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Investigation of hepatoprotective activity of *Cyathea gigantea* (Wall. ex. Hook.) leaves against paracetamol-induced hepatotoxicity in rats

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ABSTRACT

Objective: To investigate the hepatoprotective activity of methanolic leaf extract of *Cyathea gigantea* (*C. gigantea*) against paracetamol induced liver damage in rats. **Methods:** The hepatoprotective activity for plant extract was investigated for paracetamol induced hepatoxicity in rats. Wistar albino rats of either sex were divided into five groups of 6 animals each and are given orally the following treatment for seven days. The normal control group was given 1% Na.CMC 1 mL/kg bw, *p.o.* Paracetamol at dose of 1 g/kg bw, *p.o.* was given as toxic dose for inducing hepatotoxicity. Silymarin (50 mg/kg, *p.o.*) was given as reference standard. Two doses of *C. gigantea* extract *i.e.*, 100 mg/kg, *p.o.* and 200 mg/kg, *p.o.* were tested for hepatoprotective activity. The treatment was given for seven days and after 24 h of last treatment blood was collected from retro-orbital plexus and analysed for various serum parameters like serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), total bilirubin (TB) and total protein (TP) in different groups. **Results:** The paracetamol intoxication lead to histological and biochemical deteriorations. The treatment with methanolic leaf extract of *C. gigantea* reduced the elevated levels of SGOT, SGPT, ALP, TB and also reversed the hepatic damage towards normal which further supports the hepatoprotective activity of leaf extract of *C. gigantea*. **Conclusions:** The methanolic extract of leaves of *C. gigantea* at doses of 100 mg/kg bw and 200 mg/kg bw have significant effect on liver of paracetamol induced hepatotoxicity model in rats.

1. Introduction

Cyathea gigantea (*C. gigantea*) (Wall. ex. Hook.) (Cyatheaceae) is a tree fern found extensively in moist open areas of Northeastern to Southern India, Thailand, Srilanka, Nepal and Western Java. The Cyatheaceae is the scaly tree fern family and includes the world's tallest tree ferns, which reach heights up to 20 m[1]. Traditionally the fresh rhizome of *C. gigantea* mixed with black pepper seeds powdered and taken orally with milk twice a day for one week in stomach against white discharges[2].

Liver is one of the important organs of the body which plays a major role in the metabolism of proteins, carbohydrates, lipids. It is also having wide range of functions including detoxification, storage of glycogen, vitamin A, D and B12, production of several coagulation factors, growth factors (IGF-1), hormones (angiotensinogen) and biochemicals necessary for digestion (bile). Hepatic damage occurs due to its multi dimensional functions, various xenobiotics and oxidative stress leading to distortion of all of its functions[3]. Another plant from the same genus *i.e.*, *Cyathea phalerata* Mart showed antioxidant and hepatoprotective activities[4].

C. gigantea have several active constituents like triterpenes, sterols, saponins, flavonoids, hentriacontane, β-sitostenone, β-sitostanone, diploterol, sitosterol, hopan-29-ol and whole plant contains oleanolic acid[5]. The first investigation on flavonoids constituents in the genus *Cyathea* was carried out by Harada *et al*[6]. Oleanolic acid is a triterpenoid having antitumor, hepatoprotective and antiviral activity[7,8]. Oleanolic acid is found to exhibit

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strong anti-HIV activity[9]. Dietary phytosterols like β-sitosterol is having anticancer activity[10].

Herbal drugs play a major role in the treatment of hepatic disorders. In the absence of reliable liver protective drugs in modern medicine, in India, a number of medicinal plants and their formulations are used to cure hepatic disorders in traditional systems of medicine[11]. Several studies were conducted in the field of drug discovery and development but due to the side effects of modern medicine, natural remedies are considered to be effective and safe alternate treatments for hepatotoxicity.

About 600 commercial formulations with claimed hepatoprotective activity are being sold all over the world. Around 170 phyto-constituents isolated from 110 plants belonging to 55 families have been reported to possess hepatoprotective activity. In India more than 94 medicinal plants are used in different combinations in the preparations of 40 patented herbal formulations. 74% were discovered as a result of chemical studies directed at the isolation of the active constituents of plants used in traditional medicine[12]. Paracetamol induced toxicity in rats is one of the widely used experimental model to evaluate the hepatoprotective nature of herbal extracts[13,14]. However, hepatoprotective activity of *C. gigantea* has not been demonstrated *in-vivo*. The degree of protection of 70% methanolic leaf extract of *C. gigantea* against paracetamol induced hepatotoxicity was evaluated in this study.

2. Materials and methods

2.1. Plant material

Fresh matured leaves of *C. gigantea* were collected in November 2009 from the hill station of Katika village, Andhra Pradesh, India. The Voucher specimens (BGR/PMK/CG-11-09) were deposited in the herbarium, A.U College of Pharmaceutical Sciences, Andhra University.

2.2. Chemicals

All the materials used for this experiment were of analytical grade. Paracetamol (E. Merck), silymarin (Sigma Chemical Co.,) and thiobarbituric acid (Sigma Chemical Co.,). Diagnostic kits for the estimation of serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP) and serum bilirubin were manufactured by Ranbaxy Diagnostics Ltd., New Delhi, India. Standard orogastric cannula was used for oral drug administration.

2.3. Test animals

The study was carried out on Wistar albino rats (160-200 g) of either sex (Mahaveer Enterprises, Hyderabad). They were allowed to take standard pellet food (National Institute of Nutrition, Hyderabad) and water *ad libitum*. Before experiment the rats were kept in standard environmental conditions with room temperature 25-27 ℃ relative humidity (55 ± 5)% and 12 h light/12 h dark cycle. All rats received humane care in accordance to the "Guide for the Care and Use of Laboratory Animals" (National Academies Press, Washington, DC, USA, 1996).

2.4. Preparation of plant extract

The freshly collected leaves were shade dried and pulverized. The powder (450 g) was treated with petroleum ether for the removal of chlorophyll and waxy material. Then it was air dried and macerated with (70:30) methanol:water, filtered and concentrated at 45 ℃ in Buchi rotavapor. The weight of methanolic exrtact obtained was 41 g (9.1% w/w yield).

2.5. Phytochemical screening

The preliminary phytochemical screening was done by following standard qualitative chemical tests for phytoconstituents and the methanolic leaf extract of *C. gigantea* on preliminary phytochemical screening showed the presence of triterpenes, sterols, saponins and flavonoids.

2.6. Acute toxicity studies

The acute toxicity studies were performed as per OECD-425 guidelines. Selected five Wistar albino rats of uniform weight are taken. One animal was fasted overnight with access to drinking water. They were given 2 000 mg/kg of the test exract and are observed for 24 h for mortality. The animal survived and then four additional animals were tested sequentially so that a total of five animals were tested. All the animals were observed closely for 24 h and daily for 14 days, no mortality was observed. Hence, I selected 200 mg/kg (1/10th of 2 000 mg/kg) as maximum safety dose with descending dose levels with 2 fold interval *i.e.*, 100 mg/kg and 200 mg/kg body weight of the test animal.

2.7. Paracetamol-induced hepatotoxicity

Paracetamol (acetaminophen), a widely used analgesic-antipyretic drug on accidental overdosage (which may occur in alcoholics and elderly), produces acute hepatic damage. The covalent binding of an oxidation product of paracetamol *i.e.*, N-acetyl-p-benzoquinoneimine and sulphydryl groups of protein results in cell necrosis and lipid peroxidation which causes hepatotoxicity leading to increased levels of serum marker enzymes like SGOT, SGPT, ALP and total bilirubin (TB)[15,16,17,18].

2.8. Hepatoprotective studies

Wistar albino rats of either sex were divided in to five groups of 6 animals each and are given orally the following treatment for seven days. Group I served as normal control and they received 1% sodium.carboxy methyl cellulose (Na. CMC) 1 mL/kg bw, *p.o.* Group II received paracetamol at dose of 1 g/kg bw *p.o.* (paracetamol control). Group III received both silymarin (50 mg/kg bw, *p.o.*) and paracetamol dose. Group IV received paracetamol and 100 mg/kg bw, *p.o.* of *C. gigantea* leaves extract. Group V received paracetamol and 200 mg/kg bw, p.o. of *C. gigantea* leaves extract.

After 24 h of the last treatment, blood was collected from retro-orbital plexus, allowed to clot for 1 h at room temperature and serum was separated by centrifugation at 2500 rpm at 30 ℃ for 15 min. The serum was then collected and analyzed for various biochemical parameters.

2.9. Assessment of liver function

The serum collected after centrifugation was analyzed for various biochemical parameters like SGOT/AST, SGPT/ALT, ALP, TB and total protein (TP). Serum transaminase activity was measured according to the method of Rietman and Frankel[19]. The ALP and the serum bilirubin was determined by using method of Scand[20]. The TP was measured by using method of Lowry OH, *et al*[21].

2.10. Histopathological studies

Rats were sacrificed, livers excised, cleaned with saline and they were transferred into 10% neutral formalin solution, after one week liver tissues were dehydrated with a sequence of ethanol solutions, embedded in paraffin, cut into 5 μm section, stained with haematoxylin-eosin dye and then observed under photomicroscope.

2.11. Statistical analysis

All the results were expressed as Mean ± SEM. The statistical analysis was carried out by one-way ANOVA followed by Dunnett's multiple comparison tests using Graph pad Prism-5 software. $P < 0.05$ was considered as significant.

3. Results

3.1. Effects of extract on SGOT, SGPT, ALP, TB and TP levels

In paracetamol treated rats the levels of serum marker enzymes (SGOT, SGPT, ALP and TB) increased significantly when compared to control group of rats. Due to hepatic damage there was decreased production of proteins, so the TP levels of paracetamol group was less than control group. These increased and decreased levels of various serum marker enzymes were depicted in Table 1.

3.2. Histopathological observations

Histopathology observations of control group (Figure 1A) showed central vein surrounded by hepatic cord of cells (normal architecture). Liver section of paracetamol treated rats showed massive fatty changes and cell necrosis (Figure 1B). Liver section of rats treated with Paracetamol and 50 mg/kg b.w of silymarin showed almost normal liver tissue (Figure 1C). Liver section of rats treated with paracetamol and 100 mg/kg bw of methanolic leaf extract of *C. gigantea* showed less inflammatory cells around central vein and absence of necrosis (Figure 1D). Liver section of rats treated paracetamol and 200 mg/kg bw of methanolic leaf extract of *C. gigantea* showed minimal inflammatory cellular infiltration, regeneration of hepatocytes around central vein was also observed and almost near normal liver architecture (Figure 1E), indicating the hepatoprotective activity of methanolic leaf extract of *C. gigantea*.

Table 1
Effect of methanolic leaf extract of *C. gigantea* extract on various biochemical parameters in paracetamol induced hepatotoxicity in rats (Mean ± SEM).

Design of treatment (n=6)	SGOT/AST (IU/L)	SGPT/ALT (IU/L)	ALP (IU/L)	TB (mg/dL)	TP(IU/L)
I. Control (1% Na.CMC, 1 mL/kg bw, *p.o.*)	70.50 ± 1.72	44.00 ± 1.29	164.00 ± 7.52	0.31 ± 0.02	9.16 ± 0.12
II. Paracetamol (1 g/kg bw)	134.00 ± 1.65	97.83 ± 0.90	438.00 ± 10.65	0.76 ± 0.01	6.50 ± 0.15
III. Silymarin (50 mg/kg bw)	98.16 ± 4.02***	78.97 ± 1.64***	216.00 ± 2.14***	0.52 ± 0.08***	8.78 ± 0.15***
IV. LCG (100 mg/kg bw)	116.50 ± 1.76***	84.00 ± 1.23**	304.83 ± 2.91***	0.64 ± 0.02**	7.58 ± 0.38***
V. LCG (200 mg/kg bw)	105.30 ± 1.83***	82.66 ± 1.33**	261.00 ± 2.39***	0.59 ± 0.01**	8.23 ± 0.22***

LCG- Methanolic leaf extract of *C. gigantea*. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ as compared to Group II (paracetamol control).

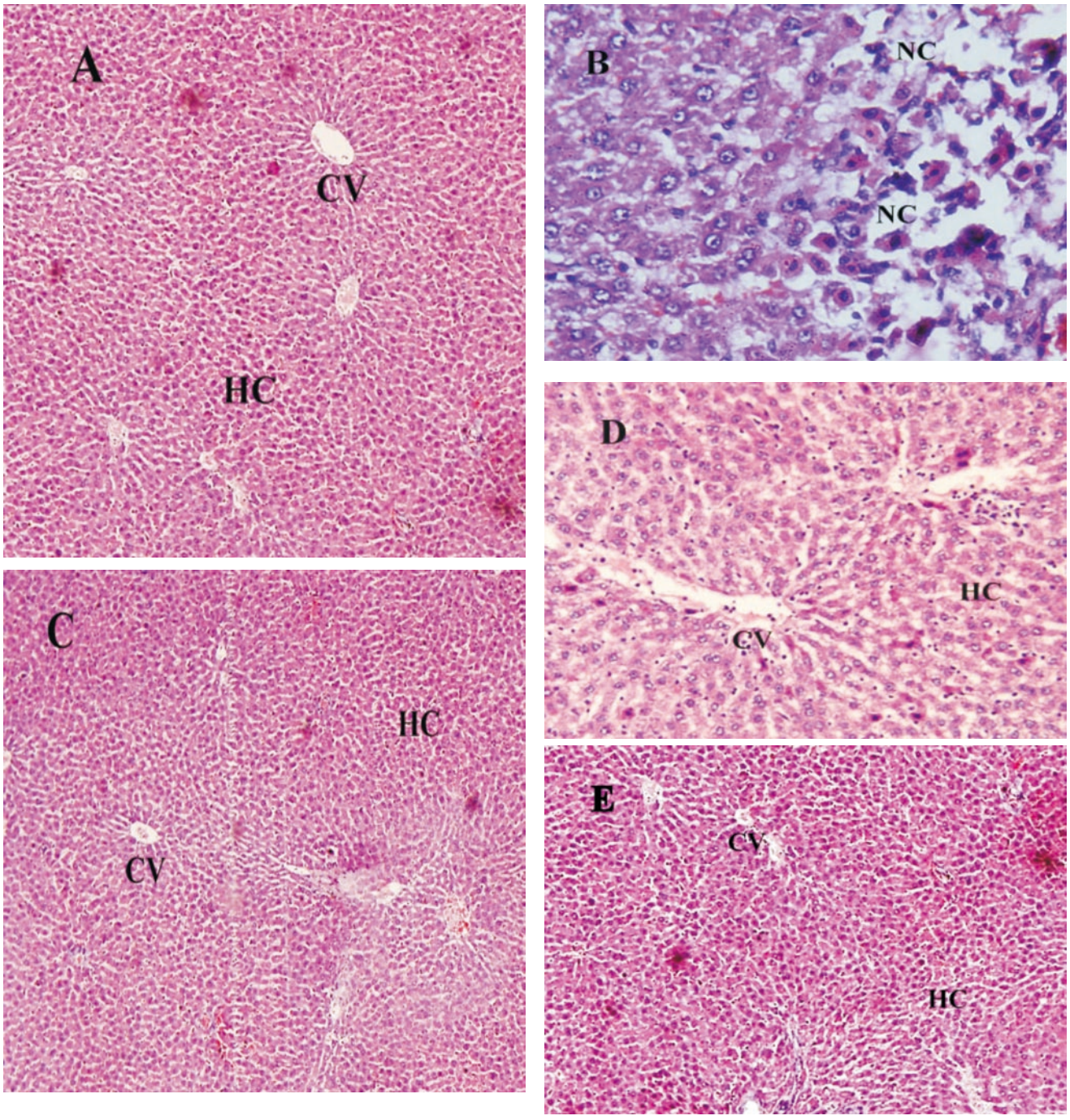

Figure 1. Sections of the livers of paracetamol-treated rats showing the central vein(CV), necrotic cells (NC) and hepatic cells (HC) with haematoxylin-eosin staining.

A) Control group; B) Paracetamol, 1g/kg bw; C) Paracetamol (1g/kg bw) + silymarin (50 mg/kg bw); D) Paracetamol (1g/kg bw) + LCG (100 mg/kg bw); E) Paracetamol (1g/kg bw)+ LCG (200 mg/kg bw).

4. Discussion

Paracetamol induced hepatotoxicity is the most commonly used screening method for testing the hepatoprotective nature of plant extracts. The normal levels of serum enzymes is SGOT [(70.5 ± 1.72) IU/L], SGPT [(44 ± 1.29) IU/L], ALP [(164 ± 7.52) IU/L] and TB [(0.31 ± 0.02) mg/dL]. The hepatic damage leads to increased serum levels of enzymes like SGOT, SGPT, ALP and TB. This is indicative of cellular damage and loss of functional integrity of cell membrane in liver[22].

Due to damage of hepatocytes *i.e.*, cell necrosis (NC) as shown in Figure 1B, there was decreased production of proteins and so the TP levels are decreased from (9.16 ± 0.12) IU/L to (6.5 ± 0.15) IU/L. The increased production of serum enzymes in blood stream was associated with central/submissive necrosis of liver which causes severe hepatic injury. The hepatoprotective effect of *C. gigantea* leaf extract may be due to presence of phyto-constituents like polyphenols[23-26]. The *in vitro* free radical scavenging activity of *C. gigantea* leaves such as DPPH radical scavenging assay, superoxide radical scavenging assay and hydroxyl radical scavenging assay suggest the ability of *C. gigantea* leaf extract to reduce biological oxidative stress[27]. Hence, the hepatoprotective effect of that leaf extract may be achieved by the scavenging free radical activity of the

oxidative stress[28]. Moreover, the increased levels of these serum enzymes were significantly decreased by treatment with methanolic leaf extract of *C. gigantea* at 100 mg/kg bw and 200 mg/kg bw, implying that the extract prevented the liver damage. The *C. gigantea* leaf extract treatment showed dose dependent activity, *C. gigantea* leaf extract at 200 mg/kg bw showed good result than 100mg/kg bw which is given in Table 1 for the measured levels of different serum enzymes. This was further confirmed by reduced amount of histopathological injuries in Figure 1D and 1E.

The phytochemical screening of methanolic leaf extract of *C. gigantea* showed the presence of triterpenes, sterols, flavonoids, phenols and saponins and these antioxidant phytochemicals of *C. gigantea* might contribute to its hepatoprotective activity. Hence, from this study, it was concluded that methanolic leaf extract of *C. gigantea* possess hepatoprotective activity against paracetamol induced hepatotoxicity in rats.

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Conflict of interest statement

We declare that we have no conflict of interest.

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