Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

# Protective effect of *Amorphophallus campanulatus* (Roxb.) Blume. tuber against thioacetamide induced oxidative stress in rats

Puthuparampil Nazarudeen Ansil, Anand Nitha, Santhibhavan Prabhakaran Prabha, Pallara Janardhanan Wills, Vahab Jazaira, Mukalel Sankunni Latha*

*Biochemistry and Pharmacognosy Research Laboratory, School of Biosciences, M.G. University, P.D. Hills. P.O, Kottayam, Kerala-686560, India*

ARTICLE INFO

*Article history:*
Received 22 August 2011
Received in revised form 15 September 2011
Accepted 15 October 2011
Available online 20 November 2011

*Keywords:*
*Amorphophallus campanulatus*
Thioacetamide
DPPH
Hepatotoxicity
Hepatoprotective
Antioxidant activity
Silymarin

ABSTRACT

**Objective:** To identify the phytochemical constituents of *Amorphophallus campanulatus* (*A. campanulatus*) tuber and to evaluate its antioxidant potential through *in vitro* and *in vivo* models. **Methods:** Phytochemical screening and *in vitro* antioxidant activities of *A. campanulatus* tuber *n*-hexane extract (ACHE) and methanolic extract (ACME) were evaluated using DPPH, hydroxyl radical, reducing power and total antioxidant capacity assays. The total phenolic and flavonoid contents were also investigated. The protective potential of two different doses of ACME (125 and 250 mg/kg) was also evaluated against thioacetamide (TAA) induced oxidative stress in rats. Silymarin used as a standard drug control. Hepatotoxicity was assessed by quantifying the serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH). The antioxidant potential of ACME were also evaluated by the estimation of catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST), reduced glutathione (GSH) and lipid peroxidation (Thiobarbituric acid reactive substances) in hepatic and renal tissues. Histopathologic changes of liver were also evaluated. **Results:** *In vitro* studies revealed that ACME has higher antioxidant and radical scavenging activity than ACHE, which may be attributed to its higher phenolic and flavonoid content. ACME significantly prevented the elevation of serum AST, ALT, ALP, LDH, and tissue malondialdehyde levels($P < 0.05$). Hepatic and renal GSH, GST, GR, GPx, and catalase levels were remarkably increased by the treatment with the extract. Quantification of histopathological changes also supported the dose dependent protective effects of ACME. **Conclusions:** The results do suggest that *A. campanulatus* tuber could be considered as a potential source of natural antioxidant.

## 1. Introduction

Oxidative stress induced by free radicals is believed to be a primary factor in various degenerative diseases such as atherosclerosis, carcinogenesis, diabetes, hypertension and aging. Generation of reactive oxygen species (ROS) beyond the body´s endogenous antioxidant balance cause a severe imbalance of the cellular antioxidant defense mechanism and results in oxidative stress which deregulates the cellular function leading to various pathological conditions[1]. Various endogenous antioxidant defense mechanisms play an important role in the elimination of ROS and lipid peroxides and therefore protect the cells against its toxic effects[2]. The antioxidant defense can be further strengthened with a diet rich in antioxidants. Plant drugs are known to play a vital role in the management of liver diseases. Hepatoprotective herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotinoids, glycosides, flavanoids, organic acids, lipids, alkaloids and xanthines.

*Amorphophallus campanulatus* (Roxb.) (*A. campanulatus*) Blume (Family: Araceae) is a perennial herb commonly known as elephant foot yam. It grows in wild and cultivate as vegetable in Asian and African countries[3]. The rounded tuberous root stock or corm is used as an important source of food in many parts of the world. Till date, different

*Corresponding author: Mukalel Sankunni Latha, Professor, School of Biosciences, M.G. University, P.D.Hills.P.O. Kottayam, Kerala - 686560. India.
Tel: +91 481 2731035
Fax: +91 481 2731009
E-mail: mslathasbs@yahoo.com
Foundation project: This study was financially supported by University Grants Commission (UGC), New Delhi, India [F.No.37-265/2009(SR)].

components including glucose, galactose, rhamnose, xylose, triacontane, lupeol, betulinic acid, stigmasterol, β-sitosterol and its palmitate have been reported in the corm of *A. campanulatus*[4]. Further, the plant is valuable as medicine especially the corm has been used traditionally for the treatment of liver diseases, abdominal pain, abdominal tumours, piles, enlargement of spleen, asthma and rheumatism[5]. Moreover, the corm has been reported to possess antibacterial, antifungal and cytotoxic activities[6]. The present investigation was undertaken to identify the phytochemical constituents of *A. campanulatus* tuber and to evaluate its antioxidant potential through *in vitro* and *in vivo* models.

## 2. Material and methods

### 2.1. Chemicals

Thioacetamide (TAA) was purchased from Loba Cheme, Mumbai, India. 5, 5′-dithiobis-(2-nitrobenzoic acid), 1-chloro-2, 4-dinitrobenzene and nitroblue tetrazolium were purchased from Sisco Research Laboratories, Mumbai, India. Silymarin and 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) were procured from Sigma Chemical Co., St. Louis, MO, USA. Assay kits for serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were purchased from Agappe Diagnostic, India. All other chemicals were of analytical grade.

### 2.2. Collection and preparation of plant extracts

*A. campanulatus* tubers were collected from the local market (Kottayam, Kerala, India) and authenticated. A voucher specimen (SBSBRL.02) is maintained in the institute. Tubers were cleaned, chopped, shade-dried and powdered. A 50 g of dried powder was soxhlet extracted with 400 mL of *n*-hexane and followed by methanol for 48 h. The extracts were concentrated under reduced pressure using a rotary evaporator and were kept under refrigeration. The yield of the *n*-hexane and methanolic extracts were 0.45% (w/w) and 9.3% (w/w), respectively. The concentrate was suspended in DMSO for *in vitro* studies and in 5% Tween 80 for *in vivo* studies.

### 2.3. Preliminary phytochemical screening

Preliminary phytochemical screening of *n*-hexane and methanolic extracts of *A. campanulatus* tuber were carried out for the detection of phytoconstituents using standard conventional protocols[7].

### 2.4. Evaluation of in vitro antioxidant activity

The antioxidant activity of *A. campanulatus* tuber *n*-hexane extract (ACHE) and *A. campanulatus* tuber methanolic extract (ACME) was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. The reduction capability of DPPH radicals is determined by the decrease in its absorbance at 517 nm[8]. Ascorbic acid was used as standard control.

The inhibitory effect of the extracts to prevent the degradation of deoxyribose by $Fe^{3+}$ ions in presence of $H_2O_2$-EDTA-ascorbate was determined in hydroxyl radical scavenging assay[9]. The reference standard used was quercetin.

The antioxidant activity of ACHE and ACME was also manifested through their reducing power. In this assay, the $Fe^{3+} \rightarrow Fe^{2+}$ transformation was established as reducing capacity[10]. Ascorbic acid was used as a standard antioxidant compound.

The amount of total phenolic compounds in the extracts was determined using the Folin-Ciocalteu method[11]. A calibration curve of gallic acid was prepared and the results were expressed as mg gallic acid equivalents (GAE)/g dry extract.

The total flavonoid content of ACHE and ACME was determined spectrophotometrically by the method described by Quettier-Deleu *et al*[12]. It was determined using a standard curve with quercetin and expressed as milligrams of quercetin equivalents (QE/g of dry extract).

The total antioxidant capacity of the extracts was determined according to the method of Jayaprakasha *et al*[13]. Ascorbic acid was used as standard and the total antioxidant capacity was expressed as the equivalent of ascorbic acid per gram of extract.

All the tests were performed in triplicate and the results were expressed with the mean values.

### 2.5. Evaluation of in vivo antioxidant activity

#### 2.5.1. Animals and diets

Male Wistar rats weighing (156±5.8) g ($n$=30) were used in this study. The animals were housed in polypropylene cages and given standard rat chow (Sai Feeds, Bangalore, India) and drinking water *ad libitum*. The animals were maintained at a controlled condition of temperature of 26-28 ℃ with a 12 h light: 12 h dark cycle. Animal studies were followed according to Institute Animal Ethics Committee regulations approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (Reg. No. B 2442009/6) and conducted humanely.

#### 2.5.2. Preparation of doses and treatments

Thioacetamide suspended in normal saline was administered (100 mg/kg body weight) subcutaneously to induce the oxidative stress in rats[14]. Silymarin at an oral dose of 100 mg/kg body weight was used as standard control in the experiment[15]. The most promising ACME was selected to study the *in vivo* antioxidant activity. It is reported that the extracts of *A. campanulatus* tubers are safe up to the dose of 2 000 mg/kg[16]. Different doses (125 and 250 mg/kg) of ACME suspended in 5% Tween 80 were also prepared for oral administration to the animals.

#### 2.5.3. Experimental design

Rats were divided into five groups with six rats in each

group and that were treated as follows:
Group Ⅰ: Control rats (vehicle only);
Group Ⅱ: Thioacetamide control (100 mg/kg, *s.c.*) ;
Group Ⅲ: Thioacetamide (as in group Ⅱ)+silymarin (100 mg/kg, *p.o.*);
Group Ⅳ: Thioacetamide (as in group Ⅱ)+ACME (125 mg/kg, *p.o.*);
Group Ⅴ: Thioacetamide (as in group Ⅱ)+ACME (250 mg/kg, *p.o.*)

All the groups except group Ⅰ received a single dose of thioacetamide (100 mg/kg, *s.c.*) suspended in normal saline on 9th day of the experiment. Group Ⅲ received silymarin and the groups Ⅳ and Ⅴ received ACME for 9 days before thioacetamide challenge[17]. Group Ⅰ animals treated as vehicle control received 5% Tween 80 and normal saline instead of drug and thioacetamide respectively. Animals were sacrificed 24 h after thioacetamide administration.

#### 2.5.4. Serum enzyme analysis

Hepatotoxicity was assessed by quantifying the serum levels of AST (EC 2.6.1.1), ALT (EC 2.6.1.2), ALP (EC 3.1.3.1) and LDH (EC 1.1.1.27) by kinetic method using the kit of Agappe Diagnostic Ltd., India. Activities of these serum enzymes were measured using semi autoanalyzer (RMS, India).

#### 2.5.5. Tissue analysis

Liver and kidney were excised, washed thoroughly in ice-cold saline to remove the blood. Ten percent of homogenate was prepared in 0.1M Tris HCl buffer (pH-7.4). The homogenate was centrifuged at 3 000 rpm for 20 min at 4 ℃ and the supernatant was used for the estimation of catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST), reduced glutathione (GSH), lipid peroxidation [Thiobarbituric acid reactive substances(TBARS)] and total protein.

Tissue CAT (EC 1.11.1.6) activity was determined from the rate of decomposition of $H_2O_2$[18]. GPx (EC 1.11.1.9) activity was determined by measuring the decrease in GSH content after incubating the sample in the presence of $H_2O_2$ and $NaN_3$[19]. GR (EC 1.6.4.2) activity was assayed at 37 ℃ and 340 nm by following the oxidation of NADPH by GSSG[20]. GST (EC 2.5.1.18) activity was determined from the rate of increase in conjugate formation between reduced glutathione and CDNB[21]. Reduced GSH was determined based on the formation of a yellow colored complex with DTNB[22]. The level of lipid peroxidation was measured as malondialdehyde (MDA), a thiobarbituric acid reacting substance (TBARS), using 1′1′3′3′ tetramethoxypropane as standard[23]. Protein content in the tissue was determined using bovine serum albumin (BSA) as the standard[24].

#### 2.5.6. Histopathological studies

Small pieces of liver tissues fixed in 10% buffered formalin were processed for embedding in paraffin. Sections of 5-6 μm were cut and stained with hematoxylin and eosin and examined for histopathological changes under the microscope (Motic AE 21, Germany). The microphotographs were taken using Moticam 1000 camera at original magnification of 100×.

Liver sections were graded numerically to assess the degree of histological features in acute hepatic injury. Centrilobular necrosis is the necrosis around the central vein characterized by prominent ballooning, swollen granular cytoplasm with fading nuclei. Bridging hepatic necrosis is a form of confluent necrosis of liver cells linking central veins to portal tracts or portal tracts to one another[25]. A combined score of centrilobular necrosis, bridging hepatic necrosis and lymphocyte infiltration was given a maximum value of 6 and descriptive modifiers such as mild, moderate, and severe was applied to activity and staging. The parameters were graded from score 0 to 6, with 0 indicating no abnormality, 1 to 2 indicating mild injury, 3 to 4 indicating moderate injury and 5 to 6 representing severe liver injury.

### 2.6. Statistical analysis

Results are expressed as mean±SD and all statistical comparisons were made by means of one-way ANOVA test followed by Tukey′s post hoc analysis and *P*-values less than or equal to 0.05 were considered significant.

## 3. Results

### 3.1. Preliminary phytochemical analysis

The preliminary phytochemical evaluation of ACHE showed the presence of steroids. But the screening of ACME revealed the presence of phytochemical constituents such as alkaloids, tannins, glycosides, phenols, flavonoids, saponins and carbohydrates.

### 3.2. In vitro antioxidant activity

#### 3.2.1. DPPH radical scavenging activity

The DPPH radical scavenging activity of extracts and standard exhibited a concentration dependent reaction trend. The $IC_{50}$ values of ascorbic acid, ACME and ACHE were 4.2, 52.4 and 1 470.5 μg/mL, respectively.

#### 3.2.2. Hydroxyl radical scavenging activity

ACME has better hydroxyl radical scavenging activity than ACHE. Extracts and quercetin, the standard antioxidant, scavenged hydroxyl radicals in a concentration dependent manner and the estimated $IC_{50}$ values of ACHE, ACME and quercetin were 29.2, 23.4 and 20.8 μg /mL, respectively.

#### 3.2.3. Determination of reducing power

Ascorbic acid used as reference compound exhibited a superior reducing power at all concentrations, compared with ACHE and ACME (Figure 1). At 0.50 mg/mL, the absorbencies of ascorbic acid, ACHE and ACME (at 700 nm) were 1.530, 0.081 and 0.101, respectively. These values reflect the following reducing capability: ascorbic acid > ACME > ACHE.

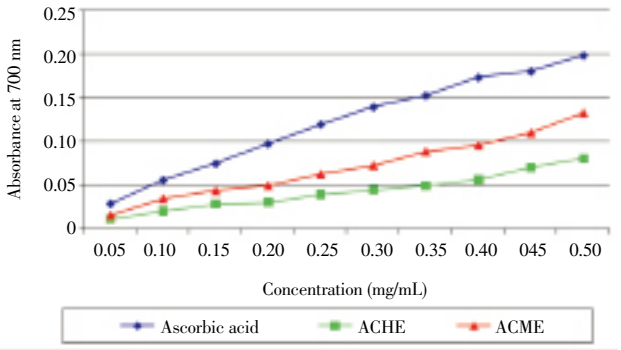


**Figure 1.** Reducing power of ACHE and ACME compared with standard antioxidant ascorbic acid.

### *3.2.4. Phenolic contents, flavonoid contents and total antioxidant activity*

ACME had a higher quantity of total phenolics [(23.00±2.30) mg GAE/g dry extract] than ACHE [(5.70±1.20) mg GAE/g dry extract]. ACME, which has a high total antioxidant activity [(90.00±2.90) mg ascorbic acid/g dry extract] also had a great quantity of flavonoids [(5.20±0.80) mg QE/g dry extract] compared to ACHE. In ACHE, the total antioxidant activity [(35.00±4.70) mg ascorbic acid /g dry extract] and flavonoid content [(0.53±0.20) mg QE/g dry extract] were comparatively low.

## *3.3. In vivo antioxidant activity*

### *3.3.1. Serum enzymes*

The serum levels of AST, ALT, ALP and LDH in group Ⅱ were significantly elevated by the administration of a single dose of TAA, when compared to normal control($P<0.05$). Treatment with ACME at a dose of 125 and 250 mg/kg showed a significant decrease of AST, ALT, ALP and LDH($P<0.05$). Standard control drug, silymarin at a dose of 100 mg/kg also prevented the elevation of serum enzymes (Figure 2). Treatment with 250 mg/kg of ACME and 100 mg/kg of silymarin exhibited a protection of 81.7% and 77.5% in AST levels, 43.0% and 29.3% in ALT levels, 38.0% and 35.2% in ALP levels and 72.5% and 65.5% in LDH levels respectively. The preventive effect of the extract in decreasing the elevated levels of serum enzymes was in a dose dependent manner.

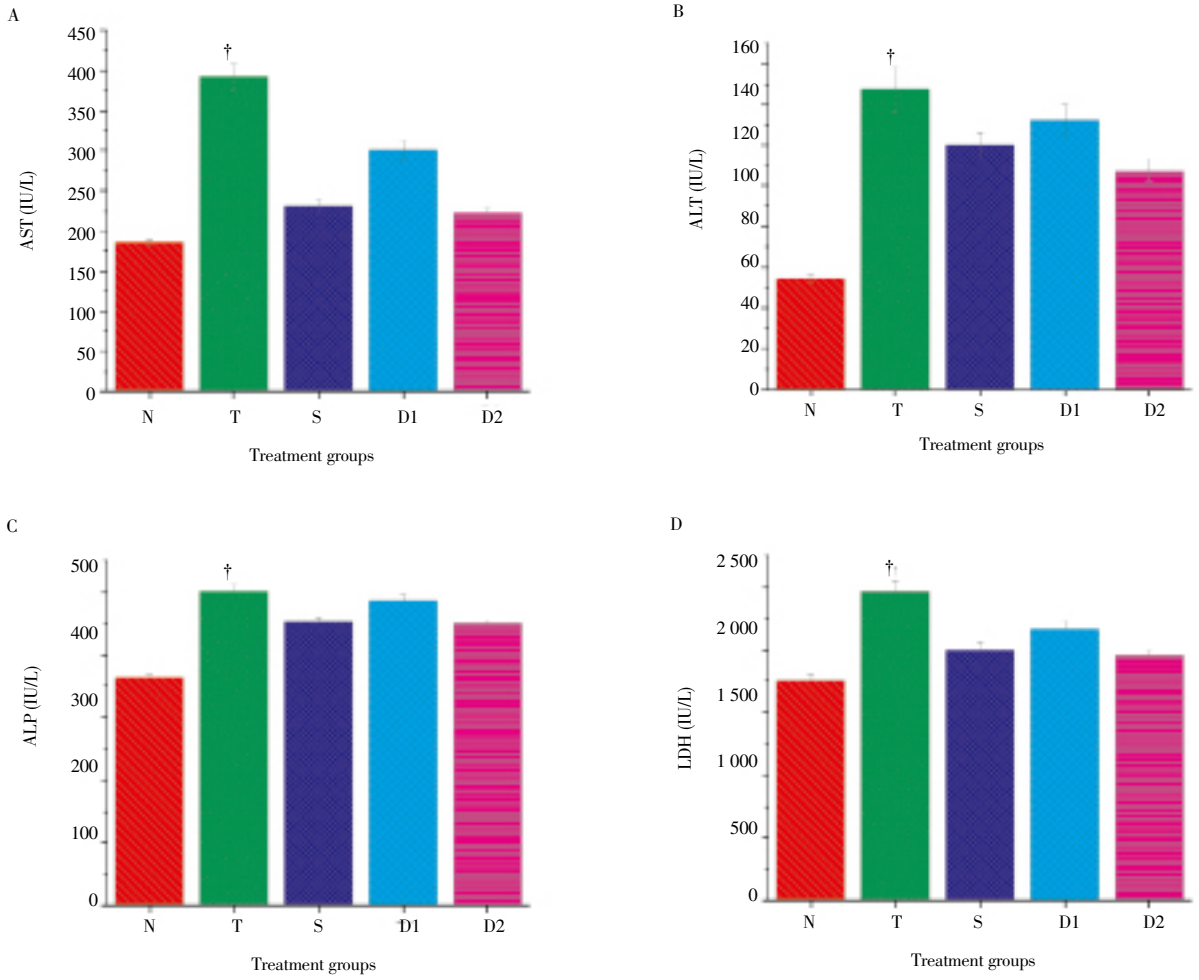


**Figure 2.** Effects of ACME on changes in serum enzyme levels of rats treated with TAA.
(A)–Aspartate aminotransferase, (B)–Alanine aminotransferase, (C)– Alkaline phosphatase and (D)– Lactate dehydrogenase; N: Normal control, T: Thioacetamide control, S: Silymarin, D1: ACME–125 mg/kg, D2: ACME–250 mg/kg; †$P<0.05$ versus normal control, *$P<0.05$ versus thioacetamide control.

**Table 1**

Protective effects of ACME against TAA induced changes in the liver antioxidant status(mean±SD, $n$ = 6).

| Treatment groups | GSH (nmol/mg protein) | GST (μmol CDNB-GSH conjugate formed/min/mg protein) | GR (nmol of GSSG utilized/min/mg protein) | GPx (nmol of GSH oxidized/min/mg protein) | CAT (U/mg protein) | MDA (nmol/g tissue) |
|---|---|---|---|---|---|---|
| Normal control | 24.8±0.4 | 74.8±0.4 | 19.8±0.7 | 298.4±7.2 | 51.5±2.5 | 45.6±0.5 |
| TAA (100 mg/kg, *s.c.*) | 15.2±0.3$^{\Delta}$ | 37.3±0.4$^{\Delta}$ | 7.7±0.5$^{\Delta}$ | 174.2±8.9$^{\Delta}$ | 35.4±1.4$^{\Delta}$ | 75.7±1.1$^{\Delta}$ |
| Silymarin (100 mg/kg)+TAA | 21.3±0.5* | 63.3±0.3* | 15.8±0.3* | 271.6±7.4* | 46.5±1.9* | 59.2±0.8* |
| ACME (125 mg/kg) +TAA | 18.2±0.3* | 59.3±0.5* | 10.6±0.3* | 209.7±9.0* | 39.1±1.0* | 63.3±1.0* |
| ACME (250 mg/kg) +TAA | 23.3±0.4* | 67.1±0.2* | 17.2±0.3* | 274.5±6.3* | 47.6$_{V}$1.2* | 52.0±0.2* |

$^{\Delta}P < 0.05$ versus normal control; *$P$<0.05 versus thioacetamide control.

**Table 2**

Protective effects of ACME against TAA induced changes in the kidney antioxidant status(mean±SD, $n$ = 6).

| Treatment groups | GSH (nmol/mg protein) | GST (μmol CDNB-GSH conjugate formed/min/mg protein) | GR (nmol of GSSG utilized/min/mg protein) | GPx (nmol of GSH oxidized/min/mg protein) | CAT (U/mg protein) | MDA (nmol/g tissue) |
|---|---|---|---|---|---|---|
| Normal control | 18.2±0.4 | 49.8±0.8 | 16.7±0.3 | 285.7±6.7 | 58.8±1.2 | 42.4±0.4 |
| TAA(100 mg/kg, *s.c.*) | 8.1±0.2$^{\Delta}$ | 27.4±0.4$^{\Delta}$ | 6.3±0.4$^{\Delta}$ | 160.3±8.1$^{\Delta}$ | 47.1±1.7$^{\Delta}$ | 72.3±0.7$^{\Delta}$ |
| Silymarin(100 mg/kg)+TAA | 14.7±0.5* | 43.3±0.3* | 12.0±0.5* | 258.9±5.3* | 56.8±1.9* | 54.6±0.3* |
| ACME(125 mg/kg) +TAA | 11.1±0.5* | 39.2±0.4* | 9.2±0.4* | 197.6±8.9* | 49.3±1.5* | 59.3±0.5* |
| ACME (250 mg/kg) +TAA | 16.1±0.5* | 47.2±0.3* | 14.2±0.3* | 261.5±5.8* | 56.3±0.5* | 49.4±0.6* |

$^{\Delta}P < 0.05$ versus normal control; *$P$<0.05 versus thioacetamide control.

### *3.3.2. Estimation of reduced GSH*

Rats administered with TAA alone were found significantly lower level of reduced GSH($P$< 0.05). Treatment with ACME exhibited significant increase in both hepatic (Table 1) and renal (Table 2) glutathione levels($P$< 0.05). In liver and kidney, 250 mg/kg of ACME showed a protection of 84.6% and 83.6%, respectively. Silymarin treated rats also prevented the lowering of GSH and the percentage of protection was 63.5% and 63.9%, respectively for liver and kidney.

### *3.3.3. Estimation of GST*

When compared to normal control the GST activity of liver and kidney tissues were significantly reduced in TAA intoxicated rats($P$< 0.05). ACME dose dependently increased the activity of GST in both the hepatic and renal tissues (Table 1 and 2) ($P$< 0.05). Treatment with 250 mg/kg ACME exhibited significant increase *ie*., 79.4% and 88.6%, respectively in hepatic and renal GST levels. In addition, silymarin treated rats also prevented the TAA induced decrease in GST activity by 69.2% and 70.9% in hepatic and renal tissues respectively.

### *3.3.4. Estimation of GR*

GR activity was significantly decreased in TAA treated animals when compared to normal control($P$< 0.05). A significant increase in the level of GR was observed in ACME (125 and 250 mg/kg) and silymarin (100 mg/kg) treated rats intoxicated with TAA($P$< 0.05). Both hepatic and renal tissues showed the same pattern of GR activity in all groups treated with ACME and silymarin (Table 1 and 2). The percentage of protection in liver and kidney tissues were 78.9% and 75.9%, respectively for 250 mg/kg of ACME. Silymarin restored the GR activity by 67.1% in liver and 54.8% in kidney.

### *3.3.5. Estimation of GPx*

Activities of hepatic and renal GPx were significantly lowered in TAA treated rats (Table 1 and 2) ($P$< 0.05). ACME dose dependently prevented the lowering of GPx in both the organs compared to TAA alone treated groups. In liver and kidney, 250 mg/kg of ACME showed a protection of 80.7% and 80.6%, respectively. Silymarin treated rats also prevented the lowering of GPx by 78.3% in hepatic and 78.6% in renal tissues.

### *3.3.6. Estimation of CAT*

Animals injected with TAA alone showed significant reduction in hepatic and renal CAT activity($P$< 0.05). ACME dose dependently increased the activity of CAT in both hepatic and renal tissues (Table 1 and 2). Treatment with 250 mg/kg of ACME exhibited significant increase *ie*., 75.8% and 78.6%, respectively in liver and kidney. In addition, silymarin treated rats also prevented the TAA induced decrease in CAT activity by 68.7% and 82.2% in hepatic and renal tissues respectively($P$< 0.05).

### *3.3.7. Estimation of Lipid peroxidation (MDA)*

A significant increase $P$< 0.05) in tissue MDA level was observed in TAA alone treated rats. However, TAA induced elevation of MDA concentration was lowered ($P$< 0.05) by 78.5% in hepatic and renal tissues of rats treated with ACME at a dose of 250 mg/kg. Silymarin also showed a protection of 54.8% in liver and 59.3% in kidney($P$< 0.05) (Table 1 and 2).

### *3.4. Histopathological analysis*

Normal architecture of the liver (Figure 3B) was completely lost in rats treated with TAA with the appearance of centrilobular necrosis, bridging hepatic necrosis and

lymphocyte infiltration with a score of 5.3±0.5 ($n$=3). The animals administered with silymarin and ACME at 250 and 100 mg/kg showed a significant protection from TAA induced liver damage($P$< 0.05) as evident from hepatic architectural pattern with mild to moderate hepatitis with scores 3.3±0.5, 3.0±1.0, and 2.6±0.5 ($n$=3) ($P$< 0.05), respectively (Figure 3C-E).

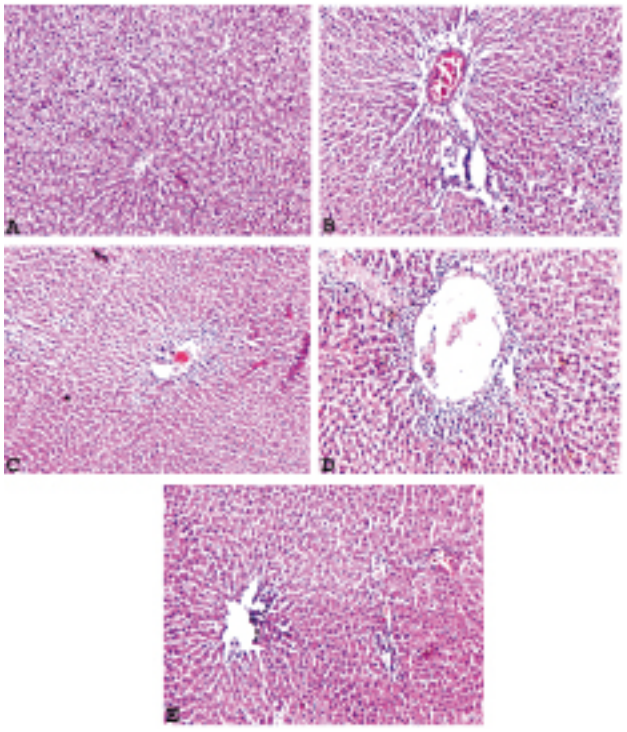

**Figure 3.** Histopathological changes occurred in rat liver after TAA intoxication and prevention by the treatment with ACME (hematoxylin and eosin, 100×).

(A) Normal control; (B) TAA control (100 mg/kg *s.c.*); (C) Silymarin (100 mg/kg) + TAA; (D) ACME (125 mg/kg) + TAA; (E) ACME (250 mg/kg) + TAA.

## 4. Discussion

Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases. In the present study, $n$-hexane and methanolic extracts of *A. campanulatus* tuber exhibited *in vitro* antioxidant activity in DPPH radical scavenging assay, hydroxyl radical scavenging assay and reducing power assay. DPPH is a stable free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule[26]. Though the DPPH radical scavenging abilities of the extracts were less than that of ascorbic acid, the study showed that ACME have potent proton-donating ability and could serve as free radical inhibitors or scavengers. The extremely reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins[27]. The potent hydroxyl radical scavenging activity of ACME may be due to its active hydrogen donating ability. The total reducing power of ACME was also comparatively higher than ACHE. The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing properties are generally associated with the presence of reductones which have shown antioxidant action by breaking the free radical chain reaction donating a hydrogen atom[28].

ACME had a higher quantity of total phenolics and flavonoids compared to ACHE. Phenolic compounds function as high-level antioxidants because they possess the ability to absorb and neutralize free radicals as well as quench reactive oxygen species. Flavonoids, one of the most diverse and widespread groups of natural compounds, are also probably the most natural phenolics capable of exhibiting *in vitro* and *in vivo* antioxidant activities. The results obtained in the present study showed that ACME can effectively scavenge reactive oxygen species including hydroxyl radical as well as other free radicals under *in vitro* conditions. The antioxidant activity of ACHE and ACME was compared with that of standard compounds and the ACME has been proven a promising antioxidant activity and hence it is chosen for *in vivo* studies.

TAA is a compound endowed with liver damaging and carcinogenic activity. It has been used to induce a model of acute liver injury in rats. Shortly after its administration, thioacetamide is metabolized to acetamide and thioacetamide-5-oxide. The latter binds to tissue macromolecules responsible for the change in cell permeability, increased intracellular concentration of $Ca^{++}$, increase in nuclear volume and enlargement of nucleoli and inhibits mitochondrial activity eventually leading to hepatic necrosis[29].

The increase in the activities of AST, ALT, ALP and LDH in serum of rats treated with TAA might be due to the increased permeability of plasma membrane or cellular necrosis leading to leakage of the enzymes to the blood stream[30] and this showed the stress condition of the TAA treated animals. In the present study, administration of a single dose of TAA significantly ($P$< 0.05) elevated the serum transaminases, ALP and LDH activities compared to the normal rats. Marked decrease in serum transaminases, ALP and LDH levels demonstrate the preventive effect of ACME in TAA intoxication.

Generation of a large amount of ROS due to TAA can overwhelm the antioxidant defense mechanism and damage cellular ingredients such as lipids, proteins and DNA; this in turn can impair cellular structure and function. The intra cellular antioxidant system comprises of different free radical scavenging antioxidant enzymes along with some non-enzyme antioxidants like GSH and other thiols. CAT, GST, GPx, and GR constitute the first line of cellular antioxidant defense enzymes. Thus, to eliminate free radicals, these cellular antioxidants play an important role and equilibrium exists between these enzymes under normal conditions. When excess free radicals are produced, this equilibrium is lost and consequently oxidative insult is established[31]. Glutathione detoxifies toxic metabolites of drugs, regulates gene expression, apoptosis and transmembrane transport of organic solutes and it is essential to maintain the reduced status of the cell/tissue[32]. In the present study, treatment with ACME and silymarin significantly ($P$< 0.05) enhanced the hepatic and renal GSH level compared to the TAA alone treated animals. The elevated GSH level could explain the dose dependent (250 and 125 mg/kg) hepatoprotective action of the extract. Our findings also show that pre-treatment with ACME prior to TAA intoxication significantly

($P< 0.05$) enhanced the GST activity, a phase Ⅱ enzyme. This was attributed to the decreased bioactivation of TAA caused by the ACME pre-treatment. GST offers protection against lipid peroxidation by promoting the conjugation of toxic electrophiles with GSH[33]. GR is also essential for the maintenance of GSH levels *in vivo*[34]. The significantly ($P< 0.05$) elevated level of GR activity in the hepatic and renal tissues of ACME pre-treated rats shows the role of extract to maintain the GSH level in these tissues. These results indicate that the protection afforded by ACME against TAA induced hepatotoxicity may be related to the increased cellular GSH content, the increased GST and GR activity. Further, GPx catalyzes the GSH dependant reduction of $H_2O_2$ and other peroxides and protects the organism from oxidative damage[32]. The significant ($P< 0.05$) restoration of GPx activity in ACME and silymarin pre-treated rats might be due to the antioxidant activity by detoxifying the endogenous metabolic peroxides generated after TAA injury in hepatic and renal tissues.

Catalase is responsible for breakdown of $H_2O_2$, an important ROS, formed during the reaction catalyzed by SOD[35]. Reduced activity of catalase after exposure to TAA in the present finding could be correlated to increased generation of $H_2O_2$. The pre-treatment of ACME significantly ($P< 0.05$) aided to maintain the CAT activity near to normal level in both hepatic and renal tissues. This evidently shows the antioxidant property of the extract against oxygen free radicals. The concentration of MDA in tissues of TAA alone exposed group was significantly ($P< 0.05$) differed from that of normal control. MDA is a major oxidation product of peroxidized polyunsaturated fatty acids and increased MDA content is an important indicator of lipid peroxidation[36-50]. Pre-treatment of rats with ACME protected the liver and kidney from increasing MDA formation. This demonstrates the antilipid peroxidative effect of the extract. The increased MDA content might have resulted from an increase of ROS as a result of stress condition in the rats with TAA intoxication. Histopathological evaluation showed negligible damage to a few hepatocytes present in the close vicinity of central vein in ACME treated rats and the improvement of histological scores proved the efficacy of the extract as an antihepatotoxic agent.

ACME contains β-sitosterol, a component reported as a hepatoprotective agent[51] and stigmasterol, a phytosterol reported as antioxidant compound[52]. The identified class of components in single or in combination with other components present in the extract might be responsible for the reduction of hepatotoxicity. In conclusion, the result of serum biochemical parameters, level of hepatic and renal lipid peroxides, glutathione antioxidant systems, CAT and histopathological studies support the dose dependent hepatoprotective and antioxidant activity of ACME. The present study also supports the traditional use of *A. campanulatus* tuber as a liver tonic. The results do suggest that *A. campanulatus* tuber might ameliorate oxidative damage induced by radicals and this can be employed as main ingredient in medical food/nutraceuticals for disorders due to oxidative stress. However, further pharmacological evidences at molecular level are required to establish the mechanism of the action of the drug which is underway.

## Conflict of interest statement

We declare that we have no conflict of interest.

## Acknowledgments

This study was financially supported by University Grants Commission (UGC), New Delhi, India [F.No.37-265/2009(SR)]. Financial assistance from UGC as Research Fellowship in Sciences for Meritorious Students to P.N. ANSIL is thankfully acknowledged.

## References

[1] Sinha D, Roy S, Roy M. Antioxidant potential of tea reduces arsenite induced oxidative stress in Swiss albino mice. *Food Chem Toxicol* 2010; **48**(4): 1032-1039.
[2] Halliwell B, Gutteridge JM, Cross CE. Free radicals, antioxidants and human disease: Where are we now? *J Lab Clin Med* 1992; **119**(6): 598-620.
[3] Das SS, Sen Malini, Dey YN, De S, Ghosh AK. Effects of petroleum ether extract of *Amorphophallous paeoniifolius* tuber on central nervous system in mice. *Indian J Pharm Sci* 2009; **71**(6): 651-655.
[4] Ramalingam R, Hima Bindu K, Bindu Madhavi B, Ravinder Nath A, David B. Phytochemical and anthelmintic evaluation of corm of *Amorphophallus campanulatus*. *Int J Pharma & Bio Sciences* 2010; Ⅵ(2): 1-9.
[5] Warrier PK, Nambiar VPK, Ramankutty C. *Indian medicinal plants, Vol* Ⅰ. Orient Longman Publisher; 2005.
[6] Khan A, Rahman M, Islam S. Antibacterial, antifungal and cytotoxic activities of tuberous roots of *Amorphophallus campanulatus*. *Turk J Biol* 2007; **31**(3): 157-172.
[7] Wagner H, Bladt S, Zgainski EM. *Plant drug analysis.* Heidelberg, Berlin: Springer Verlag; 1984.
[8] Aquino R, Morelli S, Lauro MR, Abdo S, Saija A, Tomaino A. Phenolic constituents and antioxidant activity of an extract of *Anthurium versicolar* leaves. *J Nat Prod* 2001; **64**(8): 1019-1023.
[9] Halliwell B, Gutteridge JM, Aruoma OI. The deoxyribose method: a simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals. *Anal Biochem* 1987; **165**(1): 215-219.
[10] Oyaizu M. Studies on product of browning reaction prepared from glucose amine. *Jpn J Nutri* 1986; **44**: 307-315.
[11] Yu L, Perret J, Davy D, Wilson J, Melby CL. Antioxidant properties of cereal products. *J Food Sci* 2002; **67**(7): 2600-2603.
[12] Quettier-Deleu C, Gressier B, Vasseur J, Dine T, Brunet C, Luyckx MC, et al. Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. *J Ethnopharmacol* 2000; **72**(1-2): 35-42.
[13] Jayaprakasha GK, Jaganmohan Rao L, Sakariah KK. Antioxidant activities of flavidin in different *in vitro* model systems. *Bioorg Med Chem* 2004; **12**(19): 5141-5146.
[14] Ahmad A, Pillai KK, Ahmad SJ, Balani DK, Najmi AK, Marwah R, et al. Evaluation of the hepatoprotective potential of jigrine pre-treatment on thioacetamide induced liver damage in rats. *Indian J Pharmacol* 1999; **31**(6): 416-421.
[15] Shyamal S, Latha PG, Suja SR, Shine VJ, Anuja GI, Sini S, et al. Hepatoprotective effect of three herbal extracts on aflatoxin B1-

intoxicated rat liver. *Singapore Med J* 2010; **51**(4): 326–331.
[16]Jain S, Dixit VK, Malviya N, Ambawatia V. Antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of *Amorphophallus campanulatus* Roxb. tubers. *Acta Pol Pharm* 2009; **66**(4): 423–428.
[17]Roy CK, Kamath JV, Asad M. Hepatoprotective activity of *Psidium guajava* Linn. Leaf extract. *Indian J Exp Biol* 2006; **44**(4): 305–311.
[18]Beers RF Jr, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J Biol Chem* 1952; **195**(1): 133–140.
[19]Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: Biochemical role as a component of glutathione peroxidase. *Science* 1973; **179**(73): 588–590.
[20]Beutler E. Red cell metabolism. *A manual of biochemical methods*. 3rd ed. New York: Grune and Startton; 1984.
[21]Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferase. The first enzymatic step in mercapturic acid formation. *J Biol Chem* 1974; **249**(22): 7130–7139.
[22]Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959; **82**(1): 70–77.
[23]Niehius WG Jr, Samuelsson B. Formation of Malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. *Eur J Biochem* 1968; **6**(1): 126–130.
[24]Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurements with the folin phenol reagent. *J Biol Chem* 1951; **193**(1): 265–275.
[25]Lefkowitch JH. Pathologic Diagnosis of Liver Disease. In: Zakim D, Boyer TD, editors. *Hepatology-A textbook of liver disease*. Philadelphia: W.B. Saunders Company; 1996, p. 844–871.
[26]Soares JR, Dinis TC, Cunha AP, Almeida LM. Antioxidant activities of some extracts of *Thymus zygis*. *Free Radic Res* 1997; **26**(5): 469–478.
[27]Jornot L, Petersen H, Junod AF. Hydrogen peroxide-induced DNA damage is independent of nuclear calcium but dependent on redox-active ions. *Biochem J* 1998; **335** (Pt 1): 85–94.
[28]Meir S, Kanner J, Akiri B, Philosph-Hades S. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. *J Agric Food Chem* 1995; **43**(7): 1813–1819.
[29]Bruck R, Aeed H, Avni Y, Shirin H, Matas Z, Shahmurov M, et al. Melatonin inhibits nuclear kappa B activation and oxidative stress and protects against thioacetamide induced liver damage in rats. *J Hepatol* 2004; **40**(1): 86–93.
[30]Rahman MF, Siddiqui MK, Jamil K. Acid and alkaline phosphatases activities in a novel phosphorothionate (RPR-11) treated male and female rats. Evidence of dose and time-dependent response. *Drug Chem Toxicol* 2000; **23**(3): 497–509.
[31]Manna P, Sinha M, Pal P, Sil PC. Arjunolic acid, a triterpenoid saponin, ameliorates arsenic-induced cyto-toxicity in hepatocytes. *Chem Biol Interact* 2007; **170**(3): 187–200.
[32] Lauterburg BH. Analgesics and glutathione. *Am J Ther* 2002; **9**(3): 225–233.
[33]Jakoby WB. Detoxification, conjugation and hydrolysis in liver biology and pathology. In: Arias IM, Jakoby WB, Popper H, Schachter D, Shafritz DA. *The liver: biology and pathobiology*. New York: Raven Press; 1988, p. 375–385.
[34]Carlberg I, Mannervik B. Glutathione reductase. *Methods Enzymol* 1985; **113**: 484–490.
[35]Ramanathan K, Balakumar BS, Panneerselvam C. Effects of ascorbic acid and α tocopherol on arsenic-induced oxidative stress. *Hum Expe Toxicol* 2002; **21**(12): 675–680.
[36]Freeman BA, Crapo JD. Hyperoxia increases oxygen radical production in rat lung and lung mitochondria. *J Biol Chem* 1981; **256**(21): 10986–10992.
[37]Jain M, Kapadia R, Jadeja RN, Thounaojam MC, Devkar RV, Mishra SH. Cytotoxicity evaluation and hepatoprotective potential of bioassay guided fractions from *Feronia limmonia* Linn leaf. *Asian Pac J Trop Biomed* 2011; **1**(6): 443–447.
[38]Ravikumar S, Gnanadesigan M. Hepatoprotective and antioxidant activity of a mangrove plant *Lumnitzera racemosa*. *Asian Pac J Trop Biomed* 2011; **1**(5): 348–352.
[39]Thirumalai T, Therasa SV, Elumalai EK, David E. Hypolipidaemic and antioxidant effect of *Enicostemma littorale* Blume. *Asian Pac J Trop Biomed* 2011; **1**(5): 381–385.
[40]Patel DK, Kumar R, Prasad SK, Hemalatha S. *Pedalium murex* Linn (Pedaliaceae) fruits: a comparative antioxidant activity of its different fractions. *Asian Pac J Trop Biomed* 2011; **1**(5): 395–400.
[41]Patel DK, Kumar R, Prasad SK, Sairam K, Hemalatha S. Antidiabetic and *in vitro* antioxidant potential of *Hybanthus enneaspermus* (Linn) F. Muell in streptozotocin-induced diabetic rats. *Asian Pac J Trop Biomed* 2011; **1**(4): 316–322.
[42]Sengupta M, Sharma GD, Chakraborty B. Hepatoprotective and immunomodulatory properties of aqueous extract of *Curcuma longa* in carbon tetra chloride intoxicated Swiss albino mice. *Asian Pac J Trop Biomed* 2011; **1**(3): 193–199.
[43]Devaraj VC, Krishna BG, Viswanatha GL, Kamath JV, Kumar S. Hepatoprotective activity of Hepax-A polyherbal formulation. *Asian Pac J Trop Biomed* 2011; **1**(2): 142–146.
[44]Okafor OY, Erukainure OL, Ajiboye JA, Adejobi RO, Owolabi FO, Kosoko SB. Modulatory effect of pineapple peel extract on lipid peroxidation, catalase activity and hepatic biomarker levels in blood plasma of alcohol-induced oxidative stressed rats. *Asian Pac J Trop Biomed* 2011; **1**(1): 12–14.
[45]Chakraborty SP, Mahapatra SK, Kumar SS, Pramanik P, Roy S. Antioxidative effect of folate-modified chitosan nanoparticles. *Asian Pac J Trop Biomed* 2011; **1**(1): 29–38.
[46]Lawrence R, Lawrence K. Antioxidant activity of garlic essential oil (*Allium sativum*) grown in north Indian plains. *Asian Pac J Trop Biomed* 2011; **1**(Suppl 1): S51–S54.
[47]Meenakshi S, Umayaparvathi S, Arumugam M, Balasubramanian T. *In vitro* antioxidant properties and FTIR analysis of two seaweeds of Gulf of Mannar. *Asian Pac J Trop Biomed* 2011; **1**(Suppl 1): S66–S70.
[48]Erukainure OL, Ajiboye JA, Adejobi RO, Okafor OY, Adenekan SO. Protective effect of pineapple (*Ananas cosmosus*) peel extract on alcohol-induced oxidative stress in brain tissues of male albino rats. *Asian Pac J Trop Dis* 2011; **1**(1): 5–9.
[49]Thirumalai T, Therasa SV, Elumalai EK, David E. Intense and exhaustive exercise induce oxidative stress in skeletal muscle. *Asian Pac J Trop Dis* 2011; **1**(1): 63–66.
[50]Silvia CWD, Kavya N, Kulkarni S. Evaluation of insulin sensitivity status in polycystic ovarian syndrome. *Asian Pac J Trop Dis* 2011; **1**(1): 67–70.
[51]Al-Qarawi AA, Mousa HM, Hamed Ali BE, Abdel-Rahman H, El-Mougy SA. Protective effect of extracts from dates (*Phoenix dactylifera* L.) on carbon tetrachloride-induced hepatotoxicity in rats. *Int J Appl Res Vet Med* 2004; **2**(3): 176–180.
[52]Hung CY, Yen GC. Extraction and identification of antioxidative components of Hsian-tsao (*Mesona procumbens* Hemsl.). *Lebensmittel-Wissenschaft und-Technologie* 2001; **34**(5): 306–311.