

HEPATOPROTECTIVE AND ANTIOXIDANT ACTIVITY OF *AMORPHOPHALLUS CAMPANULATUS* TUBERS EXTRACT AGAINST PARACETAMOL -INDUCED LIVER DAMAGE IN RATS

Abstract

Aims: In the present study, the methanolic and aqueous tuber extract of *Amorphophallus campanulatus* was examined for its hepatoprotective and antioxidant potential in albino rats.

Materials and Methods: Hepatotoxicity was induced by paracetamol and the biochemical parameters such as serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT) and serum alkaline phosphatase (ALP) and histopathological changes in liver were studied along with Silymarin as standard hepatoprotective agent. **Statistical Analysis**

Used: Results were analyzed by one- way ANOVA, followed by Dunnett 't' test. **Results:** The phytochemical investigations of the extracts showed presence of carbohydrates, proteins, steroids and flavonoids. Pre-treatment of the rats with paracetamol administration caused a significant reduction in the values of SGOT, SGPT, and ALP [$P < 0.01$] almost comparable to the Silymarin. The hepatoprotection was confirmed by histopathological examination of the liver tissue of control and treated animals. From the results it can be concluded that *A. campanulatus* possesses hepatoprotective effect against paracetamol-induced liver damage in rats.

Keywords: *Amorphophallus campanulatus*, Hepatoprotective, Paracetamol, Silymarin.

1. Introduction

Elephant foot yam, botanically named as *A. campanulatus* (Araceae) known as Suran is a well-known plant in the Indian traditional system of medicine and distributed throughout India. The tubers are dry, acrid, pungent; increases both appetite and taste; digestive, anthelmintic and aphrodisiac; useful in vitiated conditions of vata and kapha, elephantiasis, inflammations, haemorrhoids, haemorrhages, abdominal pain, asthma, piles, dysentery, splenopathy, amenorrhoea, seminal weakness, fatigue, anaemia and general debility ^[1,2]. The tubers are

reported to possess antibacterial, antifungal and cytotoxic activities due to presence of a diterpenoid namely salviasperanol and amblyone, a triterpenoids^[3,4].

Liver is the vital organ of metabolism and excretion. About 20,000 deaths reported every year is due to liver disorders^[5]. Paracetamol hepatotoxicity is caused by the reaction metabolite N-acetyl-p-benzo quinoneimine (NAPQI), which causes oxidative stress and glutathione depletion^[6]. It is a well-known antipyretic and analgesic agent, which produces hepatic necrosis at higher doses.

The survey of literature reveals that the *A. campanulatus* tubers are found to be used in the traditional system of medicine as tumor, rheumatoid arthritis, carminative and liver tonic. It is also used in piles and given as the restorative in dyspepsia debility, anti-inflammatory, anti-haemorrhoidal, haemostatic, expectorant, and anthelmintic^[7,8]. However, hepatoprotective activity of *A. campanulatus* has not been scientifically investigated. This study therefore, investigated the hepatoprotective properties of tuber extract of *A. campanulatus* in rats.

2. Material and Methods

2.1. Animals

Wistar rats (180-210 g body wt.) of both sexes were procured from animal house, college of Pharmacy, provided with water ad libitum and maintained at ambient room temperature (25°C ± 2°C). The study was approved by the animal ethics committee and all the ethical norms were strictly followed during the experiment. They were maintained on a standard normal diet.

2.2. Plant material and preparation of extract

The fresh tubers of *A. campanulatus* were collected in the months of Dec - Jan from the local market of Tambaram town, Chennai. Authentication of plant was done by Dr. Jairaman, Professor of Botany, Tambaram, Tamilnadu (Voucher no: Parc/112). The tubers were shade dried and defatted with petroleum ether. The defatted material was extracted with 95% ethanol and then vacuum dried. One part of powdered tubers was decocted for two hours in boiling water and the other part was cold macerated for 7 days in water with occasional stirring. The decoction and maceration were filtered and vacuum dried.

2.3. *Drugs and Chemicals*

Pure sample of Silymarin was obtained from sigma chemicals, USA, paracetamol was purchased from Remidex Pharma Pvt. Ltd. Tween80 (Polyoxy methylene sorbitan monoleate) and diethyl ether was obtained from Merck India Ltd. The assay reagents of the GOT (Glutamate oxaloacetate transaminase), GPT (Glutamate pyruvate transaminase) and ALP (Alkaline phosphatase) was obtained from Merck India Ltd. and assay reagents for GGT (Gamma glutamyl transferase) and Cholesterol was obtained from Pointe Scientific, Inc. U.S.A. and bilirubin and Total bilirubin was obtained from Ranbaxy laboratory, Mumbai, India. Analytical grades of reagents were used.

2.4. *Phytochemical analysis*

A number of qualitative phytochemical tests were carried out to detect the presence of volatile oils, alkaloids, tannins, saponins, flavanoids, glycosides, steroids, terpenoids and phenols utilizing standard methods of analysis ^[9,10,11].

2.5. *Pharmacological screening*

2.5.1. *Acute oral toxicity study*

The acute oral toxicity study was carried out as per the guidelines set by OECD, revised draft guidelines 423, received from CPCSEA, Ministry of Social Justice and empowerment, Govt. of India ^[12].

2.5.2. *Evaluation of hepatoprotective and antioxidant activity*

Rats were divided in five groups, with six animal per group (n=6). Paracetamol was suspended in 40% (w/v) aqueous sucrose soln. and administered (p.o.) at a dose of 2 g/Kg ^[12]. Group 1 received 0.5% Tween 80. Group 2 received 0.5% Tween 80 p.o. for 4 days and served as negative control. Group 3 received silymarin (100 mg/kg, p.o.) for 4 days. Group 4 received methanol extract (300 mg/kg, p.o.) for 4 days. Group 5 received aqueous extract (300 mg/kg, p.o.) for 4 days. Animals were sacrificed under mild ether anesthesia 48 hrs after paracetamol administration (except control group I) on day 5. The body wts. of each animal before beginning of experiment and just before sacrifice were recorded. After sacrifice the liver weight of each animal was also recorded ^[13].

89 **2.6. Histopathological studies**

90 One animal from the each group was utilized for this purpose. The liver specimens obtained from
91 the control and treated groups of animals were fixed in 10% buffered formalin for 24 h. The
92 formalin fixed liver samples were stained with haematoxylin-eosin for photomicroscopic
93 observations of the liver histopathological architecture ^[14].

94 **2.7. Statistical analysis:**

95 The data are presented as mean $\pm$ SEM and analyzed by one- way ANOVA, followed by
96 Dunnett‘t’ test. The results of all the extracts including the standard drug are compared with the
97 result produced by control and it is considered as significant as [P<0.05].

100 **3. Results**

101 **3.1. Phytochemical analysis**

102 Preliminary phytochemical investigation revealed the presence of carbohydrates, proteins,
103 steroids and flavonoids in both extracts.

104 **3.2. Pharmacological screening outcome**

105 *3.2.1. Evaluation of hepatoprotective activity*

106 Administration of paracetamol (2.5 g/kg, p.o.) induced a marked increase in the serum hepatic
107 enzyme levels, SGOT, SGPT, ALP and serum bilirubin as compared to normal controls
108 indicating liver damage (centrilobular necrosis). Pretreatment of the rats with methanol and
109 aqueous extract prior to paracetamol administration caused a significant reduction in the values
110 of SGOT, SGPT, ALP and serum bilirubin [P<0.01] almost comparable to the **silymerin** (Table
111 1).

112 *3.2.2. Determination of antioxidant enzymes activity*

113 CAT activity was assayed according to the method of Aebi ^[15]. The reaction time interval of the
114 absorbance was monitored at 240 nm for one minute to measure CAT activity and the data was
115 expressed as K/mg protein. SOD activity was determined as described by Beauchamp and
116 Fridovich ^[16] by measuring its ability to inhibit the photochemical reduction of nitro blue
117 tetrazolium (NBT) in absorbance at 560 nm. SOD activity of liver homogenate was determined
118 from a standard curve of the percentage inhibition of NBT reduction with standard SOD activity.

Data are expressed as SOD units/mg protein as compared with the standard. The measurement of GPx was conducted by modified methods from Flohe and Gunzler ^[17].

3.3. Histopathological results

The hepatoprotective effect of *A. campanulatus* tubers was confirmed by histopathological examination of the liver tissue of control and treated animals. The histological architecture of paracetamol treated liver sections showed normal cellular architecture with distinct hepatic cells, sinusoidal space. The histopathological profile of the rat treated with methanol extract showed no visible changes confirming the safety of the extract at selected dose (Figure 1). In the liver section of the rats intoxicated with paracetamol, there was disarrangement and degeneration of normal hepatic cells with intense centrilobular necrosis. The liver section of the rats treated with aqueous extract and intoxicated with paracetamol showed moderate hepatoprotective activity. While rats treated with silymerin and intoxicated with paracetamol showed less disarrangement and degeneration of hepatocytes, indicating marked regeneration activity.

4. Discussion

Paracetamol toxicity was due to the formation of toxic metabolites when a part of it is metabolized by cytochrome P450 ^[18]. Induction of cytochrome or depletion of hepatic glutathione was a prerequisite for paracetamol-induced hepatotoxicity. So in the present study, paracetamol was employed as toxic agent and the protective effect of *A. campanulatus* tubers against the paracetamol induced hepatotoxicity was studied. The extent of toxicity was estimated by histopathological studies and biochemical enzyme markers like SGOT, SGPT, ALP and serum bilirubin levels. The present study showed the potential hepatoprotective activity of *A. campanulatus* tubers against hepatic injury produced by paracetamol in rats. Paracetamol is a well known antipyretic and analgesic agent, which is safe in therapeutic doses, but can produce fatal hepatic necrosis in man, rats and mice with toxic doses. It is employed as an experimental hepatotoxic agent ^[19]. The hepatic cytochrome P450 enzyme system metabolizes paracetamol, forming a minor yet significant alkylating metabolite known as NAPQI. NAPQI is then irreversibly conjugated with the sulfhydryl groups of glutathione ^[20]. NAPQI depletes glutathione and initiates covalent binding to cellular proteins. These events lead to the disruption of calcium homeostasis, mitochondrial dysfunction, and oxidative stress and may eventually culminate in cellular damage and death. Reinforcing the above stated mechanisms, biochemical

parameters demonstrated significant increase in the toxic control groups in the present study. Histopathological profile also revealed a major damage in the same groups. Thus, it clearly showed that, toxicity is may be due to either of the above mechanisms such as depletion of glutathione store or free radical generation or lipid peroxidation. As expected, silymarin maintained the normal architecture with minimal injuries and better protection than *A. campanulatus* tuber extract alone. Chronic treatment with silymarin also illustrated marked recovery in serum enzymes and bilirubin levels. The methanol extract showed more significant result as compared to aqueous extract of *A. campanulatus* tubers. Report has shown flavonoids and steroids may be responsible for hepatoprotective effect ^[21-24]. Perhaps steroids present in the *A. campanulatus* tubers may be responsible for the marked hepatoprotective effects, observed in the present study.

5. Conclusion

In conclusion, the result of this study demonstrates that *A. campanulatus* has potent hepatoprotective action upon paracetamol-induced hepatic damage in rats. The present study thus justifies the traditional use of *A. campanulatus* tubers in the treatment of liver diseases and also requires that future investigation be carried out on *A. campanulatus* as a promising hepatoprotective agent. However, the exact mechanism(s) and the active compound(s) involved in these effects need to be clarified in future studies. Methanolic extract possesses significant hepatoprotective activity. This finding supports the use of the plant in ethnomedicine for the treatment of liver diseases.

Conflicts of interest

There are no conflicts of interest.

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238 **Table 1: Biochemical assessment of paracetamol induced liver injury**

GROUPS	SGOT(U/L)	SGPT(U/L)	ALP(U/L)	Serum Bilirubin (mg/dl)
Control	88.25±0.38	32.10±0.81	46.25±0.91	0.23±0.03
Paracetamol treated	236.98± 0.09	172.05±0.97	91.10±0.76	5.73±0.07
Silymarin (100mg/kg)	90.07± 0.14**	40.85±0.43**	53.14±0.78**	0.32±0.01**
Methanolic extract(300 mg/kg)	114.46±0.09**	87.45±0.85**	68.46±0.62**	0.93±0.03**
Aqueous extract(300 mg/kg)	202.71± 0.05**	130.25±1.15**	87.23±0.59**	2.72±0.02**

239 Data are presented as means ±SEM (n=6), one way **Annova** followed by Dunnett's **t test.**

240 *P<0.05, ****P**< 0.01 vs. paracetamol treated group.

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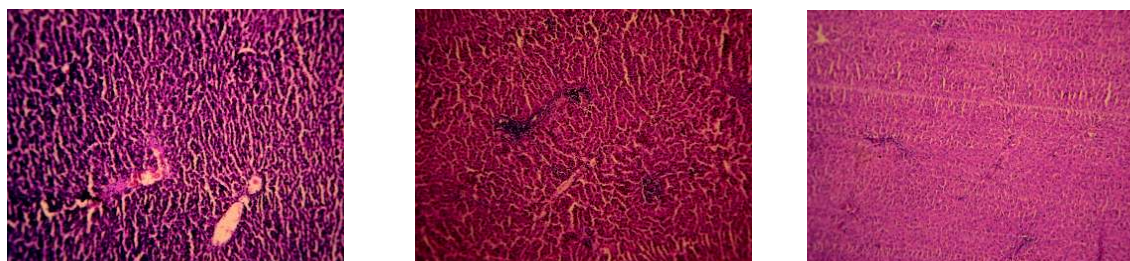
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249 **Table 2: Effect of Methanolic extract of *Amorphophallus campanulatus* antioxidant**
 250 **on liver enzymes specific activities levels in paracetamol intoxicated rats**

Groups	SOD(μ /mg protein)	CAT (K/mg protein)	GSH(mg/100g)
Control	3.57 $\pm$ 0.06	34.57 $\pm$ 1.86	45.97 $\pm$ 1.82
Paracetamol treated	1.45 $\pm$ 0.08**	20.28 $\pm$ 1.14**	20.94 $\pm$ 0.97**
Silymarin (100mg/kg)	2.87 $\pm$ 0.05**	30.80 $\pm$ 1.83*	42.87 $\pm$ 1.50***
Methanolic extract (300 mg/kg)	2.68 $\pm$ 0.07**	29.97 $\pm$ 1.23**	40.80 $\pm$ 1.37**

251 Data are presented as means $\pm$ SEM (n=6), one way Anova followed by Dunnett's t test.

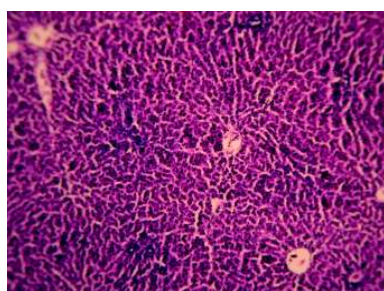
252 *P<0 .05, ** P < 0.01 vs. paracetamol treated group.



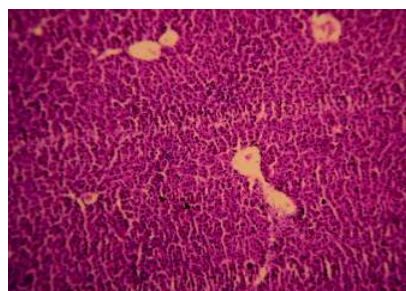
(A)

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(D)



(E)

Figure 1: Histopathology of liver tissues in different groups observed by haematoxylin-eosin staining (Light microscopy, $\times 100$)

A: Liver section of normal rat (group I); B: Paracetamol - treated group (group II); C: Paracetamol + silymarin (100 mg/kg) - treated group (group III); D: Paracetamol + methanolic extract of *Amorphophallus campanulatus* (300 mg/kg) - treated group (group IV); E: Paracetamol + Aqueous extract of *Amorphophallus campanulatus* (300 mg/kg)-treated group (group V).