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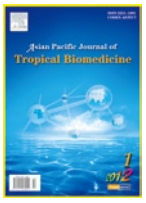

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Hepatoprotective activity of *Amorphophallus paeoniifolius* tubers against paracetamol-induced liver damage in rats

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ABSTRACT

Objective: To study the hepatoprotective activity of methanol and aqueous extracts of *Amorphophallus paeoniifolius* tubers against paracetamol induced liver damage in rats. **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 (sALP), serum bilirubin (SB) and histopathological changes in liver were studied along with silymarin and Liv-52 as standard hepatoprotective agents. **Results:** The phytochemical investigation of the extracts showed presence of carbohydrates, proteins, steroids and flavonoids. Pre-treatment of the rats with methanol and aqueous extract prior to paracetamol administration caused a significant reduction in the values of sGOT, sGPT, sALP and sB ($P<0.01$) almost comparable to the silymarin and Liv-52. The hepatoprotective was confirmed by histopathological examination of the liver tissue of control and treated animals. **Conclusions:** From the results it can be concluded that *Amorphophallus paeoniifolius* possesses hepatoprotective effect against paracetamol-induced liver damage in rats.

1. Introduction

Amorphophallus paeoniifolius Linn (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 an analgesic, liver tonic, thermogenic, anthelmentic and diuretic[1]. The roots are used to treat opthalmia and boils[2]. The plant is used for the treatment of piles, dyspnoea, spleenomegaly and cough; some important chemical constituents of the tubers include carbohydrates, sitosterol, stigmasterol, thiamin, riboflavin[3]. The plant is reported to have analgesic[4], antioxidant[5], synergistic depressant[6], antibacterial, antifungal and cytotoxic activity[7,8].

Recently there has been a large volume of work aimed at scientific validation of the efficacy of herbal drugs used in the traditional medicine. Modern medicine does not have suitable answers for many conditions such as liver disorder, asthma, cardiovascular disorder *etc*[9].

Liver is the vital organ of metabolism and excretion. About 20 000 deaths found every year due to liver disorders[10]. Hepatocellular carcinoma is one of the ten most common tumors in the world with over 250 000 new cases each year[11]. Paracetamol hepatotoxicity is caused by the reaction metabolite N-acetyl-p-benzo quinoneimine (NAPQI), which causes oxidative stress and lutathione depletion[12]. It is a well-known antipyretic and analgesic agent, which produces hepatic necrosis at higher doses.

The survey of literature reveals that the *Amorphophallus paeoniifolius* tubers are found to be used in the traditional system of medicine as a liver tonic[1]. However, hepatoprotective activity of *Amorphophallus paeoniifolius* has not been scientifically investigated. Therefore, in the present study hepatoprotective effect of methanol and aqueous extracts of *Amorphophallus paeoniifolius* tubers have been evaluated against paracetamol induced liver damage in the male Wistar albino rats.

2. Materials and methods

2.1. Drugs and chemicals

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Paracetamol was obtained as gift sample from Torrent Research Center, Ahmedabad. Silymarin was obtained as a gift sample from Cadila Pharma Ltd, India. Standard kit of serum glutamate pyruvate transaminase (sGPT), serum glutamate oxaloacetate transaminase (sGOT) and alkaline phosphatase (sALP) were obtained from Beacon Diagnostic Pvt. Ltd., Navsari. All other reagents used for the experiments were of high analytical grade.

2.2. Plant material

The *Amorphophallus paeoniifolius* tubers were collected in the month of September from Sawantvadi Dist.- Ratnagiri and authenticated by Dr. Harsha Hegde, Scientist C, Regional Medical Research Centre, ICMR, Belgaum, India and the herbarium of this plant have been deposited in RMRC, ICMR, Belgaum. (Voucher No. RMRC - 475).

2.3. Preparation of extracts

The fresh tubers were cleaned, shade dried and then powdered. The dried coarse powder of the tubers (200 g) was extracted using methanol and aqueous extraction was performed by cold maceration process. The last trace of solvent was removed by reduced pressure distillation and then the extracts were dried under vacuum. The extracts were subjected to preliminary phytochemical investigation. Suspensions of each extract were prepared using 0.5% Tween 80, Paracetamol (2.5 g/kg) suspension prepared by using 0.5% Tween 80 and subjected for hepatoprotective activity against paracetamol-induced liver damage.

2.4. Experimental animals

Male albino- Wistar rats weighing 150 to 180 g and mice weighing 25 to 30 g were housed in standard laboratory conditions of temperature [(25±2) ℃], 12 h light and dark places and with food and water *ad libitum*.

2.5. Acute oral toxicity

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[13].

2.6. Evaluation of hepatoprotective activity

The method used for evaluation of hepatoprotectivity was according to Malar *et al*[14] and Chaudhary *et al*[15], with some minor modifications. In the paracetamol-induced liver toxicity model, paracetamol (2.5 g/kg, *p.o.*) was administered on 3rd day to all animals except group 1. Silymarin (100 mg/kg, *p.o*) and Liv-52 (5 mL/kg, *p.o.*) were used as a standard. The animals were segregated in to six groups of six each. Group 1, which served as normal control and 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 Liv-52 (5 mL/kg, *p.o.*) for 4 days. Group 5 received methanol extract (300 mg/kg, *p.o.*) for 4 days. Group 6 received aqueous extract (300 mg/kg, *p.o.*) for 4 days.

The animals were sacrificed 48 h after paracetamol administration under light anesthetic ether. Blood from each rat was withdrawn by retro orbital plexus under ether anesthesia for biochemical investigation *i.e.* sGOT, sGPT, sALT and bilurubin estimation. Blood was allowed to coagulate at 370C for 30 min and the serum was separated by centrifugation at 2 500 rpm for 10 min. The liver of all the experimental animals were removed and processed immediately for histological investigation[16].

2.7. 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.

2.8. Statistical analysis

The data are presented as mean±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 $P<0.05$.

Table 1.
Biochemical assessment of paracetamol induced liver injury.

Groups	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Serum bilirumin(mg/dL)
Control	87.15±1.30	31.07±0.93	45.92±0.82	0.26±0.03
Paracetamol treated	235.9±1.101	171.3±0.99	90.55±1.36	5.26±0.06
Liv-52 (5 mL/kg)	89.02±0.74**	39.94±0.63**	52.54±0.94**	0.25±0.01**
Silymarin(100 mg/kg)	94.85±1.60**	42.93±1.03**	54.03±0.71**	0.29±0.01**
Methanolic extract (300 mg/kg)	113.4±3.14**	86.85±2.16**	67.98±1.05**	0.84±0.03**
Aqueous extract (300 mg/kg)	201.6±1.56*	129.5±2.99**	86.56±0.38*	2.50±0.07**

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.

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 3 000 mg/kg, b.w. for both extratcs. Hence 300 and 500 mg/kg b.w. *p.o.* were selected for the activity.

3.2. Hepatoprotective activity

Administration of paracetamol (2.5 g/kg, *p.o.*) induced a marked increase in the serum hepatic enzyme levels, sGOT, sGPT, sALP and SB as compared to normal controls

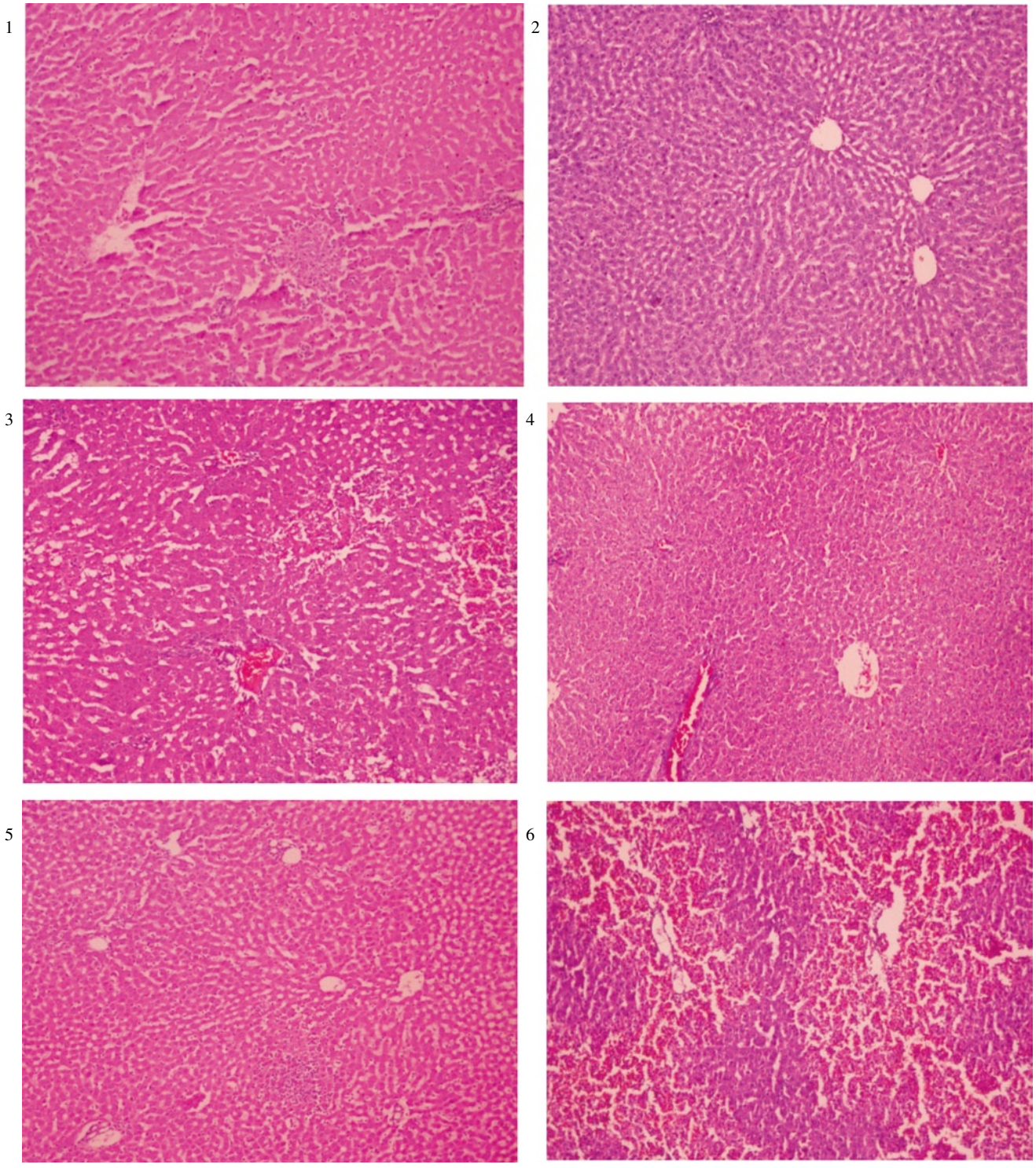

Figure 1. Histopathalogical monograph of extract and standard.
1. Normal; 2: Paracetamol (2.5 g/kg) alone; 3: Paracetamol + Liv-52 (2.5 g/kg +5 mL/kg); 4: Paracetamol + silymarin(2.5 g/kg +100 mg/kg); 5: Paracetamol + methanolic extract(2.5 g/kg+ 300 mg/kg); 6: Paracetamol + aqueous extract(2.5 g/kg+ 300 mg/kg).

indicating liver damage (centrilobular necrosis). Pre-treatment of the rats with methanol and aqueous extract prior to paracetamol administration caused a significant reduction in the values of sGOT, sGPT, sALP and sB ($P<0.01$) almost comparable to the silymarin and Liv-52(Table 1).

3.3. Histopathological results

The hepatoprotective effect of *Amorphophallus paeoniifolius* 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 (Figure 2), 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 (Figure 3). While rats treated with silymarin, liv-52 and intoxicated with paracetamol showed less disarrangement and degeneration of hepatocytes, indicating marked regeneration activity (Figure 4, 5).

4. Discussion

Paracetamol toxicity is due to the formation of toxic metabolites when a part of its metabolized by cytochrome P_{450}[17]. Introduction of cytochrome or depletion of hepatic glutathione is a prerequisite for paracetamol-induced hepatotoxicity. So in the present study, paracetamol was employed as toxic agent and the protective effect of *Amorphophallus paeoniifolius* tubers against the paracetamol induced hepatotoxicity was studied. The extent of toxicity was estimated by histopathological studies and biochemical enzyme markers like sGOT, sGPT, sALP and SB levels.

The present study reports the potential hepatoprotective activity of *Amorphophallus paeoniifolius* 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[18].

The hepatic cytochrome P_{450} 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[19]. 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 demonstrate significant increase in the toxic control groups in the present study. Histopathological profile also reveals a major damage in the same groups. Thus, it clearly states that, toxicity is due to either of the above mechanisms such as depletion of glutathione store or free radical generation or lipid peroxidation.

As expected, Liv-52 and silymarin, maintained the normal architecture with minimal injuries and better protection than *Amorphophallus paeoniifolius* tuber extract alone. Chronic treatment with Liv-52 and silymarin also illustrated marked recovery in serum enzymes and bilirubin levels.

The methanol extract showed more significant result as compared to aqueous extract of *Amorphophallus paeoniifolius* tubers. Report shows flavonoids and steroids are may be responsible for hepatoprotective effect[20-23]. Perhaps steroids present in the *Amorphophallus paeoniifolius* tubers may be responsible for the marked hepatoprotective effects, observed in the present study.

In conclusion, the result of this study demonstrates that *Amorphophallus paeoniifolius* has potent hepatoprotective action upon paracetamol-induced hepatic damage in rats. The present study thus justifies the traditional use of *Amorphophallus paeoniifolius* tubers in the treatment of liver diseases and also points out that *Amorphophallus paeoniifolius* warrants future detailed investigation 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.

Conflict of interest statement

We declare that we have no conflict of interest.

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