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IN VIVO HEPATOPROTECTIVE STUDIES ON SAPONIN AND ALKALOID RICH FRACTIONS ISOLATED FROM *COLOCASIA ESCULENTA* [L. SCHOTT] LEAVES

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Abstract

Colocasia esculenta leaves have medicinal uses as antimicrobial, antidiabetic, hypolipidemic and hepatoprotective agents. The present study was designed to investigate the effects of the saponins and alkaloid-rich fractions of *C. esculenta* leaves on thioacetamide-induced hepatic injury in rats. Hepatic damage in rats was assessed after intraperitoneal treatment with thioacetamide (TAA) following a 6–day pretreatment with silymarin, saponins (*SPF*) and alkaloid (*ALF*) rich fractions in respective groups. Serum levels of alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphate (ALP) were estimated and microscopical examination of excised liver tissues were performed. The study revealed that oral administration of *SPF* significantly prevented TAA-induced elevated levels of ALT, AST and ALP. The histology of the liver sections of *SPF*-treated rats showed marked preservation of the hepatic parenchyma comparable to the standard hepatoprotective drug, silymarin, whereas pretreatment with *ALF* showed mild preservation of the liver histoarchitecture of treated rats. The present study indicated that the saponins content of *C. esculenta* leaves could be responsible for the hepatoprotective effects of the plant.

Keywords: *Colocasia esculenta*, hepatic injury, Saponins, Alkaloids

Introduction

The metabolic and detoxification functions of the liver is well documented, and these render the hepatic tissue vulnerable to disorders which include hepatitis (inflammation), steatosis (fatty deposition), fibrosis (scarring), and cirrhosis [1]. Diseases of the liver are a worldwide problem and conventional medical treatment is currently insufficient and sometimes is associated with serious side effects. Consequently, many people rely on complementary and alternative sources of treatment [2].

Colocasia esculenta L. Schott which belongs to the family Araceae is a commonly known as cocoyam. It is grown all over the world and has common names which include Taro, Elephant ear, and Dasheen. Its leaves are used traditionally for the treatment of liver ailments, stomatitis haemorrhoids and constipation [3,4]. Anti-inflammatory, anti-hypertensive, anti-hepatotoxic, antifungal, antibacterial antidiabetic, hypoglycemic, hypolipidemic, antioxidant and anti-cancer activities of *C. esculenta* leaves have been documented [5-11].

The hepatoprotective efficacy of the crude leaves of *C. esculenta* on acetaminophen and thioacetamide induced hepatotoxicity have been demonstrated in experimental models [12,13]. The two most abundant phytochemicals present in the *C. esculenta* leaves are saponins and alkaloids [14] and their hepatoprotective effects have not been determined. The present study, therefore, seeks to evaluate the hepatoprotective potential of saponins and alkaloids isolated from the leaves of *C. esculenta* in liver damage induced by thioacetamide (a well-known hepatotoxin), as a way to determine the phytochemical responsible for the hepatoprotective activity of the plant leaves.

Materials and Methods

Plant Collection

Colocasia esculenta fresh leaves were plucked from farms in Asata, Enugu metropolis during the month of June, 2013. Plant material identification and comparison with the voucher specimen [UNH No.379[a]] deposited at the herbarium section of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka was done.

Preparation of Plant extract and Isolation of Crude Saponins and Alkaloid fractions

Two kilograms (2kg) of milled dried leaves of *C. esculenta* was defatted using 4 litres of Petroleum ether for 72 hours. The marc was dried and macerated with 10 litres of 95% methanol and shaken intermittently for 48 hours. The mixture was filtered and the filtrate evaporated to obtain a dark green semisolid mass which was preserved under refrigerated conditions. The extract was divided into two parts for the isolation of crude saponins and alkaloid fractions using conventional methods [15-17].

Isolation of Crude Saponins: One part of the methanol extract was partitioned with n-butanol and water (1:1, v/v) and was shaken thoroughly. The n-butanol layer was separated after the mixture was allowed to stay overnight. Using aliquots of n-butanol, the aqueous partition was washed five times until it became colourless. Under reduced pressure, pooled butanol partition was evaporated to yield a residue. The n-butanolic residue was dissolved in methanol and precipitated by addition of diethyl ether in excess to yield the crude saponin fraction [15].

Isolation of Crude Alkaloids: The second part of the methanol extract was used for the extraction of alkaloids using a modified version of the classic 'acid-base shakeout' method [16,17]. The extract was acidified with tartaric acid titrated to pH5. The mixture was partitioned with ethyl acetate pre-saturated with water. The aqueous acidic phase obtained was made alkaline with sodium bicarbonate and partitioned using ethylacetate. The ethyl acetate partition was evapoarted under vacuum at 45 – 50°C to yield the alkaloid fraction.

Laboratory animals

Twenty-five (25) male rats (120 - 150g) of the Wistar strain were obtained from the Animal house of 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 kept under standard environmental conditions and a 12:12 hr light/dark cycle. Water and commercially available rat pellets (Guinea Feed®, Benin Nigeria) were provided for the animals *ad libitum*. The animals were allowed to acclimatize for one week at the laboratory condition prior to the experimentation. Animal handling was in accordance with Institutional and International guidelines for care and use of Animals in Scientific Research [18].

Experimental design

The rats were randomly divided into five groups (A - E) (n=5). Group A served as the normal control and was given no treatment. Distilled water (10ml/kg), Silymarin (100mg/kg), ALF (100mg/kg) and SPF (100mg/kg) were fed orally to the last four groups of rats (B, C, D and E respectively) once daily for six days. On Day 7, intraperitoneal administration of thioacetamide (TAA) (150mg/kg b.wt.) was performed to induce liver toxicity in all rats in groups B – E. Sixteen hours post-TAA injection,

blood samples were obtained via retro-orbital punctur e from the rats for biochemical analysis.

Biochemical Analyses

Sera were separated from the blood samples after cen trifugation at 2500rpm at 30°C for 15min. Activities of AL T and AST [19] and ALP [20] were determined.

Gross and Histopathological studies

The rats were anesthetized using chloroform and the li ver tissues were removed. Necropsy was conducted to de termine macroscopical changes. The samples were blotte d with filter paper and weighed on a balance. The relative liver index [ratio of liver weight and the animal's body we ight (at the end of experiment) x 100] of each rat was calc ulated. The tissues were further fixed in 10% formal saline prior to histological processing [21]. Haematoxylin and Eo sin (H&E) staining procedure was employed to stain the li ver sections for light microscopical examination.

Statistical Analysis: The results obtained from the stud y were expressed as mean ± S.E.M. of five rats per group. Data were subjected to one-way analysis of variance (AN OVA). This was followed by Tukey-highest significant diffe rence (HSD) post-hoc test to determine the statistical sig nificance of the differences in the parameters among the groups. SPSS software package program (SPSS, Chicago, IL; version 20.0) was used for the analyses. The level of si gnificance was considered at $p < 0.05$.

Results

Biochemical parameters

The effects of pre-treatment with the different fractions (*SPF* and *ALF*) of *C. esculenta* leaves on serum levels of ALT, AST and ALP in TAA-induced hepatotoxicity are shown in Table 1. Data showed markedly increased levels of these b iochemical parameters in TAA-control rats after 16h of inj ection when compared with values from normal control ($p < 0.05$). Pre-treatment of rats with *ALF* (100mg/kg b.wt.) showed increased levels ($p<0.05$) of ALT, AST and ALP w hen compared with baseline control, whereas no significa nt change was observed when compared with the negati ve control group [TAA-only treated rats]. Conversely, the effects of pre-treatment with *SPF* [100mg/kg b.wt.] is simi lar to that of the standard hepatoprotective drug, Silymar in, both revealing significantly reduced levels of all the liv er marker enzymes assayed when compared with the neg ative control.

Liver index

Table 2 shows the mean liver weight and index of the res pective treatment groups and controls. Increased liver in dex values were observed in TAA-only treated rats [negat ive control group - B] ($p<0.05$).

Histological examination

The light microscopical findings of the control group [Fig ure 1A] shows normal tissue architecture with prominent central vein, normal hepatocytes and sinusoidal spaces. I n the TAA-intoxicated group [Figure 1B], the liver sections showed marked centrilobular necrosis and infiltration of i nflammatory cells. The histological profile of rats pre-trea ted with *ALF* showed partial preservation of hepatocytes [Figures 1D]. However, the severity of the lesions associat ed with hepatotoxicity induced by TAA was extensively re duced upon pre-treatments with Silymarin and *SPF* [Figur es 1C and 1E respectively].

Discussion

Various animal models have been used experimentally to evaluate hepatoprotective agents against hepatotoxic ants such as acetaminophen, carbon tetrachloride and thi oacetamide. Detoxification of toxins and xenobiotics is o ne of the important roles of the liver. Thioacetamide, a wi dely used hepatotoxin [22], is metabolized in the liver by Cytochrome-P450 enzymes. The very reactive metabolite s produced during the cytochrome-P450 mediated oxidati on are TAAS-oxide and TAAS-dioxide [23,24].

The mechanism of hepatotoxicity caused by TAA is not completely understood. However, previous researchers r eported that oxidative stress may play a major role in live r damage induced by TAA [25,26]. Oxidative stress is indu ced by these harmful metabolites of TAA in the liver cells (especially those at the centrilobular region) resulting in c ell permeability changes, increased nuclear volume, inhibi tion of the activity of mitochondria and eventual death [2 7,28]. Centrilobular necrosis, liver cirrhosis and inflammati on are some of the lesions produced by TAA [29,30].

In acute liver necrosis, the injury exerted on the hepat ocytes is reflected by the release of their constituents int o the blood circulation resulting in markedly elevated leve ls of the liver marker enzymes (ALT, AST and ALP). The es timation of these parameters is a useful indication of the severity and type of hepatic cell damage [31]. Accordingly , the present study showed that treatment with TAA signi ficantly elevated the serum levels of the liver marker enzy mes. Moreover, a profound reversal of these elevated he pato-specfic enzymes was observed in the group of rats p re-treated with the saponins fraction only, and this effect was similar to those of the silymarin-treated group. To ou r knowledge, this is the first study that reveals the hepato protective

efficacy of saponins fraction of *C. esculenta* leaves against TAA-induced hepatotoxicity in rats.

The significantly improved serum enzymatic parameters upon pre-treatment with saponins fraction was confirmed by the well preserved tissue parenchyma of the liver of treated rats upon microscopical examination. Several plant-derived saponins with hepatoprotective activities have been reported recently [32-34] and they have antioxidative activity which afford protection mechanisms against the oxidation reactions of free radicals in the body system [35].

Many alkaloids isolated from plants have also shown efficient hepatoprotection against hepatotoxins in experimental models [36,37]. Pre-treatment with the alkaloid fraction did not produce significant change on the biochemical parameters evaluated in the present study. However, histopathological findings did not show marked lesions as observed in TAA-only treated rats. Perhaps this may be due to the dose administered or an inability of the phytochemical to effectively prevent the toxic effects of TAA. Conversely, a profound hepatoprotection was observed with the saponins fraction which may be attributed to the direct action of the phytochemical on the bioactivation of TAA derivatives. This activity ultimately reduced the extent of TAA-induced cell disruption and offered remedial measures against the deleterious effects of TAA metabolites. Thus, this connotes an ability of the plant phytochemical to scavenge free radicals formed during TAA biotransformation. It is well established that the antioxidant activity of many herbs serves as the major mechanism behind their hepatoprotective activities [38].

In conclusion, the present study demonstrates that the saponins content of *Colocasia esculenta* leaves exhibited potent hepatoprotective effect in thioacetamide-induced hepatotoxicity in rats. This finding is supported by biochemical analyses and histological examination of the liver. The alkaloid fraction did not offer significant hepatoprotection. Perhaps, the hepatoprotection offered by the leaves of *C. esculenta* may be due to the combined effects of these phytochemicals. Further studies are required to identify and characterize the active principle and also establish the mechanisms responsible for the hepatoprotective activity demonstrated in this study.

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

GROUPS	Biochemical Parameters		
	ALT(iu/l)	AST(iu/l)	ALP(iu/l)
Group A (control)	44.20±6.03#	61.60±1.96#	120.80±2.01#
Group B (TAA only)	197.74±52.98*	274.13±21.83*	164.00±7.19*
Group C (100mg/kg Silymarin +TAA)	54.40±3.19#	65.40±2.46#	117.20±1.66#
Group D (100mg/kg ALF +TAA)	129.60±25.81	237.20±29.59*	176.70±8.78*
Group E (100mg/kg SPF +TAA)	65.80±5.35#	83.00±5.11#	121.60±3.22#
F-ratio	5.910	38.099	26.968
Sig.	0.003	0.000	0.000

Data expressed in mean ± SEM; *p<0.05 when compared to the control (Group A) and #p<0.05 in comparison to the negative control (Group B). ALT – Alanine transaminase; AST – Aspartate transaminase; ALP – Alkaline phosphatase; ALF – Alkaloid fraction; SPF – Saponin fraction; TAA – Thioacetamide.

Table 2: Effect of pre-treatment with crude alkaloids and saponins-rich fractions of *C. esculenta* leaves on liver index of treated rats upon TAA-induced hepatic injury

GROUPS	Parameters		
	Body weight (g)	**Liver weight (g)**	**Liver index**
Group I (control)	144.00±1.87	5.94±0.09	4.13±0.03$^{\#}$
Group II (TAA only)	135.00±3.16	6.63±0.64	4.88±0.37*
Group III (100mg/kg Silymarin +TAA)	134.00±2.45	5.69±0.13	4.24±0.39
Group III (100mg/kg *ALF* +TAA)	125.00±5.00	5.33±0.22	4.26±0.03
Group IV (100mg/kg *SPF* +TAA)	149.00±1.00	6.17±0.06	4.14±0.03$^{\#}$

Data expressed in mean ± SEM; *p<0.05 when compared to the control (Group A) and $^{\#}$p<0.05 in comparison to the negative control (Group B). *ALF* – Alkaloid fraction; *SPF* – Saponin fraction; TAA – Thioacetamide.

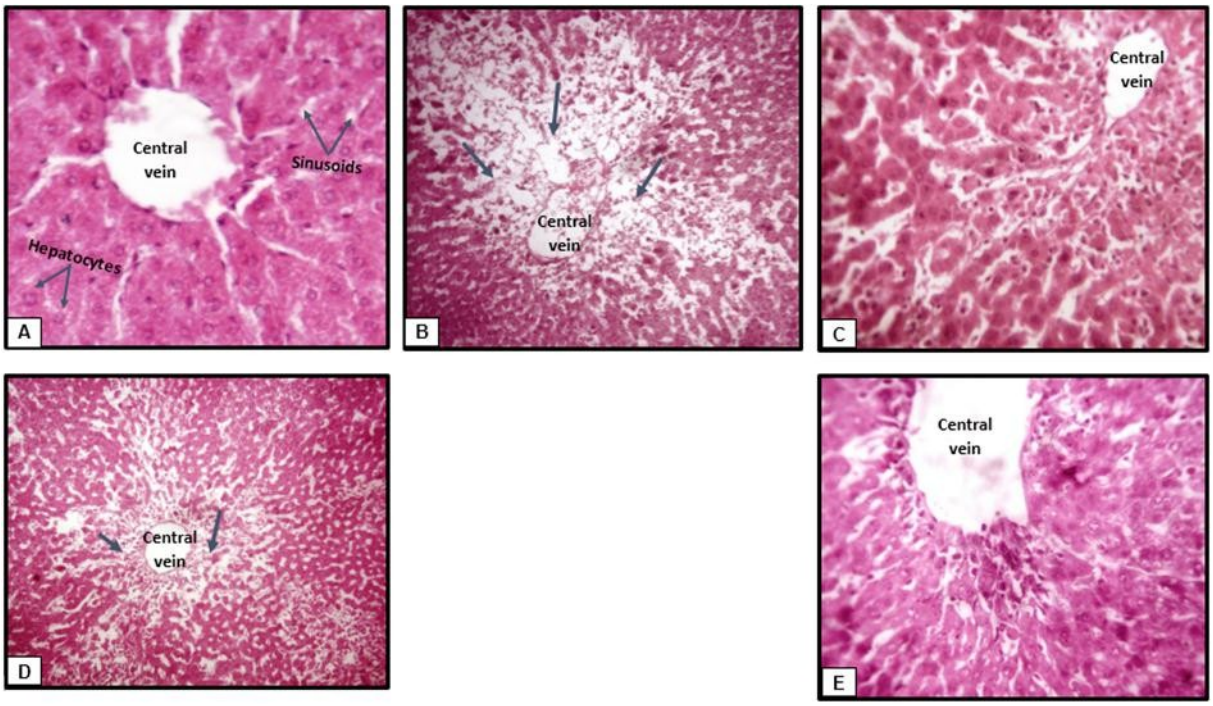

Figure 1: Photomicrographs of liver sections of rats [Stain: Haematoxylin & Eosin]; (A) **Normal control group**: Liver section shows normal histoarchitecture; normal central vein, radially distributed sinusoids and hepatocytes are shown (Mag.-x400). (B) **Thioacetamide treated group**: Extensive hepatocyte degeneration, centrilobular necrosis and mild inflammatory cellular infiltration are observed around the central vein (arrows) (Mag.-x100). (C) **Silymarin treated group**: The tissue parenchyma appears appreciably preserved (Mag.-x400). (D) **100 mg/kg ALF of *C. esculenta* treated group**: liver histoarchitecture is partially preserved, some pericentral hepatocytes appear degenerated and mild cellular infiltration are obvious (arrows) (Mag.-x100). (E) **100 mg/kg SPF of *C. esculenta* treated group**: Preservation of liver histoarchitecture is evident (Mag.-x400).