



HEPATOPROTECTIVE AND ANTIOXIDANT EFFECTS OF *AMORPHOPHALLUS CAMPANULATUS* AGAINST ACETAMINOPHEN-INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

The present study was aimed to evaluate the hepatoprotective effect of the dried tuber of *Amorphophallus campanulatus* (MAC) against acetaminophen induced hepatic injury in albino rats. Pretreatment with MAC reduced the biochemical markers of hepatic injury like serum glutamate pyruvate transaminase (SGPT), serum oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), bilirubin (BRN) and total protein shows hepatoprotective activity. There is increase in the levels of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) shows that the plant may posses hepatoprotective and antioxidant property.

Keywords: *Amorphophallus campanulatus*, Hepatoprotective, Acetoaminophen, Antioxidant.

INTRODUCTION

Acetaminophen is an extensively used analgesic and antipyretic drug and, though safe when used at therapeutic doses, is associated with significant hepatotoxicity when taken in overdose¹. Under normal conditions, acetaminophen is primarily metabolized in the liver by glucuronidation and sulfation. A small proportion of the drug is metabolized by several of the cytochrome P450 enzymes into the reactive intermediate N acetyl- p-benzoquinone imine (NAPQI), which is normally detoxified by glutathione (GSH) both non-enzymatically and enzymatically. In overdose, sulfation and glucuronidation become saturated and GSH is depleted by NAPQI. Excess of NAPQI causes oxidative stress and binds covalently to liver proteins². Although the precise mechanism by which acetaminophen causes cell injury is still unknown, it is suggested that mitochondria may play an important role in the acetaminophen-induced liver cells death³. The majority of the world's population in developing countries still relies on herbal medicines to meet their health needs in cases where synthetic medicine could not relieve patients who suffer from hard-to-cure illnesses. The tuber of *Amorphophallus campanulatus* (AC) (Araceae) is used for 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^{4,5}. Previously studies have indicated that analgesic activity⁶, Antibacterial, Antifungal and Cytotoxic Activities⁷.

MATERIALS AND METHODS

Plant material

The fresh tuber of *Amorphophallus campanulatus* were collected in the months of December from the local market of Tambram, Tamil Nadu. Authentication of plant was done by Dr. Jairaman, Tambaram, Tamil Nadu. A voucher specimen was kept for future reference (Parc/2007/112).

Preparation of extract

Tuber part of *Amorphophallus campanulatus* was cut into small pieces and dried in oven at 40 to 50 °C and after grinding coarsely was subjected to maceration in Methanol media for 4 days. The % yield was found to be 23.3% w/w.

Experimental animals

Albino Wistar rats (150-200g) were procured from Animal house, College of Pharmacy, SRM University, Kattankulathur, Tamil Nadu, India. They were kept in polyacrylic cages in group of 5 and maintained under standard housing condition (room temperature 25±1 °C and humidity 60-65%) with 12 h light and dark cycle. The food and water were available ad libitum. Experiments were

performed as per internationally followed ethical standards, after obtaining clearance from IAEC, under Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. Each group (control as well as treated) consists of 6 rats.

Drugs and chemicals

Methanol, Petroleum ether - (Loba chimie Pvt. Ltd., Mumbai, India), Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) - (Merck KgaA, 64271 Darmstadt, Germany). Acetaminophen - (Nulife pharmaceutical pvt.ltd. Pune), Silymerin - (Ranbaxy Laboratories Ltd. Dewas).

Acetaminophen induced hepatotoxicity

Albino Wistar rats (150-200g) were divided into five groups each containing six rats taken for assessing hepatoprotective activity of methanolic extract of *Amorphophallus campanulatus* tuber on Acetoaminophen induced hepatotoxicity rat model.

The groups were treated as follows:

Group 1: Control group (normal saline 10 ml/kg, p.o.)

Group 2: Negative control (Acetaminophen treated 3g/kg; p.o.)

Group 3: Acetaminophen + Methanolic extract of *Amorphophallus campanulatus* (250 mg/kg)

Group 4: Acetaminophen + Methanolic extract of *Amorphophallus campanulatus* (500 mg/kg)

Group 5: Acetaminophen + silymarin (25 mg/kg/day p.o.)

All the treatment (control, test, standard) are given to the rats once a day for seven days. But Acetaminophen was given on 7th day after the last dose of respective treatment. After 24 h of Acetaminophen intoxication, Blood samples were withdrawn by cardiac puncture when the animals had been anaesthetized with ketamine/xylazine mixture (ketamine 67 mg/kg, xylazine 6 mg/kg, i.p.). The animals were sacrificed by an overdose of pentobarbitone (Phenobarbital 200mg/kg, i.p.) or diethyl ether immediately after blood collection. Blood samples collected in heparinized tubes were centrifuged at 3000×g at 4 °C for 10 min to obtain serum. On the other hand, the liver of each rat was promptly removed histopathological study.

Estimation of biochemical parameters

The liver function markers such as serum glutamate pyruvate transaminase (SGPT), serum oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), total protein (TP) were measured with an auto-analyzer (Hitachi 7050, Tokyo, Japan).

Determination of antioxidant enzymes activity

CAT activity was assayed according to the method of Aebi⁹. The reaction time interval of the absorbance was monitored at 240 nm for 1 min to measure CAT activity and the data was expressed as K/mg protein. SOD activity was determined as described by Beauchamp and Fridovich¹⁰ 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 Gunzler¹¹. The absorbance of the reaction product was read at 412 nm and the enzyme activity was calculated as U/mg protein.

Histopathological studies

A portion of the liver was cut into two to three pieces of approximately 6mm³ size and fixed in phosphate buffered 10% formaldehyde solution. After embedding in paraffin wax, thin sections of 5µm thickness of liver tissue were cut and stained with haematoxylin – eosin. The thin sections of liver were made into permanent slides and examined under high-resolution microscope with photographic facility and photomicrographs were taken.

Statistical analysis

All data obtained were analyzed by ANOVA followed by Students't test. Values at P <0.001 were considered significant.

RESULTS

Activities of serum SGPT, SGOT and ALP (marker enzymes for liver damage) were markedly elevated in acetaminophen treated animals compared to normal control rats, indicating liver damage. Administration of Methanolic extracts of *Amorphophallus campanulatus* at doses of 250 and 500 mg/kg remarkably prevented acetaminophen-induced elevation of serum SGPT, SGOT and ALP in Table 1.

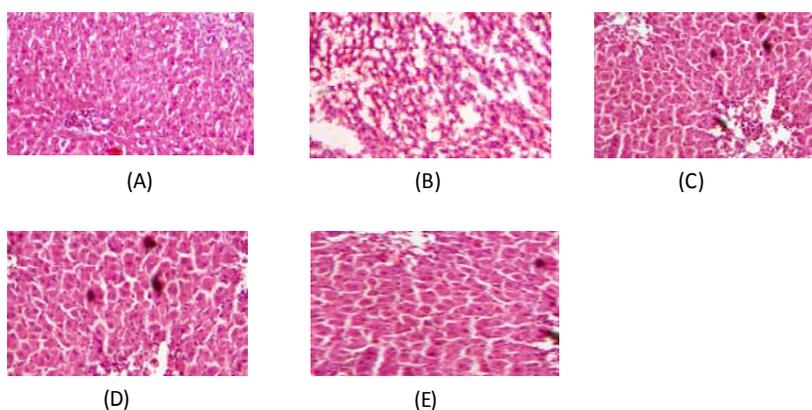
Since oxidative stress contributes to the development of acetaminophen -induced hepatotoxicity^{12,13,14}, the levels of liver antioxidant enzymes SOD, CAT, and GPx, were measured. Amounts of SOD, CAT, and GPx were significantly diminished in the negative Control group as compared with the normal control ($P < 0.001$). Pretreatment with 250 or 500 mg/kg of Methanolic extract *Amorphophallus campanulatus* of significantly raised the antioxidant enzyme levels as compared with the rats intoxicated with acetaminophen treatment. These results are presented in Table 2.

The histopathological studies also supported the protective properties of *Amorphophallus campanulatus*. The areas of necrosis and ballooning degeneration of hepatocytes were observed in the toxic group. The group of animals pre-treated with *Amorphophallus campanulatus* showed a marked protective effect with decreased necrotic zones and hepatocellular degeneration. The photomicrographs of the liver sections were given in Fig -1.

Table 1: Effect of methaolic extract *Amorphophallus campanulatus* on liver function marker in acetaminophen-intoxicated rats

0	Group	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	TP (g/dL)
1	Control	152.4±9.8	98.6±17.2	78.6±8.9	9.08±0.16
2	Negative control	368.6±11.4	285±14.6	182.3±6.4	6.86±0.13
3	ME-250mg./kg	208.2±3.68**	162.3±4.8**	104±4.2**	8.64±0.3**
4	ME-500mg/kg	164.5±3.74 **	112.8±6.4**	94.6±9.23**	8.82±0.05**
5	Silymarin (50mg/kg)	162±1.74 **	110.2±1.4**	92.4±6.41**	8.89±0.12**

Each value is represented as mean±SEM, No of animals [n] =6, ** p<0.001vs Normal control. Datas were analyzed by ANOVA followed by Students't test. Values at p <0.001 were considered significant.



Photomicrographs (original magnification 45×) of histopathological studies of livers of various groups stained with haematoxylin and eosin. (A) Normal architecture of rat liver. (B) Necrosis and hepatocellular degeneration of acetaminophen intoxicated. (C) Lesser damage of hepatocytes and low index of necrosis in ME-250mg./kg pre-treated group. (D) Very Lesser damage of hepatocytes and low index of necrosis in ME-500mg./kg pre-treated group. (E) Very Lesser damage of hepatocytes and low index of necrosis in Silymarin pre-treated group

Fig. 1: Histopathological studies

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

S. No.	Group	SOD (μ /mg protein)	CAT (K/mg protein)	GSH(mg/100g)
1	Control	3.61 \pm 0.16	35.66 \pm 1.9	46.8 \pm 1.5
2	Negative Control	1.57 \pm 0.07**	22.5 \pm 1.25**	20.8 \pm 3.3**
3	ME-250mg./kg	2.17 \pm 0.23**	26.66 \pm 1.39**	39.9 \pm 1.1**
4	ME-500mg/kg	2.79 \pm 0.24**	31.8 \pm 1.53**	41.2 \pm 3.9**
5	Silymarin (50mg/kg)	2.88 \pm 0.08**	32.88 \pm 1.25**	43 \pm 2.9**

DISCUSSION

In the present study, the Methanolic extract of *Amorphophallus campanulatus* was observed to exhibit hepatoprotective effect as demonstrated by a significant decrease in SGOT, SGPT, and ALP concentrations and in rats induced with acetaminophen hepatotoxicity. Moreover, the Methanolic extract of *Amorphophallus campanulatus* enhanced the activities of antioxidant enzymes (SOD, CAT, GPx) and diminished the amount of lipid peroxide against the acetaminophen induced hepatotoxicity in these animals, suggesting that the reduction of oxidative stress in this scenario likely plays a role in the mechanism of its hepatoprotective effects. Acetaminophen is an antipyretic and analgesic drug, which is widely used to cure fever, headache and other pains, and is readily available without prescription. When taken in at toxic doses, it becomes a potent hepatotoxin, generating fulminated hepatic and renal tubular necrosis which is lethal in humans and experimental animals^{15,16}.

The laboratory features of hepatotoxicity induced by acetaminophen resemble other kinds of acute inflammatory liver disease with prominent increase of SGOT, SGPT, and ALP levels¹⁷. In this present study, the serum level of hepatic enzymes SGOT, SGPT, and ALP were increased and reflected the hepatocellular damage in the acetaminophen induced hepatotoxicity animal model. The Methanolic extracts of *Amorphophallus campanulatus* with concentrations of 250 and 500 mg/kg, however, could lower the SGOT, SGPT, and ALP in these acetaminophen intoxicated animals.

The metabolic activation and biochemical mechanisms of hepatotoxicity induced by acetaminophen have been reviewed, and it has been shown that overdose of acetaminophen can cause centrilobular hepatic necrosis, liver function failure, and death in human as well as experimental animals¹⁸. Acetaminophen at therapeutic dosage is primarily metabolized and detoxified by glucuronidation and sulphation, and subsequently followed by renal excretion¹⁹. However, when acetaminophen is taken in at toxic doses, the compound is converted to a toxic form, NAPQI. NAPQI is an electrophilic intermediate which is oxidized by cytochrome P450 and converted to a highly reactive and toxic metabolite as in cases of acetaminophen overdose^{20, 21}.

NAPQI can rapidly react with glutathione (GSH) and lead to a 90% total hepatic GSH depletion in cells and mitochondria, which can result in hepatocellular death and mitochondrial dysfunction²². In addition, NAPQI can increase the formation of ROS and reactive nitrogen species (RNS) such as superoxide anion, hydroxyl radical, and hydrogen peroxide, and nitro oxide and peroxy nitrite, respectively. Excess levels of ROS and RNS can attack biological molecules such as DNA, protein, and phospholipids, which leads to lipid peroxidation, nitration of tyrosine, and depletion of the antioxidant enzymes (SOD, CAT, GPx) that further results in oxidative stress²³. NAPQI can also induce DNA strand breaks and promote apoptosis and necrosis in acetaminophen induced hepatotoxicity^{24, 25}.

In conclusion, the present study has demonstrated that the Methanolic extract of *Amorphophallus campanulatus* has hepatoprotective activity against acetaminophen induced hepatotoxicity in rats and it may be due to their anti-oxidant property.

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