

CAMPANULATUS TUBERS EXTRACT AGAINST PARACETAMOL -INDUCED LIVER DAMAGE IN RATS

Abstract

The present study was to evaluate the hepatoprotective and antioxidant activity of the methanolic and aqueous extracts of *Amorphophallus campanulatus* tubers against Paracetamol induced liver damage in rats. The administration of extracts at a dose 300mg/kg restored the elevated serum enzyme levels to normal. Silymarin was used as a standard reference, exhibited significant hepatoprotective activity. The biochemical and histopathological results proved that *Amorphophallus campanulatus* tubers had hepatoprotective potency to liver damage due to paracetamol. It was concluded from the results that methanolic extract possessed more potent hepatoprotective and antioxidant activity than aqueous extract.

Keywords: *Amorphophallus campanulatus* tuber, 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 used as digestive, anthelmintic, aphrodisiac and anti-inflammatory. It is also used for the treatment of haemorrhoids, abdominal pain, asthma, piles, dysentery, amenorrhoea, seminal weakness, anaemia^[1,2]. It also possesses antibacterial, antifungal and cytotoxic activities due to presence of salviasperanol and amblyone^[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 is known antipyretic and analgesic agent, at a higher dose it can produce hepatic necrosis. Paracetamol metabolite N-acetyl-p-benzo quinoneimine (NAPQI), causes oxidative stress and glutathione depletion^[6]. 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]. This study, 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 (IAEC/25/2007) 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 macerated with methanol for 5 days. One part of powdered tubers was decocted for two hours in boiling water. 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]. Both extracts were found safe (no mortality) even when given at the dose of 3000 mg/kg body weight with no signs of acute oral toxicity at respective dose. Alcoholic extract or aqueous extract was administered orally to overnight fasted wistar albino rats (n=6) at dose of 3000mg/kg body weight respectively. Animals were observed to watch the behavioral changes in interval of 4h, 6h 24h and 48 h for next 14 days. Behavioral parameters include salivation, hyperactivity, sedation, respiratory arrest, convulsion, diarrhea, skin, eye, coma, mortality.

2.5.2. Evaluation of hepatoprotective activity

Rats were divided in five groups, with six animals per group (n=6). Paracetamol was suspended in 40% (w/v) aqueous sucrose soln. and administered (p.o.) at a dose of 2.5 g/Kg ^[13]. Group-I (control) received single daily dose of 5% tween-80 (5 ml/Kg, p.o.) for 4 days & a single dose of 40% sucrose soln. (1 ml/rat, p.o.) on day 3. Group II received single daily dose of 5% tween-80 (5 ml/kg, p.o.) for 4 days & a single dose of Paracetamol suspension (p.o.) on day 3. Group-III received daily dose of silymarin (100 mg/Kg, p.o.) for 4 days & a single dose of Paracetamol suspension (p.o.) on day 3. Test Group IV received daily dose of methanolic extract suspension (p.o.) for 4 days (300mg/Kg) and a single dose of Paracetamol suspension (p.o.) on day 3. Test group V received daily dose of aqueous extract suspension (p.o.) for 4 days (300mg/kg) and a single dose of Paracetamol suspension (p.o.) on day 3. Animals were sacrificed under mild ether anesthesia 48 hrs after Paracetamol administration on day 5. The body wt. of each animal before

beginning of experiment and just before sacrifice were recorded. After sacrifice the liver weight of each animal was also recorded^[14].

2.5.3. Determination of antioxidant enzymes activity

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

2.5.4. Method for collection of blood sample

The blood (0.3ml-0.5ml) was collected into centrifuge tubes, from retro-orbital puncture under the influence of light ether anesthesia which were centrifuged at 3000 rpm at room temperature.

2.6. Histopathological studies

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

2.7. Statistical analysis:

The data are presented as mean $\pm$ SEM and analyzed by one- way ANOVA, followed by Dunnett t-test. The results of all the extracts including the standard drug are compared with the result produced by control and it is considered as significant as ($p < 0.05$).

3. Results

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

3.1. Acute toxicity

The extracts were found to be safe in the dose used and there was no mortality up to a dose of 3000 mg/kg, b.w. for both extracts. Hence 300mg/kg b.w. p.o. were selected for the activity (Table 1)

3.2. Pharmacological screening outcome

3.2.1. Evaluation of hepatoprotective activity

The serum hepatic enzyme levels, SGOT, SGPT, ALP and serum bilirubin were elevated in paracetamol treated as compared to normal control liver damage (centrilobular necrosis). Pretreatment with methanol and aqueous extracts of *Amorphophallus campanulatus* prevented significantly the paracetamol induced elevation of serum hepatic enzymes, SGOT, SGPT, ALP and serum bilirubin (Table 2).

3.3. Antioxidant study

Amounts of SOD, CAT, and GSH were significantly diminished in the negative Control group as compared with the normal control ($P < 0.01$). Pretreatment with 300mg/kg of Methanolic & Aqueous extracts of *Amorphophallus campanulatus* significantly raised the antioxidant enzyme

levels as compared with the rats intoxicated with acetaminophen treatment. Methanolic extract shows more significant effect than Aqueous extract.(Table 3)

3.4. Histopathological results

The hepatoprotective effect of *A. campanulatus* tubers was confirmed by histopathological examination of the liver tissue of control and treated animals. Normal control group showed normal hepatocytes with clear nucleus and central vein. The liver intoxicated with paracetamol showed necrosis of centrilobular hepatocytes, loss of cellular boundaries, spotty and diffuse hyaline necrosis with blood pooling in central vanules and many cells were at the end stage of death. Animals treated with 300mg/kg of methanolic and aqueous extracts showed protection because there near normal histology were seen. There was no inflammation or necrosis and the central vein architecture was about normal. The histopathological plates of the reference drug silymarin treated liver showed near normal state of liver.

4. Discussion

In toxic dose a part of paracetamol is metabolized by cytochrome P450 to form toxic metabolite NAPQI^[18]. NAPQI rapidly react with glutathione (GSH) causing depletion of glutathione in the cells and mitochondria which can cause hepatocellular death. 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. Paracetamol in therapeutic doses shows well known antipyretic and analgesic effects, but at a toxic doses can produce 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. 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. 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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Table: 1 Acute toxicity of both the extracts :

S. No .	Extracts	Salivation	Hyperactivity	Respiratory arrest	Convulsion	Diarrhea	Skin	Coma	Eye	Mortality
1	Methanolic	N	N	N	N	N	N	N	N	N
2	Aqueous	N	N	N	N	N	N	N	N	N

N=Negative

**Table: 2 Biochemical assessment of *A. campanulatus* extracts in paracetamol
toxicated rat liver.**

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**

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

* $p < 0.05$, ** $p < 0.01$ vs. paracetamol treated group.

Table: 3 Antioxidant activity of *A. campanulatus* extracts in paracetamol intoxicated rats

Groups	SOD(μ /mg protein)	CAT (K/mg protein)	GSH(mg/100g)
Control	3.57±0.06	34.57±1.86	45.97±1.82
Paracetamol treated	1.45±0.08**	20.28±1.14**	20.94±0.97**
Silymarin (100mg/kg)	2.87±0.05**	30.80±1.83*	42.87±1.50***

Methanolic extract (300 mg/kg)	2.68±0.07**	29.97±1.23**	40.80±1.37**
Aqueous extract(300 mg/kg)	2.17±0.10**	26.66±1.12**	39.9±1.1**

248 Data are presented as means ±SEM (n=6), one way Anova followed by Dunnett's t-test.

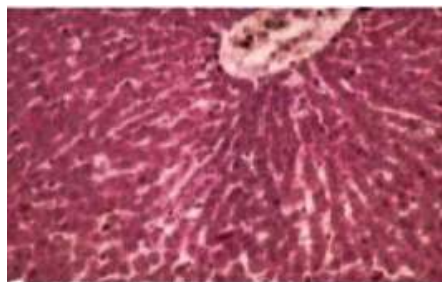
249 * $p < 0.05$, ** $p < 0.01$ vs. paracetamol treated group.

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(A)

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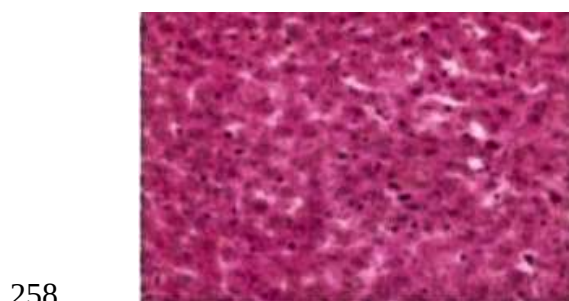
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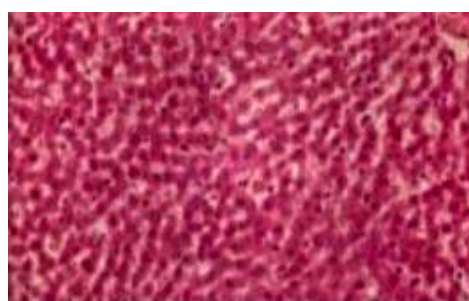


(C)

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(D)



(E)

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265 **Figure 1: Histopathology of liver**

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

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