

ANTIOXIDANT AND HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC AND AQUEOUS EXTRACTS OF *AMORPHOPHALLUS CAMPANULATUS* ROXB. TUBERS

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Abstract: In the present study, the hepatoprotective activity of ethanolic and aqueous extracts of *Amorphophallus campanulatus* (Roxb.) tubers were evaluated against carbon tetrachloride (CCl₄) induced hepatic damage in rats. The extracts at a dose of 500 mg/kg were administered orally once daily. The substantially elevated serum enzymatic levels were significantly restored towards normalization by the extracts. Silymarin was used as a standard reference and exhibited significant hepatoprotective activity against carbon tetrachloride induced hepatotoxicity in rats. The biochemical observations were supplemented with histopathological examination of rat liver sections. The results of this study strongly indicate that *Amorphophallus campanulatus* (Roxb.) tubers have potent hepatoprotective action against carbon tetrachloride induced hepatic damage in rats. The ethanolic extract was found hepatoprotective more potent than the aqueous extract. The antioxidant activity was also screened and found positive for both ethanolic and aqueous extracts. This study suggests that possible mechanism of this activity may be due to free radical scavenging potential caused by the presence of flavonoids in the extracts.

Keywords: *Amorphophallus campanulatus* Roxb; carbon tetrachloride; hepatoprotective activity; antioxidant activity; DPPH; silymarin; histopathology

In spite of tremendous advances in modern medicine no effective drugs are available, which stimulate liver functions and offer protection to the liver from the damage or help to regenerate hepatic cells (1). In absence of reliable liver-protective drugs in modern medicine, a large number of medicinal preparations are recommended for the treatment of liver disorders (2) and quite often claimed to offer significant relief. Attempts are being made globally to get scientific evidences for these traditionally reported herbal drugs. *Amorphophallus campanulatus* Roxb. (ACR) (family: Araceae), locally known as Ol Kachu, is a perennial herb with rounded tuberous root stock (corm). The plant is widely distributed in Bangladesh, India and Africa. The tuberous roots of the plant have been used traditionally for the treatment of piles, abdominal pain, tumors, enlargement of spleen, asthma and rheumatism (3). The tuberous roots of the plant have also been reported to possess tonic, stomachic and appetizer properties. Antibacterial, antifungal and cytotoxic activities of tuberous roots extract were also reported (4). To the best of our knowledge there is no scientific report

available in support of the hepatoprotective activity of ACR tubers. Therefore, to justify the traditional claims, we have assessed the hepatoprotective effect of ACR tubers using CCl₄ intoxicated rats.

EXPERIMENTAL

Animals

Wistar albino rats and mice of either sex were used for the study of the crude extracts. Institution Animal Ethics Committee has approved the project (Registration No. 1227/ac/08/CPCSEA). The animals were kept at 27 ± 2°C, relative humidity 44–56% and light and dark cycles of 10 and 14 h, respectively, for 1 week before and during the experiments. Animals were provided with water *ad libitum* and standard diet (Lipton, India) and the food was withdrawn 18–24 h before the start of the experiment. All the experiments were performed in the morning according to current guidelines for the care of the laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals (5).

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Plant resources and preparation of crude drug extract

The tubers of *ACR* were collected from Shajapur district of Madhya Pradesh (MP) state, India and identified at the Agriculture College, Indore (MP). The herbarium specimen has been submitted to Pharmacognosy Department of the college (Voucher specimen no. -001/A). 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 in boiling water and the other part was macerated for 7 days in water with occasional stirring. The decoction and maceration were filtered and vacuum dried.

Phytochemical studies

All the extracts were subjected for phytochemical study (6).

Acute toxicity studies

The acute toxicity study for ethanolic and aqueous extracts (decocted and macerated) of *ACR* tubers were performed using albino mice and rats. The animals were fasted overnight prior to the experiment and maintained under standard conditions. All the extracts were administered orally with increasing doses and found safe up to dose of 2000 mg/kg for all extracts.

CCl₄ induced hepatotoxicity

Rats were divided into six groups (n = 6). Group I (control) animals were administered a single dose of water (1 mL/kg, *p.o.*) daily for 5 days and received liquid paraffin (1 mL/kg, *s.c.*) on day 2 and 3. Group II (CCl₄) received water (1 mL/kg body weight, *p.o.*) once daily for 5 days and received CCl₄ : liquid paraffin (1:1, 2 mL/kg body weight, *s.c.*) on day 2 and 3. Group III received standard drug silymarin (50 mg/kg, *p.o.*) once daily for 5 days. Test groups animals (Groups IV-VI) were administered orally a dose of 500 mg/kg of aqueous (maceration, decoction) and ethanolic extracts, respectively, in the form of aqueous suspension once daily for 5 days. Groups III-VI animals were administered simultaneously CCl₄ : liquid paraffin (1:1, 2 mL/kg body weight, *s.c.*) on day 2 and 3 after 30 min of administration of the silymarin and extracts. Animals were sacrificed 24 h after the last treatment. Blood was collected, allowed to clot and serum was separated at 2500 rpm for 15 min and biochemical investigations were carried out. Liver was dissected out and used for histopathological studies and biochemical investigations (7).

Biochemical determinations

The biochemical parameters like serum enzymes: aspartate aminotransferase (AST), serum glutamate pyruvate transaminase (SGPT) (8), serum alkaline phosphatase (SALP) (9) and total bilirubin (10) were assayed using assay kits (Span Diagnostic, Surat).

1,1-Diphenyl-2-picrylhydrazyl [DPPH] scavenging activity

The free radical scavenging activity of all the extracts was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH (11). A solution of DPPH (0.1 mM) in ethanol was prepared and 1.0 mL of this solution was added to 3.0 mL of all the extracts solution in water at different concentrations (100-1000 µg/mL). Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Ascorbic acid was used as a standard drug.

Estimation of MDA, hydroperoxides, GSH, SOD, CAT

Liver homogenates (5% w/v) were prepared in cold 50 mM potassium phosphate buffer (pH 7.4) using a Remi homogenizer. The unbroken cells and cell debris were removed by centrifugation at 1000 rpm for 10 min using a Remi refrigerated centrifuge. The supernatant was used for the estimation of GSH (12), malondialdehyde (MDA) (13), hydroperoxides (14), superoxide dismutase (SOD) (15) and catalase (16) levels.

Histopathological studies

The liver tissue was dissected out and fixed in 10% formalin, dehydrated in gradual ethanol (50-100%), cleared in xylene, and embedded in paraffin. Sections were prepared and then stained with hematoxylin and eosin (H-E) dye for photomicroscopic observation, including cell necrosis, fatty change, hyaline regeneration, and ballooning degeneration.

Statistical analysis

The data are expressed as the mean ± S.E.M. The difference among the means has been analyzed by one-way ANOVA. A value of $p < 0.05$ was considered as statistically significant.

RESULTS

Phytochemical study

All extracts subjected for phytochemical study showed the presence of alkaloids, carbohydrates,

Table 1. Effects of extracts of *Amorphophallus campanulatus* on biochemical parameters of liver in rats

Groups	Treatment	AST (U/L)	SGPT (U/L)	Total bilirubin (mg/dL)	ALP (IU/L)
Group I	Control	50.02 ± 1.135	99.67 ± 2.12	1.19 ± 0.131	127.43 ± 1.050
Group II	CCl ₄	252.51 ± 8.933	466.5 ± 4.49	5.19 ± 0.190	285.34 ± 7.199
Group III	Silymarin + CCl ₄	91.05 ± 6.022***	211.8 ± 4.04***	2.153 ± 0.167***	115.11 ± 9.559***
Group IV	Maceration + CCl ₄	237.60 ± 2.124 ^{ns}	424.66 ± 3.172***	4.95 ± 0.169 ^{ns}	259.41 ± 6.482 ^{ns}
Group V	Decoction + CCl ₄	204.85 ± 2.84***	352.04 ± 3.80***	4.16 ± 0.117**	180.60 ± 2.278***
Group VI	Ethanolic Extract + CCl ₄	139.71 ± 1.413***	212.99 ± 6.247***	3.16 ± 0.1358***	137.145 ± 6.615***

Values are the mean ± SEM of six rats. Symbols represent statistical significance.

*** p < 0.001, ** p < 0.01, ns – not significant, as compared to CCl₄ intoxicated group.

Table 2. DPPH scavenging activity of extracts at various concentrations

Concentration (µg/mL)	DPPH scavenging (% inhibition)		
	Maceration	Decoction	Ethanolic Extract
100	1.02 ± 1.15*	1.47 ± 0.99*	2.09 ± 0.89*
200	9.36 ± 3.75**	10.21 ± 0.42*	12.54 ± 1.73**
300	15.76 ± 3.78**	19.64 ± 0.51*	21.84 ± 0.92*
400	21.83 ± 3.57**	26.87 ± 0.47*	29.09 ± 0.89*
500	27.38 ± 1.97*	33.97 ± 0.61*	37.54 ± 1.65**
600	34.13 ± 0.74*	41.48 ± 1.83*	46.14 ± 0.43*
700	41.43 ± 1.44*	46.79 ± 0.59*	55.09 ± 0.31*
800	46.14 ± 0.91*	52.37 ± 1.44*	62.54 ± 1.47*
900	49.37 ± 1.44*	57.94 ± 0.74*	67.14 ± 0.91*
1000	52.78 ± 0.73*	61.63 ± 0.91*	74.25 ± 1.13*
Ascorbic acid (IC ₅₀)		127 ± 7.16	

Data represent the mean ± S.E.M. of triplicate analysis. * p < 0.05 and ** p < 0.001 compared to control.

proteins, amino acids, phenolic compounds, glycosides and flavonoids.

Acute toxicity studies

The ethanolic and aqueous extracts did not show any sign and symptoms of toxicity and mortality up to 2000 mg/kg dose.

Effects of extracts on AST, SGPT, ALP and total bilirubin

The results of hepatoprotective effect of extracts on CCl₄-intoxicated rats are shown in Table 1. In the CCl₄ intoxicated group (II) serum AST, SGPT, ALP and total bilirubin were increased to 252.51 U/L, 466.5 U/L, 285.34 IU/L and 5.19 mg/dL, respectively, whereas these values were 50.02 U/L, 99.67 U/L, 127.43 IU/L and 1.19 mg/dL in control group (I), respectively.

The elevated levels of serum AST, SGPT, ALP, and total bilirubin were significantly reduced in the animals groups treated with various extracts. Treatment with ethanolic extract showed highly significant activity (p < 0.001) with maximum inhibition. So, the ethanol extract treated group was superior to the other extracts but not as effective as the silymarin. Among the aqueous extracts, decoction showed more significant (p < 0.01) and better inhibition.

DPPH scavenging activity

Table 2 illustrates a significant decrease in the concentration of DPPH radical due to scavenging ability of the extracts. The results indicate that the ethanolic extract has better scavenging activity that was enhanced with increasing concentration. The IC₅₀ of ascorbic acid was 127 ± 7.16 µg/mL.

Table 3. Effects of extracts on liver MDA, hydroperoxides, GSH, SOD and CAT

Groups	Treatment	MDA (nmol/mg)	Hydroperoxides (mmol/100 g)	GSH (mg/100 g)	SOD (units/mg)	CAT (units/mg)
Group I	Control	197.7 ± 2.1	69.1 ± 2.9	46.8 ± 1.5	6.78 ± 0.24	74.9 ± 1.29
Group II	CCl ₄	460.2 ± 4.5*	98.1 ± 6.6**	20.8 ± 3.3**	3.45 ± 0.08**	45.8 ± 1.27**
Group III	Silymarin + CCl ₄	235.7 ± 1.3*	78.3 ± 1.5**	41.2 ± 3.9*	5.73 ± 0.22*	68.5 ± 1.03*
Group IV	Maceration + CCl ₄	367.3 ± 3.4*	87.2 ± 2.1**	27.5 ± 1.6**	4.17 ± 0.24**	48.4 ± 2.6**
Group V	Decoction + CCl ₄	334.7 ± 4.3*	83.2 ± 1.7**	33.2 ± 1.7**	5.01 ± 0.56**	54.1 ± 1.9**
Group VI	Ethanollic Extract + CCl ₄	294.4 ± 3.6*	78.6 ± 2.08**	39.9 ± 1.1*	5.21 ± 0.25*	62.2 ± 2.3**

Values are the mean ± SEM of six rats. Symbols represent statistical significance. * p < 0.05, ** p < 0.01, as compared to control group.

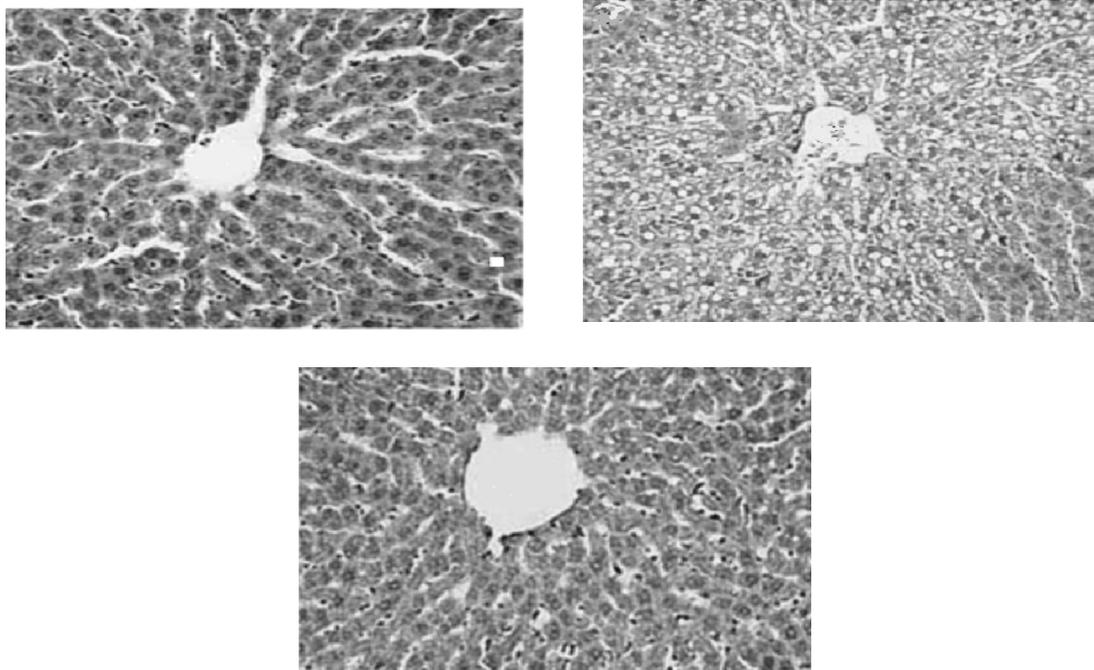


Figure 1. **A.** Liver sections of normal control rats showing: normal hepatic cells with well preserved cytoplasm; well brought out central vein; prominent nucleus and nucleolus. **B.** Liver section of CCl₄ treated rats showing: massive fatty changes, necrosis, ballooning degeneration and broad infiltration of the lymphocytes and the loss of cellular boundaries. **C.** Liver section of rats treated with CCl₄ and the extracts showing: well brought out central vein, hepatic cell with well preserved cytoplasm, prominent nucleus and nucleolus.

Effects of extracts on MDA, hydroperoxides, GSH, SOD and CAT levels

The results are presented in Table 3. They clearly revealed an increase in the levels of MDA and hydroperoxides in CCl₄-intoxicated rats compared to control group. Treatment with the extracts significantly prevented this raise in levels. GSH, SOD and CAT content have significantly increased in the extract treated groups whereas CCl₄-intoxicat-

ed group has shown a significant decrease in levels compared to control group. The ethanolic extract has shown maximum protection followed by decoction and maceration.

Histopathological observations

Histology of the liver sections of control animals (Group I) showed normal hepatic cells with well preserved cytoplasm, prominent nucleus,

nucleolus and visible central veins. The liver sections of CCl_4 -intoxicated rats showed massive fatty changes, necrosis, ballooning degeneration and broad infiltration of the lymphocytes and the loss of cellular boundaries. The histological architecture of liver sections of the rats treated with aqueous and ethanolic extracts showed more or less normal lobular pattern with a mild degree of fatty change, necrosis and lymphocyte infiltration almost comparable to the control and silymarin treated groups (Fig 1).

DISCUSSION AND CONCLUSION

In the present study the aqueous and ethanolic extracts of *ACR* tuber were evaluated for the hepatoprotective activity using hepatotoxicity induced by CCl_4 in rat model and finding out the therapeutically better efficacious extract. An attempt was made to find out the correlation between antioxidant and hepatoprotective activity. This study also gives some scientific evidences on effect of extraction solvents and method of extraction. CCl_4 is being used extensively to investigate hepatoprotective activity on various experimental animals (17). A major defense mechanism involves the antioxidant enzymes, including SOD, catalase and glutathione peroxidase (GPx), which convert active oxygen molecules into non-toxic compounds. The lipid peroxidation is accelerated when free radicals are formed as the results of losing a hydrogen atom from the double bond in the structure of unsaturated fatty acids. Scavenging of free radicals is one of the major antioxidant mechanisms to inhibit the chain reaction of lipid peroxidation.

The free radical scavenging activity of the crude drug extracts was evaluated by DPPH assay. DPPH is known to abstract the labile hydrogen (18, 19). Scavenging of DPPH radical is related to the inhibition of lipid peroxidation (20, 21). Simultaneously, the hepatoprotective effects of the extracts were compared with those treated with silymarin, which is an active constituent of the fruit of the milk thistle (*Silybum marianum*, Compositae). The *in vivo* antioxidant activity was assayed by estimation of MDA, hydroperoxides, GSH, SOD and CAT levels. Liver damage was assessed by biochemical studies (AST, SGPT, ALP and total bilirubin) and by histopathological examinations. CCl_4 produces an experimental damage that histologically resembles viral hepatitis (22). Toxicity begins with the change in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures (23). The toxic metabolite, $\text{CCl}_3\cdot$ radical, is produced and further reacts

with oxygen to give trichloromethyl peroxy radical. Cytochrome P450 2E1 is the enzyme responsible for this conversion. This radical binds covalently to the macromolecule and causes peroxidative degradation of lipid membrane of the adipose tissue. In this view, the reduction in levels of AST and SGPT by the extracts is an indication of stabilization of plasma membrane as well as a repair of hepatic tissue damage caused by CCl_4 . This effect is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes (24). The alkaline phosphate is the prototype of these enzymes that reflects the pathological alteration in biliary flow (25). The CCl_4 induced elevation of this enzymatic activity in the serum is in line with high level of serum bilirubin content. The ethanolic extract induced suppression of the increased SALP activity with the concurrent depletion of raised bilirubin suggests the possibility of the extracts to have ability to stabilize biliary dysfunction in rat liver during hepatic injury by CCl_4 . Thus, administration of ethanolic and aqueous extracts of leaves revealed hepatoprotective activity of *ACR* tubers against the toxic effect of CCl_4 , which was also supported by histological studies. The preliminary phytochemical analysis of the extracts has shown the presence of flavonoids and phenolic compounds, which have been known for its antioxidant and hepatoprotective activities (26). The result of DPPH scavenging activity of the extracts suggests that *ACR* tubers contain a free radical scavenging factor which could exert a beneficial action against pathological alterations caused by the generated free radical CCl_4 . Scavenging of free radicals is one of the major antioxidation mechanisms to inhibit the chain reaction of lipid peroxidation. The reduced lipid peroxidation was revealed by significant decrease in MDA and hydroperoxides level in the extracts treated groups. Simultaneously, a significant increase in GSH, SOD and CAT content of liver suggested antioxidant activity of *ACR* tubers extracts and silymarin. Thus, it can be concluded that the possible mechanism of hepatoprotective activity of *ACR* tubers may be due to its free radical scavenging and antioxidant activity, which may be due to the presence of flavonoids and phenolic compounds in the extracts. However, further studies are needed to confirm an involvement of cytochrome P450 enzyme inhibition. It is further concluded that the ethanolic extract with maximum inhibition of free radicals is the most potent extract among the extracts tested. Decoction has better activity than maceration. Further studies are in progress for better

understanding of the mechanism of action and it's anti hepatitis activity.

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