1	Control (Saline)	10ml/kg	2.70±0.062	25±1.29	21±1.29	7.5±0.866	0.35±0.065	0.125±0.017	0.225±0.039
2	Paracetamol	2.5g/kg	5.05^{a}±0.124	63.5^{a}±1.707	47^{a}±1.29	29^{a}±1.291	1.5^{a}±0.129	0.095^{d}±0.026	1.405^{b}±0.123
3	Silymarin	25	3.15^{a}±0.053	25.5^{ns}±1.5	21.5^{ns}±0.957	6.5^{d}±0.5	0.375^{ns}±0.063	0.1^{d}±0.085	0.275^{ns}±0.058
4	Ethanolic extract	200	4.01^{b}±0.095	31^{b}±1.290	25^{b}±1.29	9.25^{b}±0.479	0.65^{b}±0.029	0.095^{d}±0.033	0.555^{d}±0.099
5	Aqueous extract	200	4.63^{a}±0.007	33.5^{a}±0.957	26^{c}±0.816	10.25^{b}±0.479	0.8^{a}±0.408	0.15^{d}±0.014	0.65^{d}±0.047
6	Total aqueous extract	200	4.35^{a}±0.045	32^{a}±0.816	25.5^{d}±0.957	9.5^{d}±0.289	0.7^{a}±0.408	0.085^{ns}±0.018	0.615^{d}±0.071
7	Ethanolic extract	400	3.51^{d}±0.030	27^{d}±1.290	22^{ns}±0.816	7.25^{ns}±0.479	0.45^{d}±0.064	0.11^{ns}±0.013	0.34^{ns}±0.331
8	Aqueous extract	400	3.84^{b}±0.048	13^{c}±1.826	24^{ns}±0.816	8.5^{d}±0.866	0.6^{c}±0.041	0.12^{ns}±0.041	0.475^{d}±0.059
9	Total aqueous extract	400	4.05^{c}±0.157	29^{d}±1.290	23^{d}±1.291	8^{ns}±0.408	0.55^{d}±0.065	0.125^{ns}±0.018	0.425^{ns}±0.057

i) [a]P<0.001, [b]P<0.01,[c]P<0.02,[d]P<0.05, n.s – non significant *Vs* Control

ii) Values are Mean ± S.E.M

iii) One way ANOVA followed by Dunnet's *t* - test.

iv) n=6 (Number of animals in each group)

v) Values in the parenthesis indicate the normal range of biochemical parameters.

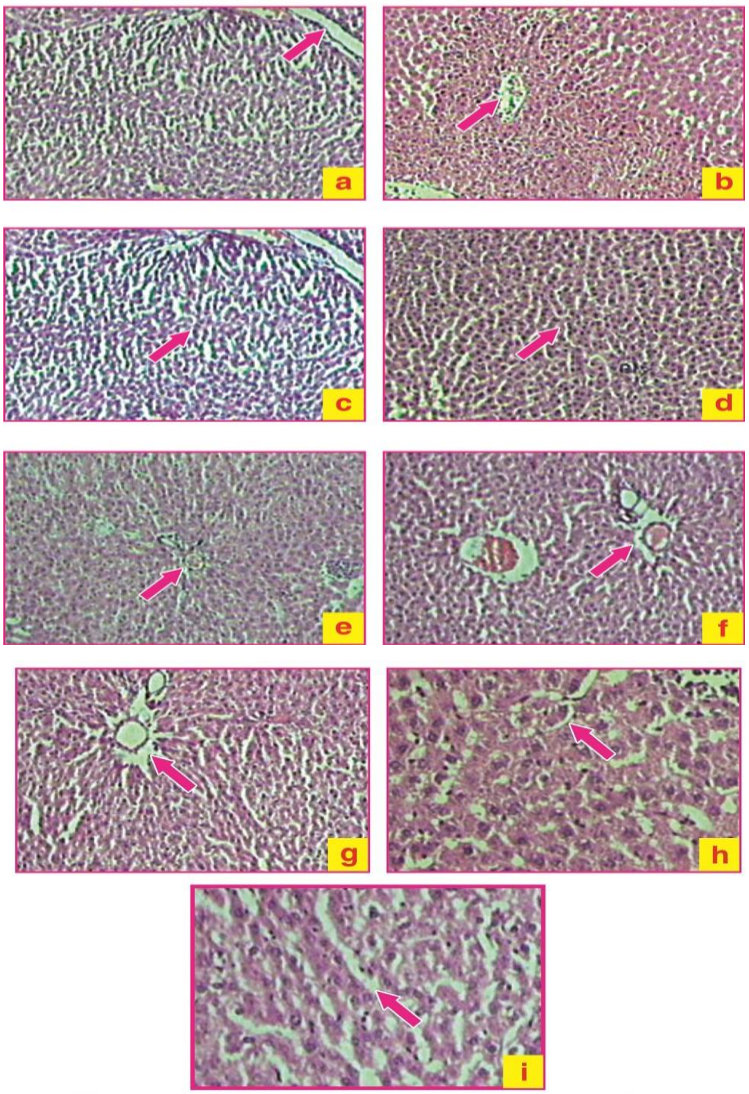

Fig. 1 a - i : Photomicrographs of liver section

Fig. 1 -a : Normal control rat : Section of liver showing normal architecture, hepatocytes and portal tracts.

Fig. 1 -b : Paracetamol treatment (2.5 g / kg.) : Section of liver showing portal triad with moderate fibrosis, inflammation, centrilobular fatty degeneration, cloudy swelling and necrosis of hepatic cells

Fig. 1 -c : Silymarin treated rat ; Section of liver showing normalcy of liver architecture, hepatocytes, central vein and portal triad.

Fig. 1 -d : Ethanolic extract 200mg/kg treated : Section of liver showing miled portal inflammation, spotty and focal necrosis with acidophil bodies.

Fig. 1 -e : Aqueous extract 200mg / kg treated : Section of liver showing a single focus of necrosis.

Fig. 1 -f : Total aqueous extract 200mg / kg treated : Section of liver showing normal architecture and spotty necrosis.

Fig. 1 -g : Ethanolic extract 400mg/kg treated : Section of liver showing normal architecture, hepatocytes, and portal tracts.

Fig. 1 -h : Aqueous extract 400mg / kg treated : Section of liver showing centrizonal necrosis. and a neutrophil infiltrate.

Fig. 1 -i : Total aqueous extract 400mg / kg treated : Section of liver showing normal architecture, hepatocytes, and portal tracts. There is mild sinusoidal dilation.

CONCLUSION

The results obtained in this study provide a scientific support to the traditional folkloric use of *Cayratia carnosa* as a hepato-protective agent. Further, isolation of active principles will be advantageous to produce novel bioactive constituents from these extracts which may possess more significance in the treatment of liver diseases and to elucidate its exact mechanism of action. Attempts are being made to isolate and characterize the active principle to which the hepato-protective activity can be attributed.

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