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HEPATOPROTECTIVE ACTIVITY OF *COLOCASIA ANTIQUORUM* AGAINST EXPERIMENTALLY INDUCED LIVER INJURY IN RATS

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Colocasia antiquorum (Araceae) commonly known as 'taro', was claimed to have medicinal properties to treat hepatic ailments in ancient literature. The aim of this work was to study the hepatoprotective effect of crude ethanolic extracts of C. antiquorum. Ethanolic extract obtained from the corms of C. antiquorum (L.) Schott (Araceae), was evaluated for hepatoprotective activity using paracetamol and carbon tetrachloride (CCl_4) intoxicated rats as experimental models. The protective effect was evident from serum biochemical parameters and histopathological analysis. Ethanolic extract of C. antiquorum significantly ($P<0.5$) prevented the elevation of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) in paracetamol and CCl_4 treated rats as compared to silymarin, which was used as a positive control. These biochemical observations were supplemented by histopathological examination of liver sections showing the prevention of disarrangement and degeneration of hepatic cells induced by paracetamol and CCl_4. The activity may be a result of the presence of anthocyanin compounds. Furthermore, acute toxicity studies showed no signs of toxicity up to a dose level of 1000 mg/kg. Thus, it may be concluded that ethanolic extract of C. antiquorum possesses significant hepatoprotective properties.

Keywords: *Colocasia antiquorum, Carbon tetrachloride, Paracetamol, Silymarin, Hepatoprotective*

INTRODUCTION

Colocasia antiquorum (L.) Schott (Syn. *Colocasia esculenta*, Family Araceae) is commonly known as 'taro' in Tahitian or other Polynesian languages. It is an ancient crop grown throughout the humid tropics. Taro (taloes) was probably first native to the lowland wetlands of Malaysia. *C. antiquorum* is widely used throughout the world; Africa, Asia, the West Indies, and South America. Its edible corms and leaves are traditionally used for hepatic ailments (Miller 1971). Phytochemical investigations of taro have shown the presence of the following constituents and their effects:

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cyanoglucoside possesses hypoglycemic property (Phillip *et al.* 2002); digalactosyl and monogalactocyl diacylglycerols were identified for anti-hyperlipedemic activity (Tanaka *et al.* 2005); cystatin was shown to possess antifungal activity (Yang and Yeh 2005); arabinogalactan possesses hypolipidemic action (Boban, Nambisan and Sudhakaran 2006).

Tubers of *C. antiquorum* have amylase, trypsin and chymotrypsin inhibitory activities (Prathibha, Nambisan and Leelamma 1998). *C. antiquorum* is good for people, who are allergic to milk or cereals and can be consumed by children, who are sensitive to milk (Roth, Worth and Lichton 1967). The leaves and corms can be boiled and consumed by women experiencing difficult childbirths. The corms of *C. antiquorum* contain anthocyanins such as cyanidin-3-glucoside, pelargonidin-3-glucoside and cyanidin-3-rhamnoside which were reported to have antioxidant and anti-inflammatory properties (Cambie and Ferguson 2003). Traditionally, *C. antiquorum* is also used to alleviate stomach swelling and pain, and act as an antipyretic.

Hepatic dysfunction due to ingestion or inhalation of hepatotoxins such as acetaminophen, cadmium chloride, ethanol, carbon tetrachloride (CCl_4) and allyl alcohols are increasing worldwide (Wolf 1999). Paracetamol is a common antipyretic agent which can produce fatal hepatic necrosis by formation of metabolite *N*-acetyl -*p*-benzoquinoneimine in the liver (Wong *et al.* 1981; Savides and Oehme 1983).

CCl_4 is catalysed by cytochrome P450 in the liver cell endoplasmic reticulum leading to the generation of an unstable complex of CCl_3 radical, which reacts rapidly with O_2 to yield highly reactive hepatotoxic trichloromethyl peroxy radical (Packer, Slater and Wilson 1978; Recknagel *et al*. 1989). These free radicals attack microsomal lipids leading to its peroxidation and also covalently bind to microsomal lipids and proteins, ultimately initiating a site of secondary biochemical processes (Rao and Recknagel 1969).

This paper deals with the evaluation of the hepatoprotective effect of the extract of leaves of *C. antiquorum* on rat liver toxicity induced by paracetamol and CCl_4.

METHODS

Plant Material

C. antiquorum L. (Schott.) corms were collected from local flora areas near Pune, India and authenticated by the Botanical Survey of India, Regional Centre, Pune. Voucher specimen (BSI/WC/Tech./2008/1086) was deposited in the Herbarium of Botanical Survey of India, Regional Centre, Pune.

Preparation of Extract

The shade dried corms of about 1 kg were subjected to size reduction to coarse powder. The powder was defatted with petroleum ether (60°C –80°C) then extracted with 90% ethanol maceration process for 7 days. The ethanol extract of corms was concentrated under vacuum to get the residues. The percentage yield of ethanolic extract was found to be 2.5% (w/w). The extract was tested for specific qualitative biochemical test (Cambie and Ferguson 2003).

Animals

Wistar albino rats of either sex weighing 200–250 g and Swiss albino mice of either sex, weighing 22–25 g, maintained under standard laboratory conditions (temperature 23±2°C, relative humidity 55±10%) were used for the experiments. Animals were allowed to take standard laboratory feed (Amrut standard feed, Pune, India) and pure water. The experiments were conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) issued by Government of India and all protocols were approved by Institutional Animal Ethical Committee (approval no. CPCSEA/IAEC/PC-08/09-2K7).

Drugs and Chemicals

Paracetamol was procured from Vaishali Incorporation, New Delhi; CCl_4 was procured from Cosmochem, Pune; silymarin was procured from Sanjivani Herbals, Mumbai; olive oil and 1% gum acacia were procured from Vijay Chemicals, Pune.

Behavioral Effect and Toxicity

Albino mice (Swiss strain) were divided into 5 groups of 10 animals. The animals were then treated with graded dose (200, 400, 600, 800 and 1000 mg/kg) of the extract intraperitoneally (i.p.). The mice were observed continuously for 1 h for any gross behavioural changes and death, if any, intermittently for the next 6 h and then again at 24 h after dosing.

Paracetamol-induced hepatotoxicity

The paracetamol model described by Jafri *et al.* (1999) was employed. Twenty rats (Wistar strain) of either sex were distributed into four groups of five animals each (n=5). Animals received silymarin (100 mg/kg; p.o.) as positive control or ethanolic extract of *C. antiquorum* (125, 250 and 500 mg/kg/day; p.o.) in 1% gum acacia for seven days and following which paracetamol was administered in a single dose of 3 g/kg (p.o.) on the 8^{th} day. Wherein, individual treatment group was compared with paracetamol control. Control group received gum acacia 1%; (p.o.) in saline, taken as normal for comparing the paracetamol control group.

Carbon tetrachloride-induced hepatotoxicity

The CCl_4 model described by Handa and Sharma (1990) was employed. Twenty five rats of either sex were distributed into five groups of five animals each (n=5). Hepatotoxicity was induced by 0.8 mL/kg of CCl_4: olive oil (1:1); p.o. for 7 days and compared with saline treatment group (olive oil). Silymarin (100 mg/kg; p.o.) or ethanolic extract of *C. antiquorum* (125, 250 and 500 mg/kg; p.o.) was administered for 7 days along with 0.8 mL/kg of CCl_4:olive oil (1:1). All animals were observed daily for toxicity interventions for 24 h after the last dose of *C. antiquorum* extract (500 mg/kg; p.o.).

Assessment of Liver Function

Blood samples of the rats were withdrawn from ratino bulber venous plexus with the help of a tapering plastic capillary under light ether anesthesia and were kept at room temperature for 2 h so that the process

of coagulation gets completed. The blood samples were centrifuged and the separated serum was used to estimate serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT). Animals were then sacrificed by excessive anesthesia (ether inhalation) and cervical dislocation. Their livers were taken out, washed with water, dried gently with filter paper and preserved in 10% formol-saline. SGOT and SGPT were estimated by Reitman and Frankel (1957) method using autoanalyser (300 TX, E. Merck-Micro Labs, Mumbai).

Histopathological examination of the liver

The liver samples fixed for 48 h in 10% formol-saline were dehydrated by passing successively in different mixtures of ethyl alcohol–water (50%, 80%, and 95% and finally in absolute alcohol), cleared in xylene and embedded in paraffin. Sections (4–5 mm thick) were prepared and then stained with hematoxylin and eosin dye for microscopic observation of cell necrosis, fatty change, hyaline degeneration, ballooning degeneration and infiltration of kupffer cells and lymphocytes.

Statistical Analysis

The the mean values ± S.E.M. were calculated for each parameter. For the determination of significant inter-group difference, each parameter was analysed separately and one-way analysis of variance (ANOVA) (Gennaro 1995) was carried out and the individual comparisons of the group mean values were done using Duncan test.

RESULTS AND DISCUSSION

In the Indian system of medicine, certain herbs are claimed to provide relief against liver disorders. The claimed therapeutic reputation has to be verified in a scientific manner. In this study, silymarin was used as a standard drug. It has been shown to provide liver protection in rat models with liver damage induced by CCl_4 and paracetamol (Mourelle *et al*. 1989). The drug prevents lipid peroxidation and normalises the lipid profile of hepatocyte membranes. Our results showed the

hepatoprotective effect of ethanolic extract of *C. antiquorum* in the paracetamol and CCl_4 models of hepatic treatment in rats. Phytochemical investigations on the extracts have shown the presence of anthocyanins such as cyanidin-3-glucoside, pelargonidin-3-glucoside and cyanidin-3-rhamnoside, which have antioxidant activities (Noda *et al.* 2002; Cambie and Ferguson 2003; Kowalczyk *et al*. 2003). Therefore, anthocyanins may be responsible for the hepatoprotective activity.

Behavioral Effect and Toxicity

The extract of *C. antiquorum* was found to be safe for further biological studies, as no lethality was observed even at 1000 mg/kg given orally in mice. No behavioral changes were observed in any dose tested in rats.

Paracetamol-induced hepatotoxicity

Enzymic assays

The hepatic damage produced by paracetamol (3 gm/kg; p.o.) was reflected by increased levels of SGOT and SGPT as compared to control (p<0.001). Pretreatment of rats with *C. antiquorum* extract (500 mg/kg/day; p.o.) and silymarin (100 mg/kg; p.o.) for 7 days before paracetamol administration, resulted in significant (p<0.001) reversal of elevated levels of SGOT and SGPT as compared to paracetamol-treated group (Fig. 1).

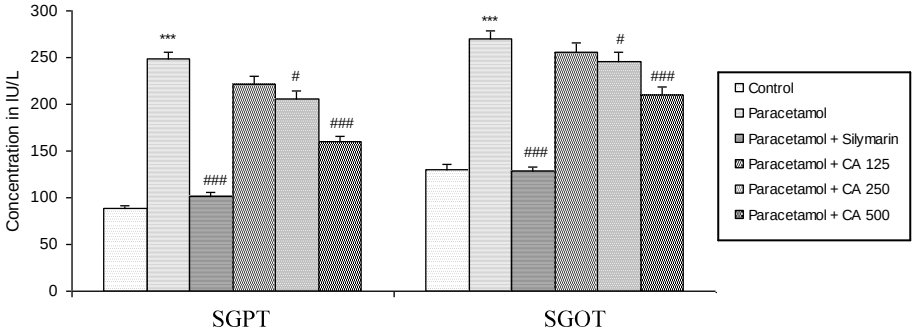

Fig. 1: Effect of ethanolic extract of *C. antiquorum* (CA) on paracetamol induced hepatotoxicity in rats. ***p<0.001, paracetamol vs. control, whereas ###p<0.001, *C. antiquorum* extract and silymarin vs. paracetamol. Values are mean±S.E.M. (n=5).

Histopathology

The liver samples of paracetamol-treated rats showed gross necrosis of the centrilobular hepatocytes characterised by nuclear pyknosis, karyolysis and eosinophilic infiltration [Fig. 3(b)]. Other histopathological examinations are shown in Figure 3(c) (pretreated with silymarin 100 mg/kg; p.o.) and in Figure 3(d) (pretreated with *C. antiquorum* extract 500 mg/kg/day; p.o.). Rats pretreated with *C. antiquorum* extract, when challenged with paracetamol showed minimal necrosis in centrilobular and regeneration of hepatocytes.

Paracetamol is a common antipyretic agent that can produce fatal hepatic necrosis if given in the dose of 3 g/kg; (p.o.) to animal and it is mainly metabolised in liver to excretable glucuronide and sulphate conjugates (Wong *et al*. 1981). Hepatotoxicity of paracetamol has been attributed to the formation of toxic metabolites activated by hepatic cytochrome P-450 to a highly reactive metabolite *N*-acetyl -*p*-benzoquinoneimine (Savides and Oehme 1983). The transport function of hepatocytes gets disturbed, resulting in the disintegration of plasma membrane, thereby causing an increased enzyme level in the serum (Seeff and Lewis 1989). SGOT and SGPT levels are required to be maintained for a substance to show hepatoprotection (Dwiwedi *et al.* 1991; Visen *et al.* 1993; Singh and Handa 1995). In this study, hepatoprotective activity of the ethanolic extract of *C. antiquorum* was found to be substantiated by significant attenuation of the increased levels of SGOT and SGPT in rats intoxicated with paracetamol.

Carbon tetrachloride-induced hepatotoxicity

Enzymic assays

CCl_4 intoxication significantly (p<0.001) elevated the levels of SGOT and SGPT indicating acute hepatocellular damage and biliary obstruction as compared to control group (olive oil) (Fig. 2). However, rats treated with ethanolic extract of *C. antiquorum* and silymarin (100 mg/kg; p.o.) had significantly (p<0.001) decreased levels of SGOT and SGPT as compared to CCl_4 group (Fig. 2).

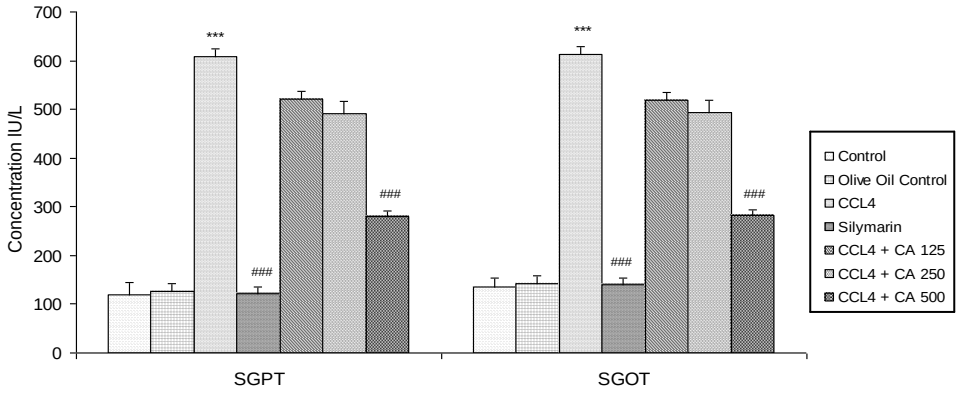

Fig. 2: Effect of ethanolic extract of *C. antiquorum* (CA) on CCl_4 induced hepatotoxicity in rats. ***p<0.001, CCl_4 (in olive oil as vehicle) vs. control (olive oil), whereas ### p<0.001, *C. antiquorum* extract and silymarin vs CCl_4. Values are mean±S.E.M. (n=5).

Histopathology

Histopathological examination of the liver sections of control animals [Fig. 3(a)] showed normal hepatic architecture with prominent nucleus and well brought out central vein. The liver sections of CCl_4-intoxicated rat [Fig. 3(e)] showed disarrangement and degeneration of normal hepatic cells with centrilobular necrosis, swelling of hepatic cytoplasm and vacuolisation of periportal vein extending to mid zone and dilation. CCl_4-intoxicated group showed the most disorganised structural characteristics than any other group. *C. antiquorum* extract (500mg/kg; p.o.) pretreated rats when challenged with CCl_4 [Fig. 3(g)] showed less vacuole formation and revealed the absence of necrosis. Overall no visible change was observed as compared to silymarin-treated group [Fig. 3(f)].

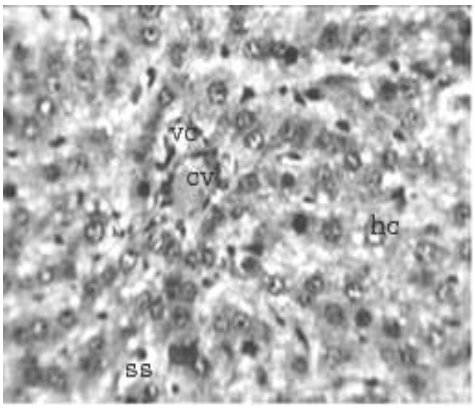

Fig. 3(a): Normal rat liver section, 400×, haematoxylin-eosin stain.

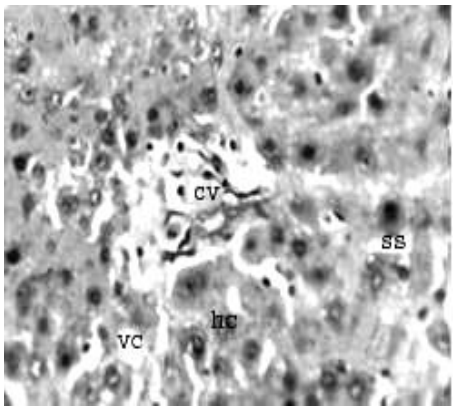

Fig. 3(b): Liver section intoxicated with paracetamol, 400×, haematoxylin-eosin stain.

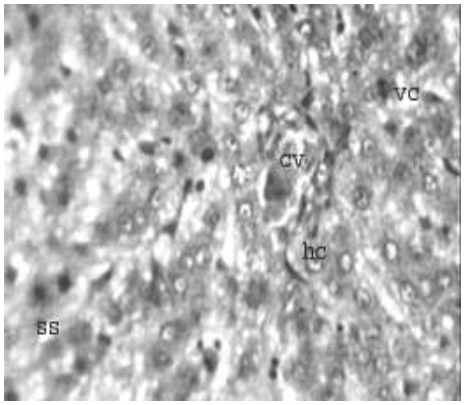

Fig. 3(c): Liver section of rat treated with silymarin and intoxicated with paracetamol, 400×, haematoxylin-eosin stains.

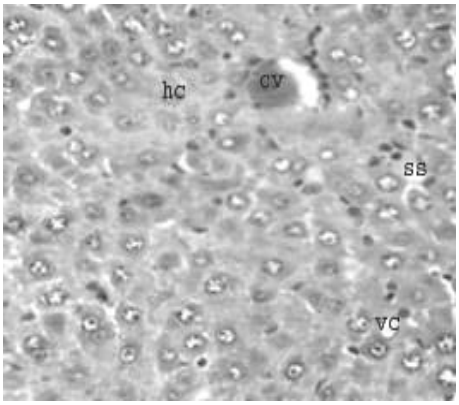

Fig. 3(d): Liver section of rat treated with ethanolic extract and intoxicated with paracetamol, 400×, haematoxylin-eosin stain.

Notes: cv, central rein; hc, hepatocyte; ss, sinusoidal space; vc, vacuole

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Fig. 3: *(continued)*

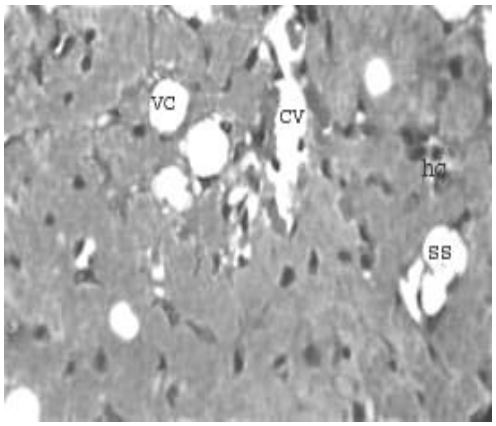

Fig. 3(e): Liver section of rat intoxicated with CCl_4, 400×, haematoxylin-eosin stain.

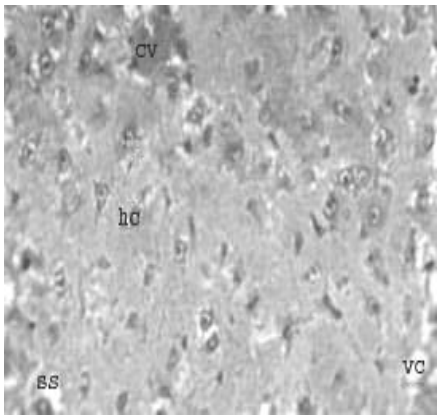

Fig. 3(f): Liver section of rat treated with silymarin and intoxicated with CCl_4, 400×, haematoxylin-eosin stain.

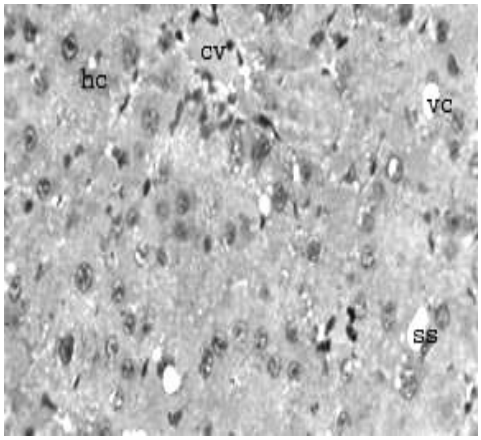

Fig. 3(g): Liver section of rat treated with ethanolic extract and intoxicated with CCl_4, 400×, haematoxyline-eosin stain.

Notes: cv, central rein; hc, hepatocyte; ss, sinusoidal space; vc, vacuole

The hepatotoxicity of CCl_4 might be due to the formation of the highly reactive trichloro free radical, which attacks polyunsaturated fatty acids. It produces hepatotoxicity by altering liver microsomal membranes in experimental animals (Ashok, Somayaji and Bairy 2001).

In the present study rats treated with chronic dose of CCl_4 developed significant hepatic damage, which was observed through a substantial increase in the concentrations of SGOT and SGPT. The increased levels of SGOT and SGPT in serum are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Drotman and Lowhorn 1978).

Silymarin, which was used in this study as the standard showed hepatoprotective effects, which is known to be accomplished via several mechanisms including antioxidation (Wagner 1981), inhibition of lipid peroxidation (Bosisio, Benelli and Pirola 1992), enhanced liver detoxification via inhibition of Phase I detoxification and enhanced glucuronidation (Halim *et al.* 1997; Baer-Dubowska, Szaefer and Drajka-Kuzniak 1998), and protection of glutathione depletion (Campos *et al.* 1989). In addition to anti-inflammatory effects, silymarin has been shown to increase hepatocyte protein synthesis, thereby promoting hepatic tissue regeneration (Sonnenbichler and Zetl 1986) and reduces the conversion of hepatic stellate cells into myofibroblasts, slowing or even reversing fibrosis (Fuchs, Weyhenmeyer and Weiner 1997). In agreement with these reports, the present study also revealed hepatoprotection against CCl_4 and paracetamol-treated groups (Mourelle *et al.* 1989). Treatment of rats with ethanolic extract of *C. antiquorum* concomitant with the challenge of CCl_4 reduced the hepatic injury to a considerable extent, which was reflected by lowered serum enzymes level. The hepatoprotective effect of the *C. antiquorum* was further confirmed by histopathological examination of the livers of control group.

CONCLUSION

The ethanolic extract of *C. antiquorum* corms possesses hepatoprotective effect against paracetamol and CCl_4-treated rats, which may support the ethnic claim.

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