

Evaluation of hepatoprotective activity of *Colocasia esculenta* (L. Schott) leaves on thioacetamide-induced hepatotoxicity in rats

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Abstract: The hepatoprotective effect of orally administered leaf aqueous extract of *Colocasia esculenta* (CCLE) in thioacetamide-induced liver toxicity in rats was investigated in this study. Adult male Wistar rats (weight range: 120-150g) were divided into 5 groups (n=5) and received no treatment (normal control), distilled water (negative control), 50mg/kg silymarin (positive control) and CCLE (250 and 500mg/kg) respectively once daily for 3 consecutive days. Thioacetamide (TAA) (150mg/kg b.w.) was administered intraperitoneally on the 4th day to rats in all groups except the normal control. Evaluations were made for serum levels of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphate (ALP) and serum albumin. Histopathological examination was performed on the excised liver tissues. TAA-induced hepatotoxicity increased ALT, AST, ALP and decreased serum albumin. Histopathological results revealed extensive disruption of the liver histoarchitecture when compared to the normal control liver sections. Pre-treatment with CCLE showed protective effects by normalizing the liver enzymes markers. These results were supported by the histopathological observations. The activity of the CCLE was comparable to that of the standard hepatoprotective drug, silymarin (50mg/kg). Overall findings suggest that CCLE possesses *in vivo* hepatoprotective activity against thioacetamide in rats.

Keywords: *Colocasia esculenta*, thioacetamide, hepatoprotection, liver.

INTRODUCTION

Liver disease may be referred to any disorder of the liver, which includes fatty deposition (steatosis), scarring (liver fibrosis), inflammation (hepatitis), cirrhosis and liver carcinoma (Kaner *et al.*, 2007). Medical intervention in the management of liver disorders is currently insufficient, and therapy to prevent its progression, despite its increasing occurrence, morbidity and mortality, has been inefficient. Plants are used in traditional practices for the treatment of liver disorders (Schuppan *et al.*, 1999). In recent years, there has been a great interest in plants as alternative therapeutic agents. Many plants have been reported to possess protective effects on experimentally induced liver-injury in animal models by different hepatotoxins (Neetu *et al.*, 2011; Kumar, 2012; Shaik *et al.*, 2012). The central focus for hepatoprotective studies is a continuous search for plant-based drugs with antioxidant activity.

Colocasia esculenta L. Schott belongs to the family Araceae. It is a common aroid grown all over the world and is popularly known as cocoyam. Other common names include: Taro, Elephant ear and Dasheen. *C. esculenta* is used both as a food source and as an ornamental plant. It originated in the swampy regions of the South-eastern Asia and has been cultivated as food crop for thousands of years (Cable, 1984). The leaves of

C. esculenta are rich and high in nutrients like vitamins (A, B and C) and minerals (calcium, iron, phosphorus and potassium) (Lewu, 2009). Previous reports have shown the antidiabetic, hypoglycemic, hypolipidemic, anti-inflammatory, antioxidant, anti-cancer, antifungal and antibacterial activities of the plant (Yang *et al.*, 2005; Boban *et al.*, 2006; Ravikumar *et al.*, 2011; Eleazu *et al.*, 2013).

In vitro antihepatotoxic activity of *C. esculenta* leaf juice has been demonstrated (Patil and Ageely, 2011). However, there is paucity of available information regarding hepatoprotective activity of the aqueous leaf extract of *C. esculenta* (CCLE) in TAA-induced liver injury in experimental models. Thioacetamide (TAA), a well-known potent hepatotoxicant widely used to induce liver damage in animal models. Since the leaves of *C. esculenta* are used in folklore-based practices for the treatment of hepatic ailments (Miller, 1971), the present study, therefore, was performed to assess the hepatoprotective activity of *C. esculenta* leaf extract against TAA-induced liver damage in rats as a way to validate its use.

MATERIALS AND METHODS

Plant collection and extraction

Colocasia esculenta fresh leaves were plucked from farms in Army Barracks region in Enugu metropolis during May of 2013. A sample of the plant material was

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identified and compared with the voucher specimen (UNH No.379^a) deposited at the herbarium section of the Department of Botany, University of Nigeria, Nsukka.

Plant extraction

Dried leaves of *C. esculenta* (approximately 200g) were initially pulverized in a mechanical grinder and soaked in 800ml of distilled water. The homogenate was filtered after 24 hours using a muslin cloth. The extractive value of 102mg/ml was obtained.

Laboratory animals

Twenty-five (25) adult male rats (120-150g) of the Wistar strain were obtained from the Animal house attached to the Department of Physiology, University of Nigeria. The animals were kept in clean cages in the College of Medicine Animal House, University of Nigeria, Enugu Campus. The animals were acclimatized for two weeks at the laboratory condition prior to the experimentation. The facility was under standard environmental conditions and a 12:12 hr light/dark cycle. Clean water and commercially available rat pellets (Guinea Feed[®]) were provided for the animals *ad libitum*. Animal handling was in accordance with Institutional and International guidelines for care and use of Animals in Scientific Research (APS, 2002)

Experimental protocol

Rats were divided into five groups (I-V) (n=5). Silymarin (50mg/kg), CCLE (250 and 500mg/kg) and were fed orally to the last three groups of rats (III, IV and V) respectively once daily for three days before thioacetamide (TAA) (150mg/kg b.wt.) was administered intraperitoneally on the 4th day. Group I served as the normal control and was given no treatment while group II received distilled water and same dose of the TAA administration.

Biochemical analyses

Twenty-four hours after the TAA injection, the animals were anaesthetized-using chloroform and blood samples were obtained. The samples were kept undisturbed for 30 minutes for clotting and thereafter centrifuged for 15 minutes at 3000rpm to separate the serum for biochemical assay. The sera obtained were used to estimate the levels of alanine and aspartate transaminases (Reitman and Frankel, 1957), alkaline phosphatase (Hawk, 1954) and albumin (Bartholomew and Delaney, 1966).

Histopathological studies

The liver tissues were excised from the rats in each group and fixed in 10 percent formal saline. Further histological processing was done using the method described by Drury and Wellington (1967). The tissues were sectioned at 5 μ m thickness and stained with haematoxylin and eosin (Baker *et al.*, 2001). Histopathological changes of the liver tissues were observed using a compound microscope and their photomicrographs were captured.

STATISTICAL ANALYSIS

Data obtained were expressed as mean \pm S.E.M. One-way analysis of variance (ANOVA) and post-hoc Tukey-highest significant difference (HSD) test were used to determine the differences among the groups. SPSS software package program (SPSS, Chicago, IL; version 20.0) was used for the analyses. The value of significant difference was considered at $p < 0.05$.

RESULTS

Thioacetamide intoxication significantly increased the serum levels of hepatospecific enzymes (ALT, AST and ALP) in negative control rats (TAA only) when compared with values obtained from the normal control rats ($p < 0.05$). However, there was no significant difference in the value of albumin upon TAA treatment only. Pre-treatment with Silymarin, reduced the levels of ALT, AST and ALP significantly ($p < 0.05$) to values similar to those obtained in normal control rats. Doses of 250 and 500mg/kg b.wt of CCLE also significantly decreased ALT and AST levels ($p < 0.05$) when compared with the negative control (Group II). However, significant decrease and increase in ALP levels ($p < 0.05$) were observed upon pre-treatments with 250 and 500mg/kg b.wt of CCLE respectively when compared with the negative control (table 1).

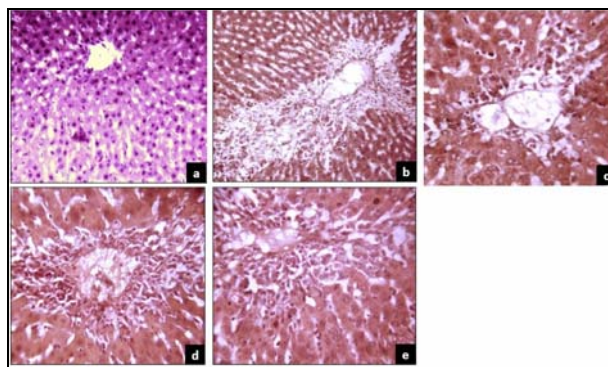


Fig. 1: Photomicrographs of liver sections of rats [Haematoxylin & Eosin: X 400]; (a) Normal control group: Liver section shows normal histoarchitecture; normal hepatocytes with central nucleus, radially distributed sinusoidal spaces and central vein are shown. (b) Thioacetamide treated group: showing hepatocyte degeneration, centrilobular necrosis and inflammatory cellular infiltration. (c) Silymarin treated group: the liver tissue parenchyma is preserved; mild cellular infiltrates are evident. (d) 250mg/kg *C. esculenta* treated group: liver histoarchitecture is maintained, less injury and cellular infiltration at pericentral region are shown. (e) 500mg/kg *C. esculenta* treated group: evidence of minimal injury; few surrounding hepatocytes appear degenerated and inflammatory cellular infiltration are shown.

Table 1: Effect of pre-treatment with crude *C. esculenta* aqueous leaves extract on some biochemical parameters [AST, ALT, ALP and Albumin] upon TAA-induced hepatic injury.

Treatment Groups	Biochemical Parameters			
	ALT (iu/l)	AST (iu/l)	ALP (iu/l)	Albumin (mg/dL)
Group A (control)	34.2±1.8 ^b	44.0±2.3 ^b	121.8±1.3 ^b	2.6±0.2
Group B (distilled water +TAA)	114.0±4.0 ^a	129.4±6.3 ^a	147.6±7.6 ^a	2.0±0.0
Group C (100mg/kg Silymarin +TAA)	45.6±4.4 ^b	45.6±0.7 ^b	115.4±2.5 ^b	3.0±0.0 ^b
Group D (250mg/kg CEAE +TAA)	76.2±2.0 ^{ab}	68.0±5.2 ^{ab}	116.8±2.6 ^b	2.8±0.0 ^b
Group E (500mg/kg CEAE +TAA)	57.0±4.4 ^{ab}	53.0±5.9 ^b	165.4±7.2 ^a	2.0±0.0

Data expressed in mean ± SEM, ^ap<0.05 when compared to the control (Group A) and ^bp<0.05 in comparison to the negative control (Group B). ALT -Alanine transaminase; AST -Aspartate transaminase; ALP -Alkaline phosphatase

The findings from histopathological studies of liver tissues from rats in all groups are shown in fig. 1. The normal control rat liver which received no treatment showed intact central vein, sinusoids and hepatocytes (fig. 1A), whereas, TAA intoxicated rats revealed hepatocyte degeneration, centrilobular necrosis and infiltration of inflammatory cells (fig. 1B). However, pre-treatments with the Silymarin and CCLE reduced the severity of the lesions exerted by TAA treatment to great extents (figs. 1C-D).

DISCUSSION

In the present study, the hepatoprotective activity of CCLE against thioacetamide-induced liver injury in rats was investigated. TAA is a well-established tool used to induce hepatotoxicity in experimental animal models (Alkiyumi *et al.*, 2012; Zheng *et al.*, 2012). The hepatic cells contain a host of enzymes, which are involved in a number of metabolic processes. Detoxification of xenobiotics and toxins is one of the major functions of the liver (Mitra *et al.*, 1998). Exposure to hepatotoxicants elicits hepatic injury via various mechanisms and the affected hepatocytes release their constituents into the blood circulation. The major intracellular liver-specific enzymes are alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) (Bhadoria *et al.*, 2008). AST and ALT are found in the cytoplasm but ALT is the most specific marker enzyme for the liver. ALP is located around the bile canaliculi and is excreted normally by the liver through bile, but increased serum levels indicate bile duct epithelial damage following exposure to toxic agents (Talwar and Srivastava, 2002; Wallace and Meyer, 2010).

After treatment of experimental rats with TAA in this work, profound deleterious effects on serum biochemistry and histopathology of the liver tissues of treated rats were observed. Accordingly, the acute liver damage registered a significant increase in ALT, AST and ALP activities and decrease in serum albumin (AB) levels in the TAA-only treated group, and this may be regarded as an indicator of hepatic damage (Zafar and Mujahid Ali, 1998). Similar findings have been described earlier by other authors

(Galisteo *et al.*, 2000). Histopathological examination of the liver confirmed these findings by revealing pathomorphological alterations.

The mechanisms underlying TAA-induced hepatotoxicity still remains unclear. Shortly after TAA administration and within 24 hours in rats, TAA undergoes an extensive oxidative metabolism to acetamide and to the toxic metabolite called thioacetamide S-oxide (TAASO) by a mixed function oxidase system (Kim *et al.*, 2000). By means of cytochrome P450 enzymes, which are present in the liver microsomes, TAASO is further metabolized by oxidative stress (de David *et al.*, 2011) to further products, including a very reactive polar product, thioacetamide-S-dioxide, which is thought to be a sulfene (Bruck *et al.*, 1999). This oxidative pathway, which generates free radicals cause lipid per oxidation, reduces levels of glutathione and increases the activities of liver marker enzymes in the serum (Perez-Tortosa *et al.*, 2012). Thus, the binding of the toxic metabolite to tissue may be responsible for the extensive liver tissue necrosis observed in the present study.

Pre-treatment with the doses of the CCLE (250 and 500mg/kg b.w.) were effective to counteract the TAA-induced hepatic injury by both reducing the increased levels of the liver enzymes and elevating serum albumin levels to normal functioning status. This activity was similar to that conferred by the standard hepatoprotective drug, Silymarin. However, a somewhat potentiated increase in serum ALP levels was observed by treatment with the high dose (500mg/kg b.w) of the extract. The hepatoprotective potency of the extract, notwithstanding, cannot be questioned since the ALT and AST levels were decreased including the relatively preserved histoarchitecture of the liver parenchyma and most especially the portal regions of treated rats. It is well known that markedly increased levels of ALP is not only observed in hepatobiliary obstruction and disorganized histoarchitecture of the hepatic tissue (Witthawaskul *et al.*, 2003), however other tissues such bone, intestines and kidneys are known to produce some amounts of the enzyme (Badgu and Merugu, 2013). More so, a previous report has communicated reduced serum ALT and AST

levels but unchanged serum ALP values after administration of a hepatoprotective drug in TAA-induced hepatotoxicity (Zheng *et al.*, 2012). In spite of the better improved serum levels of ALT and AST produced by pre-treatment with the higher dose of CCLE (500 mg/kg) than that of the lower dose (250mg/kg), the lower dose may be considered a better antihepatotoxicant given that its administration significantly improved all liver enzymatic parameters assayed compared with the negative control (TAA-only) group.

In agreement with the findings in this work, extracts of *C. esculenta* leaves have been shown in previous studies to offer protection on hepatocytes (in-vitro and in-vivo) against injurious effects of carbon tetrachloride (CCl₄) and paracetamol (Patil and Ageely, 2011; Azubike *et al.*, 2013). The authors reported that the hepatoprotection observed may have been elicited by the hindrance of free radicals formation from the hepatotoxicants. More so, the hepatoprotection offered by CCLE in our work may be attributed to its ability to interfere with the biotransformation of TAA to form harmful metabolites responsible for hepatic damage. In our previous work, phytochemical screening of *C. esculenta* leaves revealed the presence of alkaloids, saponins, flavonoids, terpenoids and steroids (Azubike *et al.*, 2012) which are known antioxidants. The mechanism behind hepatoprotective potential of most herbs is due to their antioxidant activity (Sasidharan *et al.*, 2010; Ghori *et al.*, 2014). Therefore, it is anticipated that the hepatoprotective activity of *C. esculenta* is due to a single or combination of these bioactive components responsible for scavenging the free radicals formed during the biotransformation of TAA.

In conclusion, the findings in this study show that *Colocasia esculenta* leaf extract possess the ability to offer significant protection to the liver of albino Wistar rats against thioacetamide-induced hepatotoxicity.

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