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Hepatoprotective and antioxidant potential of corm of *Amorphophallus paeoniifolius* (Dennst) in rats

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ABSTRACT

The plant *Amorphophallus paeoniifolius* (Dennst) is commonly known as Suranah in Indian system of medicine. In the present study ethyl acetate and ethanolic extracts of corm of *A. paeoniifolius* were investigated for hepatoprotective and *in vivo* antioxidant activity against CCl_4 (0.7 ml/kg in olive oil, 1:1, i.p.) induced liver damage in rats. Various biochemical parameters such as SGOT, SGPT, ALP, serum bilirubin and cholesterol levels were determined. Ethyl acetate and ethanol extracts (100 and 200mg/kg, p.o) exhibited significant ($P<0.001$) hepatoprotective activity by reducing elevated levels of SGOT, SGPT, ALP, serum bilirubin and cholesterol in CCl_4 induced hepatotoxic animals. To assess the *in vivo* antioxidant activity, levels of liver detoxificating enzymes were determined. Pretreatment of rats for successive seven days with ethyl acetate and ethanol extracts (100 and 200mg/kg, p.o) along with CCl_4, preserved catalase, superoxide dismutase (SOD), and peroxidase activity as compared to control and CCl_4 group, thus providing protection against CCl_4 toxicity. Histopathological studies of rat livers revealed regeneration of hepatocytes and maintained hepatic enzyme levels which are normally involved in combating reactive oxygen species (ROS).

KEYWORDS

Amarphophallus paeoniifolius;
Carbon tetrachloride;
Silymarin;
Hepatoprotective;
Antioxidant activity.

INTRODUCTION

The liver, because of its strategic anatomical location and its large capacity for metabolic conversions, is exposed to many kinds of xenobiotics and therapeutic agents. Moreover, the rapidly growing morbidity and mortality from liver diseases is largely attributable to the increasing number of chemical compounds and enviornmental pollution.

In the modern era of medicine there is no specific treatment to counter the menacing impact of these dreaded diseases[1]. The therapeutic regimen followed in all these cases up to the present moment is by and large symptomatic & at best palliative, but it still confronts the medical practioner with formidable task. Due to this fact efforts to find suitable curative agents for treatment of liver diseases in natural products of plants and mineral origin are being made[2]. Liver injury induced by carbon tetrachloride is the best characterized system of xenobiotics induced hepatotoxicity in human beings. It is a volatile organic chemical and causes liver and kidney damage through free radical mediated pro-

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cess. Though the modern medicinal system has advanced phenomenally, there are no potential drugs which can completely cure all liver disorders[3]. Hence, scientists are looking for the traditional systems of medicine for possible remedies to treat various hepatic disorders.

Amorphophallus paeoniifolius Dennst. (Family: Araceae), commonly known as Suranah in Indian system of medicine. It is an herbaceous plant with underground hemispherical depressed dark brown corm, cultivated throughout India. Traditionally the corm is used as expectorant, carminative, aphrodisiac, haemostatic, anthelmintic and in treatment of liver disorders[4]. The corm extract is reported to possess analgesic[5], enzyme inhibitory in tuber crops[6] and antiobesity activity[7]. The corm of the plant contains steroids[8], flavonoids[9] and tannins[10]. Tannins being polyphenolic in nature are also known to possess antioxidant property[11]. Hence, the present investigation was designed to evaluate the hepatoprotective and antioxidant effect of *A. paeoniifolius* in CCl_4 induced toxicity in rat liver.

MATERIALS AND METHODS

Chemicals

All the solvents and chemicals used were of analytical grade. Standard kits for SGOT, SGPT, and Bilirubin (Teco Diagnostic, USA) and Cholesterol (Span Diagnostics, India), Standard drug silymarin (Micro Laboratory, India) were used in the present study.

Plant material and extraction

The dried corm of *A. Paeoniifolius* was collected from the local market of Dharwad in the month of September 2006. The collected material was authenticated by Prof. Ganesh Hegde, Taxonomist, Department of Botany, Karnataka University, Dharwad, India. A voucher specimen has been kept in the herbarium of Department of Pharmacognosy (SETCPD/Ph.cog/herb/34/2006). Two kilogram of dried powder of corm was exhaustively extracted with ethyl acetate and ethanol using soxhlet extractor. The extracts were concentrated using rotary flash evaporator and crystals were obtained. The average yield of the ethanol extract was 25.0% and ethylacetate extract was found to be 4.5% w/v. Suspensions of each extract were prepared using Tween-80 and distilled water (2:8). The suspensions were used to assess hepatoprotective and *in vivo* antioxidant activity in CCl_4 induced liver toxicity.

Animals

Adult male swiss mice (25-30g) and wistar rats (180-200g) were used in the present study. The animals were procured from disease free animal house, BLDEA medical college, Bijapur, Karnataka, India. All the animals were kept in quarantine for 10 days under standard husbandry conditions.(27.3^{0}C, RH-65^{0}C $\pm$10%) for 12h in dark and light cycle respectively and were given standard food (Hindustan lever) and water *adlibitum*. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) and care of laboratory animals was taken as per CPCSEA guidelines.

Acute toxicity study

Acute toxicity study was conducted on Swiss albino mice weighing between 20-25g using stair case/up and down method. The extracts were orally administered to different groups of mice at doses of 50-2000mg/kg. Convulsion, sedation, body temperature, mortality and behavioral changes if any were observed[12]. Corm of *A.Paeoniifolius* did not produce any toxicity up to dose level of 2000 mg/kg .

Experimental protocol

Rats were divided into seven groups comprising of 6 animals in each group. Group I served as normal control and received normal saline (5ml/kg, p.o.) for seven days. Group II was administered with $CC1_4$ in olive oil (0.7m1/kg, 1:1, v/v, i.p, on alternate days)[13-14]. Group III was administered with Silymarin (100mg/kg, p.o.) simultaneously with toxicant[15]. Group IV and Group V were treated with ethyl acetate extract (100 and 200mg/kg p.o.). Group VI and VII were administered with ethanol extract (100 and 200mg/kg p.o.).

Assessment of hepatoprotective activity

On the Seventh day after administration of last dose of extracts, the rats were anaesthetized by light ether anesthesia and blood was collected by making intracardiac puncture. It was allowed to coagulate for 30min and serum was separated by cold centrifugation at 2500 rpm. The centrifugate was used to estimate the serum glutamate pyruvate transaminase (SGPT) serum

TABLE 1: Effect of *A.paeoniifolious* on biochemical parameters

Group	Dose mg/kg	SGOT (μ/1)	SGPT (μ/1)	ALP (μ/1)
Normal	5ml	170.3±3.66	74.85±4.40	201.1±7.99
$CC1_4$	0.7	696.0±11.87[a]	320.5±10.18[a]	382.9±7.18[a]
Silymarin $^{+}CC1_4$	100	292.9±2.12[b]	77.64±0.92[b]	234.4±3.03[b]
Ethyl acetate Extract+$CC1_4$	100	308.6±2.82[b]	135.8±3.58[b]	242.9±2.80[b]
Ethyl acetate Extract+$CC1_4$	200	301.3±2.16[b]	131.3±3.21[b]	238.2±2.34[b]
Ethanolic Extract+$CC1_4$	100	312.5±4.33[b]	152.0±3.09[b]	251.0±3.10[b]
Ethanolic Extract+CCl_4	200	306.2±4.12[b]	146.2±3.01[b]	246.1±3.02[b]

Values are mean ± SE from 6 animals in each group; [a]p* <0.001 as compared with Control group; [b]p* <0.001 as compared with $CC1_4$ and Control group

TABLE 2: Effect of *A.paeoniifolious* on $CC1_4$- induced Hepatotoxicity in rats

Group	Dose mg/kg	Total bilirubin (mg/d1)	Direct bilirubin (mg/d1)	Serum cholesterol (mg/d1)
Normal	5ml	1.208±0.63	0.4783±0.02	62.96±0.69
$CC1_4$	0.7	2.237±0.12[a]	0.9400±0.07[a]	110.2±2.51[a]
Silymarin$^{+}CC1_4$	100	0.8867±0.04[b]	0.60±0.011[b]	74.14±1.37[b]
Ethyl acetate Extract+$CC1_4$	100	1.51±0.03[b]	0.60±0.02[b]	66.79±0.85[b]
Ethyl acetate Extract+$CC1_4$	200	1.48±0.01[b]	0.59±0.01[b]	64.35±0.64[b]
Ethanolic Extract+$CC1_4$	100	1.622±0.02[b]	0.60±0.02[b]	7.79±0.85[b]
Ethanolic Extract+ CCl_4	200	1.599±0.02[b]	0.58±0.02[b]	69.35±0.56[b]

(Values are mean ± SE from 6 animals in each group); [a]p*<0.001 as compared with Control , [b]p* <0.001 as compared with $CC1_4$ and Control group

TABLE 3: Effect of *A.paeoniifolious* on antioxidant Enzymes in $CC1_4$ induced hepatotoxicity

Group	Dose mg/kg	Catalase (units/mg of protein)	Superoxide(SOD) (Units/mg of protein)	Peroxidase (Units/mg of protein)
Normal	5ml	461.7±6.36	21.0±0.89	165.8±3.36
$CC1_4$	0.7	109.8±1073[a]	7.66±0.42[a]	35.73±2.54[a]
Silymarin +$CC1_4$	100	435.4±5.63[b]	18.6±0.53[b]	158.2±2.42[b]
Ethyl acetate Extract+$CC1_4$	100	418.4±10.54[b]	16.87±1.46[b]	148.0±3.56[b]
Ethyl acetate Extract+$CC1_4$	200	424.4±10.62[b]	17.92±1.68[b]	152.2±3.64[b]
Ethanolic Extract+$CC1_4$	100	403.7±6.84[b]	15.42±0.79[b]	150.0±3.34[b]
Ethanolic Extract+CCl_4	200	409.3±6.92[b]	16.67±0.83[b]	153.2±3.61[b]

(Values are mean ± SE from 6 animals in each group); [a]p* <0.001 as compared with control; [b]p* <0.001 as compared with $CC1_4$ and Control group

glutamate oxaloacetate transaminase (SGOT)[16], alkaline phosphatase (ALP)[17], Total and direct bilirubin[18] and cholesterol[19] levels were determined.

Assessment of *in vivo* antioxidant activity

The liver was dissected out, immediately washed in ice-cold saline and a 5% homogenate was prepared in Tris–Hcl: Sucrose buffer for the estimation of enzymes. Catalase, Superoxide dismutase, and catalase activity were estimated[20-22] and the results were expressed as units/mg of protein.

Histopathological studies

For histpathological observation, sections were taken from each lobe of liver immediately. The tissue were fixed in 10% neutral formalin, dehydrated in graded ethanol and embedded in paraffin, cut in to 4-5m thick sections and stained with Haematoxylin-Eosin for photomicroscopic assessment[19].

Statistical analysis

The data were expressed as mean ± SEM (n=6). The data were analyzed using one way ANOVA followed by Dunnet's multiple comparison tests. $p<0.05$ were considered statistically significant[23].

RESULTS

Phytochemical analysis

Preliminary phytochemical tests and thin layer chromatographic studies of the corm ethyl acetate and ethanol extract of *A.Paeoniifolius* indicated the presence of steroids, tannins, flavonoids, carbohydrates and alkaloids.

Acute toxicity studies

A.paeoniifolius extracts did not show any toxicity and behavioral changes in mice up to 2000mg/kg. Hence, the doses selected were 100 and 200mg/kg.

Hepatoprotective and antioxidant activity

Rats treated with CCl_4 (0.7 ml/kg in olive oil, 1:1, i.p.) suffered from hepatotoxicity. The serum levels of SGOT, SGPT, ALP, (TABLE 1), bilirubin (Total and direct) and cholesterol levels were significantly elevated (TABLE 2). Ethylacetate and ethanolic extract (100 and 200mg/kg, p.o.) of *A.Paeoniifolius* exihibited significant activity ($p<0.001$) by decreasing the elevated enzymes levels against $CC1_4$ induced hepatotoxicity

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(TABLE 1). Pretreatment of rats with ethanol and ethylacetate extracts at a dose of (100 and 200 mg/kg p.o.) significantly ($P<0.001$) preserved catalase, superoxide dismutase and peroxidase activity compared to control and CCl_4 treated group (TABLE 3). Results were also comparable with standard drug silymarin (100 mg/kg, p.o).

Histopathological observations

Histopathology of normal rat liver shows prominent central vein, normal arrangement of hepatic cells (Figure 1), microscopical examination of $CC1_4$ treated liver section (Figure 2) shows centrilobular necrosis, kupffer cells around the central vein and fatty degeneration. Liver section treated from silymarin(Figure 3) protected the structural integrity of hepatocyte cell membrane and showed mild recovery of hepatic cells. Ethanol extract(Figure 4) and ethylacetate extract(Figure 5) treated groups showed regeneration of hepatocytes, no fatty degeneration, no centrilobular necrosis and exhibited significant protection against $CC1_4$ induced liver damage in rats.

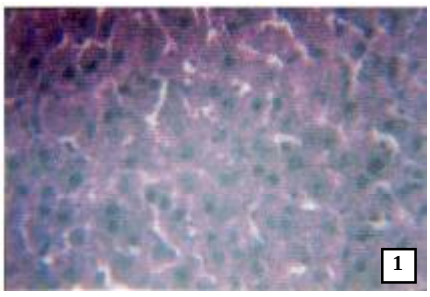

Figure 1: Photograph showing histopathology of normal (control) group showing characteristic features of the hepatic lobules

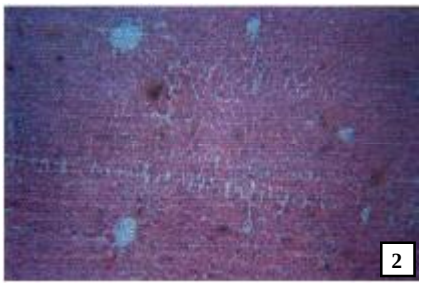

Figure 2: Photograph showing histopathology of CCl_4 treated group showing centrilobular necrosis, fatty degeneration and broad infiltration of lymphocytes and kupffer cells around the central vein

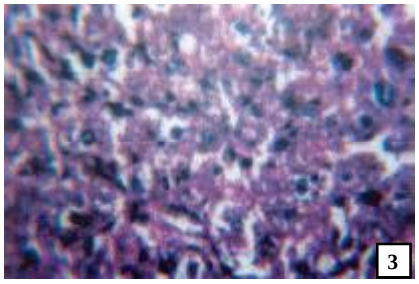

Figure 3: Photograph showing histopathology of silymarin and CCl_4 treated group showing regeneration of hepatocytes

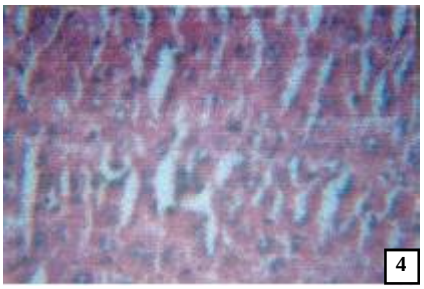

Figure 4: Photograph showing histopathology of ethanolic and CCl_4 treated group showing significant recovery of hepatocytes

DISCUSSION

CCl_4 induced hepatic damage is due to its cytochrome P-450 enzyme system catalysed hepatic conversion into highly reactive trichloromethyl radical (CCl_3) that upon reaction with oxygen radical gives trichloromethyl peroxide radical ($OOCCl_3^{\circ}$). This radical forms covalent bond with sulfohydroxyl group of several membrane molecules like glutothione, which is considered as the initial step in the chain of events leading to lipid peroxidation and hepatic tissue destruction [24-27]. The degree of hepatotoxicity developed by $CC1_4$, can be observed by elevated levels of biochemical parameters which is attributed to the generation of trichloromethyl free radical during metabolism by hepatic microsomes which in turn cause peroxidation of lipids of cellular membrane[28]. Hepatocellular necrosis lead to very high level of GOT, GPT released from liver in the blood. Among the two, GPT is a better index of liver injury, as liver GPT activity represents 90% of total enzyme present in the body[29]. ALP activities on the other hand are related to the functioning of the hepatocytes, increase in its activity is due to increased synthe-

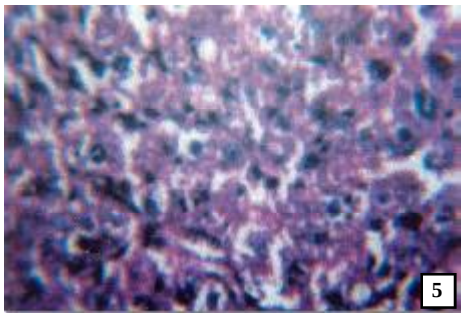

Figure 5 : Photograph showing histopathology of ethyl acetate and CCl_4 treated group showing significant recovery of hepatocytes, no fatty degeneration and centrilobular necrosis. (figures 1-5, magnification ×100)

sis in presence of increased biliary pressure[30]. Reduction in the levels of GOT and GPT towards the respective normal value is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damages caused by carbon tetra chloride. The serum levels of transaminases return to normal with healing of hepatic parenchyma and regeneration of hepatocytes. Suppression of increased ALP activity with concurrent depletion of raised bilirubin level suggests the stability of the biliary dysfunction in rat liver during Chronic hepatic injury with $CC1_4$[31].

Free radicals can be formed either by univalent pathway of oxygen reduction or as a consequence of enzymic/non-enzymic reactions. The enzymatic free radical defense system is comprised of superoxide dismatase, catalase and peroxidase. SOD converts superoxide into hydrogen peroxide. Catalase and peroxidase may trigger dangerous pathway of peroxidative damage. Peroxidative damage brought about by free radicals has been shown to be involved in pathogenesis of several diseases. The inhibition of oxidative stress may be due to free radical scavenging activity by reducing the extent of damage and elevation of liver enzymes due to its ability to increase the levels of free radical scavengers[32].

The Suspensions of ethyl acetate and ethanolic extracts (100 and 200mg/kg p.o) significantly decreased the $CC1_4$ induced elevated enzyme levels suggests the protection of structural integrity of hepatocyte cell membrane or regeneration of damaged liver cells by the extracts. The antioxidant property of ethyl acetate and ethanolic extracts (100 and 200mg/kg p.o) of *A. paeoniifolius* may prevent the formation of free radical there by inhibit the lipid peroxidation and offer hepatoprotection against CCl_4 induced hepatotoxicity.

CONCLUSION

The ethanol and ethylacetate extracts of corm of *A.paeoniifolius* are beneficial in the prevention of formation of fatty liver and thereby protect the liver and hepato-enzymes which are commonly involved in combating reactive oxygen species. Hence corm of *A.paeoniifolius* can be of enormous use in the management of oxidative stress and in treatment of various liver disorders. Isolation and characterization of various phyto-constituents of *A.paeoniifolius* possessing hepato protective and antioxidant potential is under progress in our laboratory.

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