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Research Article

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Sub-acute toxicity of *Caladium bicolor* (Aiton) leaf extract in Wistar rats

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ABSTRACT

Herbal medicinal plants constitute an integral component of plant biodiversity whose application for therapeutic purposes has been a common practice since antiquity. However, studies have reported tissue pathologies following exposure to herbal medicinal plants or extracts. This study was carried out to assess the possible pathological effects of sub-acute exposure of methanol extract of *Caladium bicolor* on the hepatic histomorphology of experimental Wistar rats. Twenty four Wistar rats were divided into four groups A-D. Group A was administered with distilled water (2 ml/kg b.w) and groups B-D administered with 100 ml/kg, 200ml/kg and 300ml/kg b.w. of the extract respectively for twenty eight days. The bodyweight of experimental animals was recorded at regular intervals during the study period. After the treatment period, hepatic tissue was harvested, weighed and processed for histopathological study. The results showed that exposure to the extract causes significant ($p<0.05$) body and organ weight reduction, a significant increase in serum level of ALT and AST as well as prominent hepatic histopathological changes such as inflammation, necrosis and steatosis. Sub-acute exposure to methanol extract of *C. bicolor* thereby causes dose-independent hepatopathy in experimental animals. Hence, its application for therapeutic purposes needs to be re-evaluated and preferably discontinued to avoid these associated hepatic tissue pathologies.

KEYWORDS: Medicinal plant toxicity, Caladium bicolor, Hepatopathy, Wistar rats

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INTRODUCTION

Herbal medicinal plants constitute an integral component of natural plant biodiversity whose application for therapeutic purposes has been a common practice since antiquity. Notably, their applications have greatly increased in recent years resulting into significant expansion of the field of phytomedicine. In particular, the prominence of herbal medicinal plants usage in developing countries is due to factors such as accessibility and affordability as well as greatly reduced adverse effects relative to orthodox medicines [1-3].

Some documented pharmacological applications of medicinal plants include antioxidant [4], anti-inflammatory [5], hypolipidemic [6], antidiarrheal [7], anti-proliferative [8,9], antihypertensive [10], neuroprotective [11], hepatoprotective [12], nephroprotective [13,14], antidiabetic[15], anti-ulcer [16,17], gastroprotective [18,19], haematopoietic [20] and many more.

However, some studies have reported tissue toxicity or pathology following exposure to herbal medicinal products during generic or specific therapeutic procedures [21,22]. Specifically, studies by Elvin-Lewis [23] and Mbaka et al [24] have reported toxic effects resulting from applications of certain herbal medicinal plant derivatives. Indeed, there has been an increase in organ toxicity arising from application of natural plant products or herbal formulations [25]. According to a study by Patrick-Iwuanyanwu et al [26], some medicinal plants possess inherent toxic properties due to their phytochemical constituents and exert toxic effects when exposed to internal body organs for prolonged duration and/or at high dosage.

Caladium bicolor (Aiton) is a medicinal plant belonging to the Araceae family and commonly cultivated in and around residential homes due to its ornamental value. It is commonly called Angel's wings, elephant's ear or heart-of-Jesus. It has a characteristic bi-coloured, heart-shaped leaves [27]. Some of the reported therapeutic applications of *C. bicolor* include anti-diarrheal, anti-ulcer, topical treatment of wounds and boils [28,29]. The therapeutic properties of *C. bicolor* can be attributed to the presence of secondary metabolites such as tannins, alkaloids and saponins [30]. However, phytochemical analysis of *C. bicolor* also showed the presence of toxic calcium oxalate which has been implicated in pathology of internal organs such as the gastrointestinal tract and kidney [31,32]. This study was therefore carried out to assess probable pathological effect of sub-acute exposure of methanol extract of *C. bicolor* on hepatic histomorphology of experimental Wistar rats.

MATERIALS AND METHODS

Study Plant Material

Fresh whole *C. bicolor* plant was obtained from Isihor community in Benin City, Nigeria. The identification of plant sample was done at the Department of Pharmacognosy, Igbinedion University, Okada, Edo State, Nigeria (6^0 20′ 17.34″ N and 5^0 37′ 32.70″ E). Thereafter, bulk quantity sufficient for the study was collected for the subsequent process of extraction.

Preparation of Plant Extract

The leaves of collected study plant were detached, air-dried at room temperature ($23^0C \pm 2^0C$) and pulverized into powdered form using a mechanical grinder. 500 g powdered leaf material was macerated in methanol for 72 hours with intermittent agitation. Afterwards, the preparation was filtered, weighed and evaporated using rotary evaporator (regulated at 40^0C). The residue was cooled, weighed and percentage yield determined (as 8.5%). 40 g of the methanolic extract was dissolved in 100 ml distilled water to obtain the stock solution and the treatment regimen was determined relative to dosages and weight of experimental animals.

Experimental Animals

In this study, twenty four male albino Wistar rats, weighing between 165–175 g and sourced from the Central Animal House Facility, Igbinedion University, Edo State, Nigeria were employed. The study was carried out within the facility, wherein study animals were housed in animal cages under hygienic conditions, fed on standard animal feed, allowed free access to drinking water *ad libitum* and exposed to 12 hour light/dark cycle.

Experimental Design

The experimental rats were randomly divided into four groups (A-D) which include control group A and treatment groups B-D each comprising 6 animals. During the 28-day treatment period, group A was administered with distilled water [2 ml/kg body weight (b.w.)] and groups B-D administered with 100 ml/kg, 200ml/kg and 300ml/kg b.w. of the methanolic extract respectively. All administrations were done once daily via the oral route using an orogastric canula coupled to calibrated hypodermic syringe. The body weight of the experimental animals was monitored at days 0, 7, 14, 21 and 28 of treatment.

Biochemical Assay

At the end of the treatment period, just before the experimental animal were sacrificed, blood sample was collected through retro-orbital sinus, centrifuged at 4000 rpm for 15 minutes to obtain clear serum that was used to evaluate levels of hepatic marker enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) following the method by Reitman and Frankel [33].

Study Tissue Collection, Processing, Sectioning and Staining

The experimental animals were sacrificed after the treatment period, their hepatic tissue harvested and weighed to evaluate the post-treatment organ weight. The hepatic tissues were subsequently processed for histopathological study as follows: fixation in 10% Neutral Buffered Formalin, dehydration with increasing grades of alcohol (70%, 90% and absolute), clearing in Xylene and embedding in paraffin wax to form tissue blocks. The hepatic tissue blocks obtained by tissue processing were sectioned to generate 5 μ thick sections which were mounted on microscope slides and stained by Haematoxylin and Eosin (H & E) technique [34]. The stained tissue sections were subjected to microscopic examination to for histopathological assessment.

Histopathological Assessment

During microscopic examination, photomicrographs of tissue sections were generated using digital camera for microscope. The observable histopathological changes within the hepatic parenchyma which include inflammation, necrosis and steatosis were evaluated using the image-J software (NIH, Bethesda, MA, USA).

Statistical Analysis

Experimental data obtained was statistically analyzed using IBM-SPSS version 20 (IBM Corp, NY, USA) and presented as mean ± standard error of mean (SEM). Statistical results were compared using T-test and one-way analysis of variance (ANOVA) and $p<0.05$ was regarded as the level of significant difference.

RESULTS AND DISCUSSION

Evaluation of Body Weight and Hepatic Tissue Weight of Experimental Animals

The mean values of body weight, organ weight and relative organ weight of experimental animals in Groups A-D were given in Figure 1. Relative to the control Group A, the mean values of body weight, organ weight and relative organ weight showed significant ($p<0.05$) difference in all treatment groups (B-D).

Biochemical Assay

The mean values of serum ALT and AST levels in control group A and treatment groups B-D animals were given in Figure 2. The serum levels of ALT and AST in treated Groups C-F animals were non-significantly elevated but significantly ($p < 0.05$) elevated in test control group B in comparison to levels in the normal control Groups A.

Hepatic Histopathological Results

Some histopathological changes were observed during microscopic examination of hepatic tissue sections of

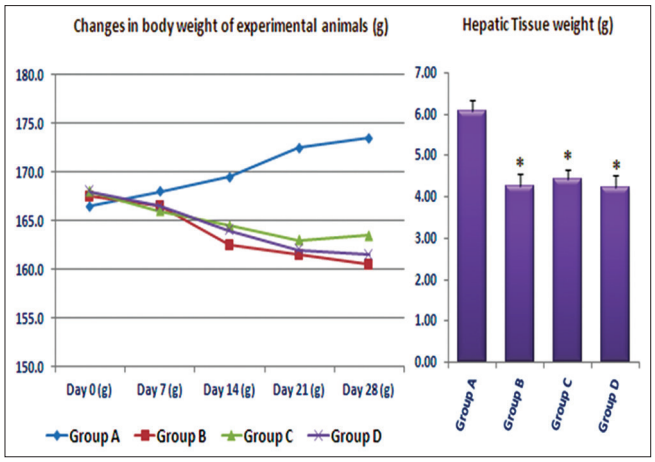

Figure 1: Evaluation of changes in body weight and hepatic tissue weight of experimental animals in Groups A–D. (Group A = Distilled water, groups B-D = 100mg/kg, 200 mg/kg and 300 mg/kg methanolic extract of *C. bicolor* respectively). * indicates significant difference from control group A at P < 0.05

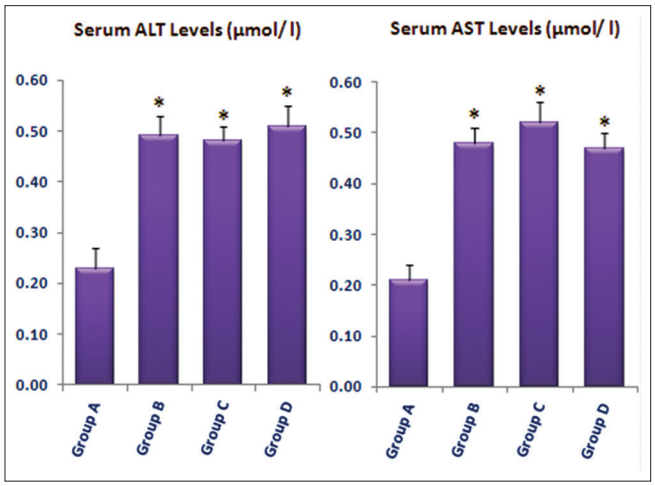

Figure 2: Evaluation of serum ALT and AST levels in groups A–D experimental animals. (Group A = Distilled water, groups B-D = 100mg/kg, 200 mg/kg and 300 mg/kg methanolic extract of *C. bicolor* respectively). * indicates significant difference from control group A at P<0.05.

experimental animals. These include inflammation, necrosis and steatosis within hepatic parenchyma of experimental animals in Groups A-D (Figure 3). Evaluation of these histopathological changes showed their significant prominence within the hepatic parenchyma of treatment groups B-D relative to control group I (Figure 4).

The hepatic tissue is a very versatile organ involved in metabolism during which organic or synthetic compounds whether naturally-occurring or synthetic are constantly exposed including those with variable toxic effects on hepatic parenchyma such as necrosis, lesions, fibrosis or tumor [35,36]. Generally, some herbal medicinal preparations have been reported to contain phytochemical constituents which potentially exhibit toxic effects including hepatopathy [37,38].

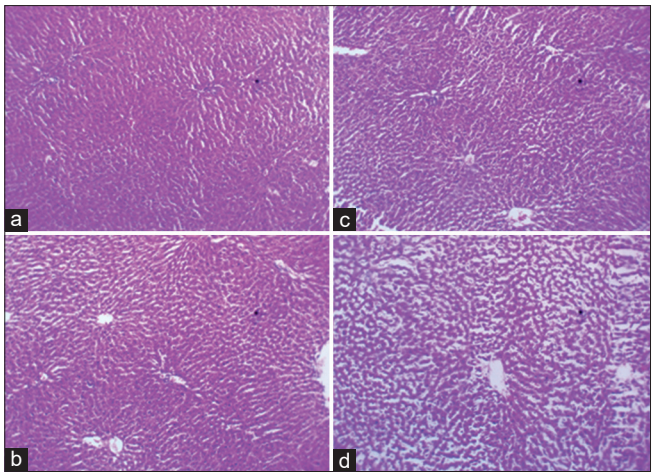

Figure 3: Photomicrographs showing prominent histopathological changes such as inflammation, necrosis and steatosis within hepatic parenchyma of experimental animals in Groups A-D (H & E X100) (Group A = Distilled water, groups B-D = 100mg/kg, 200 mg/kg and 300 mg/kg methanolic extract of *C. bicolor* respectively)

According to the findings of this study, exposure to methanol extract of *C. bicolor* causes significant ($p<0.05$) comparative reduction in the body and hepatic tissue weight of experimental animals (Figure 1). Previous studies have reported that such weight changes can be associated with the deleterious effect of exposure of xenobiotics including certain phytochemical constituents [39,40].

Typically, exposure to potential cytotoxic substances including those derived from herbal medicinal preparations can cause generation and activation of reactive oxygen species which in turn results into membrane lipid peroxidation and the associated impairment of the antioxidant defence system culminate to tissue pathologies [41]. Hepatic tissue pathologies following exposure to hepatotoxins and impairment of antioxidant defence system are indicated by endogenous enzyme markers including ALT and AST. From the findings of this study, exposure to methanol extract of *C. bicolor* causes significant ($p<0.05$) elevation in serum concentration of ALT and AST (Figure 2) which is indicative of induced hepatopathy.

The findings of this study further showed prominent histopathological changes within the hepatic parenchyma of experimental animals following exposure to methanol extract of *C. bicolor* (Figure 3). The evaluation of these changes including inflammation, necrosis and steatosis showed significant increase in all the treatment groups relative to the control group (Figure 4) which may indicate of induced hepatopathy due to exposure to the extract.

As earlier posited, some herbal medicinal plants may exhibit deleterious effects. Such effects may usually follow prolonged, unregulated use especially at high dosage levels [42]. *C. bicolor* extract has been reported to contain calcium oxalate, a toxic compound that causes irritations in oral tissues and toxicity in gastrointestinal organs when ingested [32,43].

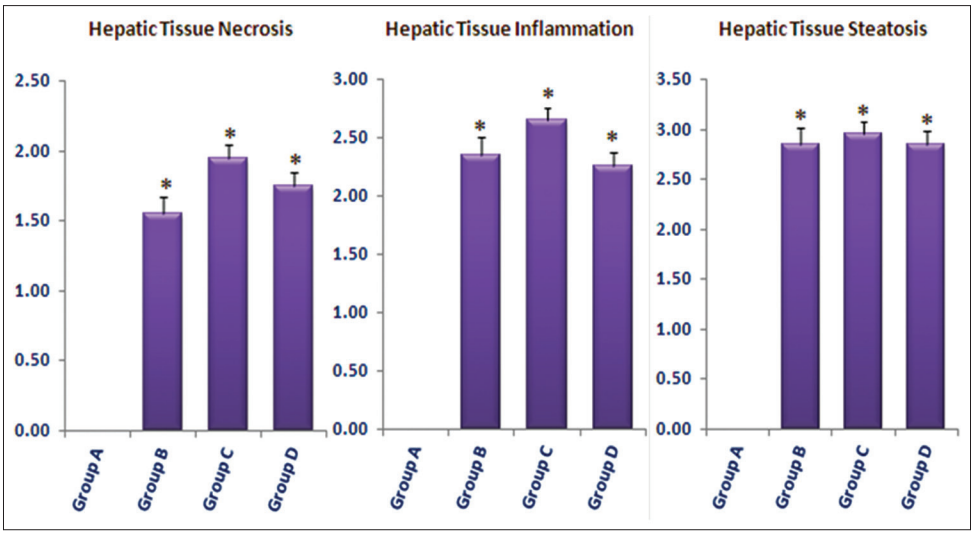

Figure 4: Evaluation of hepatic histopathological changes such as inflammation, necrosis and steatosis within hepatic parenchyma of experimental animals in Groups A-D (Group A = Distilled water, groups B-D = 100mg/kg, 200 mg/kg and 300 mg/kg methanolic extract of *C. bicolor* respectively)

Another study by Cruzan et al, [44] showed that exposure to ethylene glycol leads to accumulation of toxic calcium oxalate crystals within renal parenchyma leading to renal tissue or tubular degeneration. Invariably, the calcium oxalate-mediated tissue toxicity may be widespread and based on the findings of this study may account for the prominent histopathological changes in the hepatic tissue of experimental animals following sub-acute exposure to methanol extract of *C. bicolor*.

CONCLUSION

Sub-acute exposure to methanol extract of *C. bicolor* causes dose-independent hepatopathy in experimental animals indicated by prominent histopathological changes within the hepatic parenchyma of experimental animals. Hence, its application for therapeutic purposes needs to be re-evaluated and preferably discontinued to avoid these associated hepatic tissue pathologies.

ETHICAL APPROVAL

This study was approved by the Research and Ethics Committee of Igbinedion University, Okada, Edo State, Nigeria. All protocols used in this study complied with International guidelines for handling and use of experimental animals.

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AUTHORS' CONTRIBUTIONS

Omotoso DR conceptualized the study, designed study protocols, interpreted data, reviewed and edited the original draft. Okojie IG and Brown I performed the experiment, wrote the original draft. All authors read and validated the final draft.

COMPETING INTERESTS

The authors declare no competing interests in this study.

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