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Histopathological Changes in Gastrointestinal Tissues of Wistar Rats Administered with Methanolic Leaf Extract of *Caladium bicolor* (*Araceae*)

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Authors' contributions

This study was carried out in collaboration among all authors. Author DRO designed the study, performed the statistical analysis and wrote the protocol. Author IB wrote the first draft of the manuscript. Authors AOA and IB managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

To assess the effect of methanolic leaf extract of *Caladium bicolor* on the histomorphology of gastrointestinal tissues of experimental animals.
Twenty four Wistar rats (weighing between 175-190 g) were randomly and equally divided into four groups which include one control group (CG) and three treatment groups (TG I, TG II and TG III). The CG was administered with distilled water [2 ml/kg body weight (b.w.)] while TGs I, II and III were administered with 100 ml/kg, 200 ml/kg and 300 ml/kg (b.w.) of *C. bicolor* extract respectively. All administrations were done orally and once daily for a period of thirty days. The body weight of all animals was recorded at the beginning and end of study. After the period of study, gastric and small intestinal tissues of experimental animals were harvested, processed, converted to tissue blocks and sectioned. Tissue sections were stained using Haematoxylin and Eosin (H&E)

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technique. Thereafter, stained sections microscopically examined for observable histopathological changes within study tissues.
The results of this study showed that exposure to *C. bicolor* extract causes significant ($p < 0.05$) body weight loss in TGs I-III compared to CG. In addition, prominent histopathological changes were observed in gastrointestinal tissues of experimental animals in TGs I-III including gastric mucosal surface erosion and intestinal villi degeneration compared to normal gastrointestinal histomorphology of CG animals.
These histopathological changes may be associated with toxic effect of phytochemicals constituents of the extract. Therefore, its application for therapeutic purposes needs to be thoroughly re-validated or perhaps disallowed where alternative therapeutic agents with minimal toxic potential exist.

Keywords: Caladium bicolor; histopathology; gastrointestinal tissues; experimental animals.

1. INTRODUCTION

Herbal medicinal plants refer to members of natural plant biodiversity that can be applied for therapeutic or pharmacological purposes in order to treat illnesses and diseases [1,2]. In essence, these medicinal plants exhibit therapeutic properties through some or all of their parts as direct function of their constituent phytochemical compounds which can hereby be harnessed for therapeutic purposes [3-6].

Currently, there is a drastic global increase in the application of medicinal plants for therapeutic purposes basically due to their comparative safety, accessibility and affordability [7,8]. Further dependence of individuals, especially in developing countries, on medicinal plants as major source of health care had also been projected [9]. However, variable tissue pathologies have been linked to application of some medicinal plants or herbal preparations for specific or generic therapeutic purposes [10,11].

The *Caladium bicolor* Aiton (*C. bicolor*) plant is a member of *Araceae* family commonly called Angel's wings, elephant's ear, heart-of-Jesus, mother-in-law and so on. It has bi-coloured and variegated, heart-shaped leaves and is commonly cultivated in and around domestic residences due to its horticultural value [12-14]. According to study by Ekanem et al. [15], *C. bicolor* contain phytochemicals including glycosides, flavonoids, saponins, tannins, steroids, oxalates and phytates which confer variable activities on the plant extract. In addition, *C. bicolor* leaf or rhizome extracts have been reported to exhibit antioxidant activity, possess therapeutic properties such as anti-diarrheal and anti-ulcer and also applied for topical treatment of pain, skin wounds, infected sores and boils [16-19].

However, previous study had reported that *C. bicolor* leaf extract can stimulate nephropathic changes within the renal parenchyma of experimental animals following its sub-acute exposure [20]. In line with further need for toxicological profiling of medicinal plants in general and *C. bicolor* in particular, this study was carried out to assess the effect of methanolic leaf extract of *C. bicolor* on histomorphology of gastric and intestinal tissues of experimental animals.

2. MATERIALS AND METHODS

2.1 Study Plant Material

The *C. bicolor* plant was obtained from the suburb of Isihor community in Benin City, Nigeria. Following its identification at the Department of Pharmacognosy, Igbinedion University, Okada, Edo State, Nigeria, bulk quantity needed for the study was collected for extraction.

2.1.1 Preparation of plant extract

The leaves of the study plant collected were detached, dried at room temperature (24°C ± 2°C) and pulverized by mechanical grinder. In the powdered form, study leaf material was infused in methanol for 72 hours with intermittent agitation. Thereafter, the plant preparation was filtered, weighed and evaporated to dryness. The extraction residue was cooled, weighed and prepared as methanolic extract for the study.

2.2 Experimental Animals

Twenty four Wistar rats employed in this research study, weighing between 175-190 g, were sourced from the Central Animal House

Facility, Igbinedion University, Edo State, Nigeria wherein they were bred for about 5 - 6 weeks before the study period. Throughout the study period, experimental animals were housed in animal cages within the Facility under hygienic conditions and exposed to 12 hour light/dark cycle. They were fed with standard animal feed and allowed free access to drinking water *ad libitum*.

2.3 Experimental Design

In this study, experimental animals were randomly divided into four groups which include one control group (CG) and three treatment groups (TG I, TG II and TG III) each comprising of six animals. The CG was administered with distilled water (2 ml/kg b.w.) while the TGs I, II and III were administered with 100 ml/kg, 200 ml/kg and 300 ml/kg (b.w.) of methanolic leaf extract of *C. bicolor* respectively based on a previous study [20]. Administrations of reagent and extract were done orally and once daily for a period of thirty days with the aid of orogastric canula coupled to hypodermic syringe. The body weight of experimental animals in control and treatment groups were evaluated and recorded at the beginning and end of the treatment period.

2.4 Study Tissue Collection and Processing

At the end of the treatment period, experimental animals were sacrificed through cervical dislocation without anaesthesia and their gastric and small intestinal tissues harvested after an abdominal incision and processed for histopathological study. The tissue processing protocol involved fixation in 10% Neutral Buffered Formalin followed by dehydration in ascending grades of alcohol (70%, 90% and absolute alcohol). Xylene was used to clear the dehydrating agent and processed tissues were embedded in paraffin wax to produce tissue blocks.

2.5 Tissue Sectioning and Staining

The manually-operated rotary microtome was used to produce 5-*micron* thick tissue sections from tissue blocks and mounted on microscope slides. Histological staining of tissue sections was done by H&E technique using the following procedures: Tissue sections were dewaxed in xylene, hydrated with descending grades of alcohol (absolute alcohol, 90% and 70%) and distilled water, stained with haematoxylin, washed under running water, differentiated in 1% acid alcohol, blued in Scott's tap water, rinsed in water, stained with eosin, rinsed in water, dehydrated with ascending grades of alcohol, cleared in xylene and mounted with DPX [21].

2.6 Histopathological Study

Microscopic examination of stained tissue sections for all experimental groups was carried out by histopathologist to assess histopathological changes within gastric and intestinal tissues of experimental animals. Photomicrographs of tissue sections were generated and used to compare observable histopathological changes among TGs I-III relative to the normal histomorphology of CG.

2.7 Statistical Analysis

Experimentally derived values during this study were statistically analyzed using IBM-SPSS (version 20) (IBM Corp, NY, USA). Statistical results were presented as mean ± standard error of mean (SEM) and comparison of statistical results was done using *t*-test and the significant probability level was set at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Effect of Methanolic Leaf Extract of *C. bicolor* on the Body Weight

The mean values of body weight of experimental animals in CG, TG I, TG II and TG III measured at the beginning and end of the treatment period were presented in Fig. 1. At the end of the study period, mean values of body weight of experimental animals comparatively showed significant ($p < 0.05$) reduction in TGs I-III relative to the CG.

3.2 Effect of Methanolic Leaf Extract of *C. bicolor* on Histomorphology of Gastric and Intestinal Tissues

Microscopic examination of tissue sections revealed various histopathological changes in the gastric and intestinal tissues of experimental animals (Figs. 2 and 3). These include gastric mucosal surface erosion and degeneration of intestinal epithelium and villi.

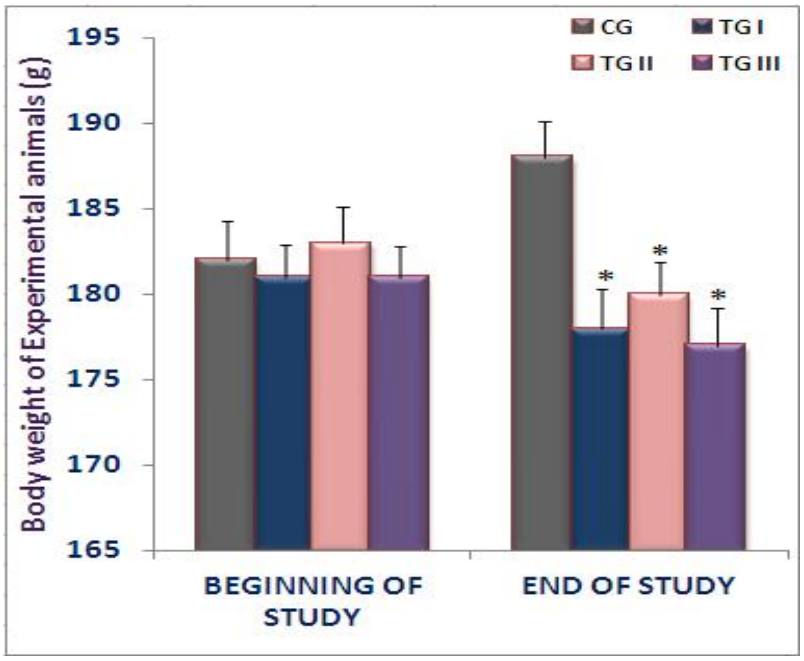

Fig. 1. Mean values of body weight of experimental animals in control group (CG) and treatment groups (TGs I-III) recorded at the beginning and end of the study period

** indicates significant difference from CG at P < 0.05). CG = Distilled water, TG I = 100 mg/kg extract, TG II = 200 mg/kg extract, TG III = 300 mg/kg extract*

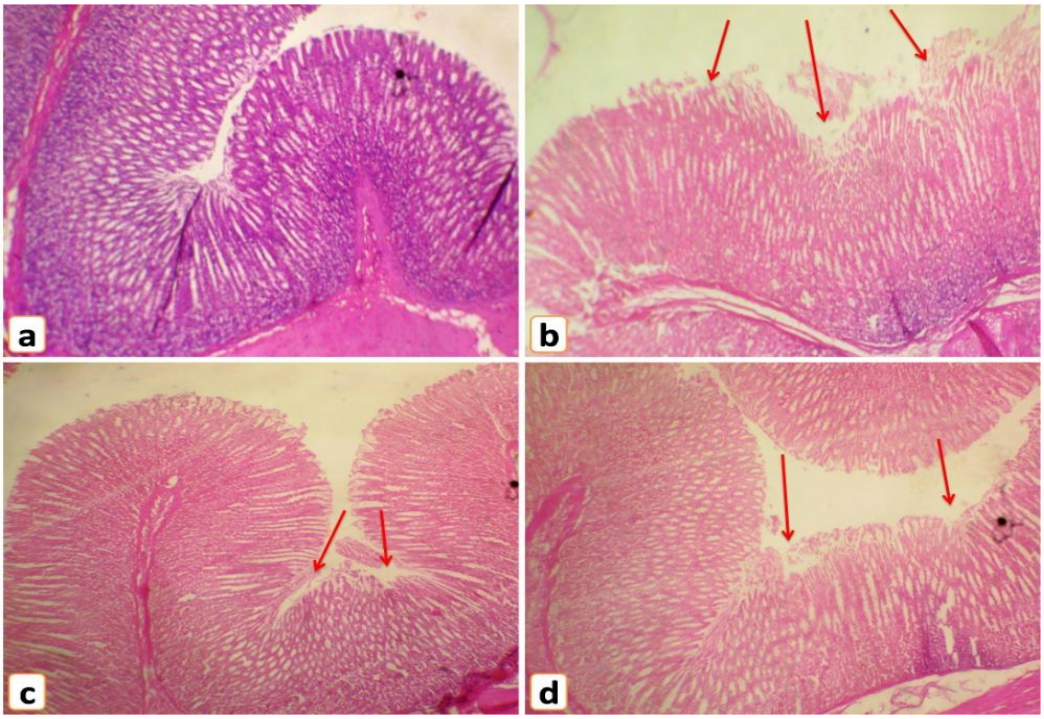

Fig. 2. Photomicrograph of gastric tissue of experimental animals (H&E X100). The figure shows prominent gastric mucosal surface erosion (red arrow) in TG I (b), TG II (c) and TG III (d) relative to normal histomorphology of CG (a)

CG = Distilled water, TG I = 100 mg/kg extract, TG II = 200 mg/kg extract, TG III = 300 mg/kg extract

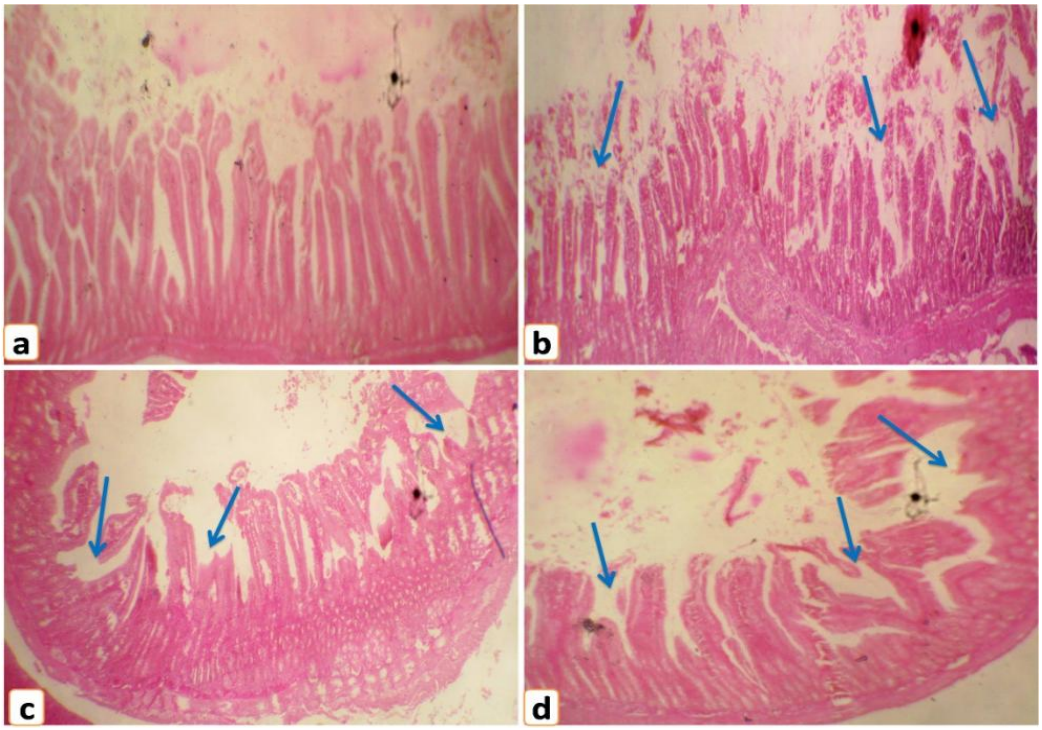

Fig. 3. Photomicrograph of small intestinal tissue of experimental animals (H&E X100). The figure shows prominent degeneration of intestinal epithelium (blue arrow) in TG I (b), TG II (c) and TG III (d) relative to normal histomorphology of CG (a)

CG = Distilled water, TG I = 100 mg/kg extract, TG ii = 200 mg/kg extract, TG III = 300 mg/kg extract

Generally, studies have suggested that widespread toxic effect of toxic substances in the body may cause distinct organ weight loss which can culminate into cumulative body weight reduction [22,23]. Accordingly, the significant reduction in body weight among treatment groups (I-III) compared to the control group can be associated with adverse effects of the plant extract on experimental animals in this study.

Furthermore, herbal medicines are commonly regarded as safe and non-toxic but some have been reported to possess toxic properties or exert toxic effects on internal body organs after prolonged or unregulated use and at high dosages [24-26]. Basically, substances introduced into the body from exterior including food, water and air in excessive quantity can exert adverse effects on tissues structures and even lead to fatality [27].

Generally, medicinal plants have been regarded as potential source of toxins depending on their origin or nature with some medicinal plant preparations exhibiting harmful effects on the human health [28,29]. Particularly, the *C. bicolor* plant like most members of *Araceae* family, contains in all its parts Calcium oxalate which is a toxic substance that can cause toxic effects in oral tissues and internal organs especially gastrointestinal tract when ingested [15,30-32].

Based on the findings of this study, exposure to methanolic leaf extract of *C. bicolor* causes histopathological changes in gastrointestinal tissues of treated animals. In comparison with control group, gastric tissues showed prominent mucosal surface erosion which may indicate potential ulcerogenic effect of the extract while small intestinal tissues showed degeneration of surface epithelium and intestinal villi in experimental animals of treatment groups I-III (Figs. 2 and 3). These outcomes are characteristic of histopathological changes usually observed in gastric and intestinal tissues following exposure to tissue toxicants [33,34].

Accordingly, pathological changes in gastric and intestinal tissues of experimental animals following exposure to methanolic leaf extract of *C. bicolor* as observed in this study can be associated with toxic effects of its constituent

phytochemicals particularly the calcium oxalate. The findings of this study were in consonance with results obtained from studies by Omotoso et al. [20] and Akhigbemen et al. [35] wherein the findings of their study showed that exposure to *C. bicolor* extracts induces deleterious effects on tissues of experimental animals. Also, in affirmation of findings by Nasri and Shirzad [36] about medicinal plants, *C. bicolor* can potently exert toxic effects characterized by variable tissue pathologies when its application is prolonged and not regulated.

4. CONCLUSION AND RECOMMENDATION

4.1 Conclusion

Based on findings of this study, the methanolic leaf extract of *C. bicolor* causes prominent histopathological changes in gastrointestinal tissues of experimental animals. These histopathological changes may be associated with toxic effects of some constituent phytochemical compounds in the plant extract.

4.2 Recommendation

The applicability of *C. bicolor* extracts for therapeutic purposes should be re-validated with particular focus on therapeutic dosage and duration or perhaps disapproved when alternative therapeutic agents with minimal toxic potential exist. Moreover, there is a continuous need for an effective regulatory control on the use of herbal medicines and medicinal plant products for therapeutic purposes in order to avoid their possible toxic effects.

CONSENT

It is not applicable.

ETHICAL APPROVAL

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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