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Nephropathic Changes in Renal Parenchyma of Wistar Rats Following Sub-chronic Exposure to Methanol Extract of *Caladium bicolor* (Aiton)

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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 study protocol. Authors IGO and IB wrote the first draft of the manuscript. Authors ODO and OO 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 nephropathic potential of *Caladium bicolor* methanolic extract within the renal parenchyma of experimental Wistar rats. Twenty four albino Wistar rats (weighing between 180-195 g) were divided into four groups which include Group I treated with distilled water (2 ml/kg b.w.), Group II treated with methanolic extract of *C. bicolor* (100 ml/kg b.w.), Group III treated with methanolic extract of *C. bicolor* (200 ml/kg b.w.), Group IV treated with methanolic extract of *C. bicolor* (300 ml/kg b.w.). All administrations were done orally and once daily for a period of thirty five days. Body weight of animals was recorded during days 0, 7, 14, 21, 28 and 35 of study. After the study period, kidney tissue of study animals was harvested, weighed and processed for histopatho logical study. Staining of renal tissue sections was done using H & E technique, examined under microscope for observable histopathological changes that were scored using image-J software.

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Results of this study showed that sub-chronic exposure to methanolic extract of *C. bicolor* caused reduction in body and renal tissue weight. Moreover, exposure to the extract increases significantly ($p < 0.05$) renal histopatho logical changes including inflammation, necrosis, glomerular congestion and tubular dilatation within the renal parenchyma of study animals.
Therefore, methanol extract of *C. bicolor* exhibited dose-independent nephropathic effect on the renal parenchyma following a sub-chronic exposure in experimental Wistar rats.

Keywords: Caladium bicolor; nephropathy; renal parenchyma; wistar rats.

1. INTRODUCTION

Caladium bicolor (Aiton) Vent is a member of Araceae family widely believed to originate from South and Central America but globally used as an ornamental plant. Due to its horticultural value, it is commonly cultivated in and around homes in different regions and climate of the world [1]. *C. bicolor* has diverse nomenclature in different parts of the world. Some of its common names include Angel's wings, elephant's ear, heart-of-Jesus, mother-in-law, caladio, cananga, capotillo, lagrimas-de-Maria, corazon-de-cabrito, Couer saignant, Buntblatt, Kaladie and many more. It has variegated (often bi-coloured), heart-shaped leaves which exhibit specie-specific variation in colouration. This accounts for the existence of various hybrids or cultivars of *C. bicolor* which include Aaron, Candidum, Fannie munson, Frieda hemple, Florida cardinal, June bride, Lord derby, Red flash, rosebud, Texas beauty, White Christmas and so on [2].

Studies have reported therapeutic properties or applications of some parts of *C. bicolor* particularly their leaves and rhizomes to include anti-diarrheal, anti-ulcer, topical treatment of wounds and boils [3,4]. Generally, most herbal medicines are regarded as safe and non-toxic but some possess inherent toxic properties and exert toxic effects on internal body organs after prolonged use or at high dosage [5,6].

There has been an increase in reported cases of organ toxicity arising from application of natural plant products or herbal formulations [7,8]. *C. bicolor* for instance contain in all its parts (including leaf, stem and root) a toxic substance (Calcium oxalate) which can cause irritation or injury in internal organs when ingested [9]. The oxalate crystal is a typical constituent of Araceae family of plants which can cause toxicity of internal organs especially gastrointestinal tract and kidney [10,11].

This study was therefore carried out to determine possible nephropathic effect that sub-chronic exposure of methanol extract of *C. bicolor* (MECB) may exert on renal parenchyma of experimental Wistar rats.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh *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. Thereafter, bulk quantity sufficient for the study was collected for the subsequent process of extraction.

2.2 Preparation of Plant Extract

The leaves of collected study plant were carefully detached, air-dried at room temperature (23°C ± 2°C) and pulverized into powdered form using a mechanical grinder. The powdered leaf material was infused in methanol for 72 hours with agitation at regular intervals. Afterwards, the preparation was filtered, weighed and evaporated using rotary evaporator (regulated at 40°C). The extraction residue was cooled, weighed and applied as the methanol extract for the study.

2.3 Experimental Animals

In this study, twenty four male albino Wistar rats, weighing between 180–195 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.

2.4 Experimental Design

The experimental rats were randomly divided into four groups which include: one control and three

treatment groups comprising of 6 animals each. The control group I was administered with distilled water [2 ml/kg body weight (b.w.)] while treatment groups II, III and IV were administered MECB 100 ml/kg, 200 ml/kg and 300 ml/kg b.w, respectively. All administrations were done once daily for a period of thirty-five days via oral route using an orogastric canula. The body weight of all experimental animals was monitored at regular interval on days 0, 7, 14, 21, 28 and 35 of study

2.5 Study Tissue Collection and Processing

After the 35-day treatment period, the animals were sacrificed and their kidney tissue harvested, grossly examined and weighed. For all experimental animals, the average organ weight was calculated as average value of right and left kidney tissue weight. The relative organ weight was calculated as ratio of average organ weight to final body weight and expressed as percentage. Kidney tissue of experimental animals was subsequently processed for histo-pathological study using the following protocol: Fixation in 10% Neutral Buffered Formalin, Dehydration in ascending grades of alcohol (70%, 90% and absolute alcohol), Clearing in xylene and Embedding in paraffin wax to form kidney tissue blocks.

2.6 Study Tissue Sectioning and Staining

With the aid of rotary microtome, kidney tissue blocks were used to generate 5 μ thick sections which were mounted on microscope slides. Histological staining of tissue sections was subsequently done by Haematoxylin and Eosin (H & E) technique. After staining, tissue sections were allowed to dry and prepared for microscopic examination.

2.7 Histopathological Study

After staining, tissue sections were examined under microscope to determine pathological changes within renal parenchyma of experimental animals and photomicrographs of tissue sections were produced using digital camera for microscope. All observable histopathological changes which include interstitial inflammation, epithelial necrosis, glomerular congestion and tubular dilatation within the renal parenchyma of experimental animals were scored using image-J software (NIH, Bethesda, MA, USA).

2.8 Statistical Analysis

All data obtained during this study were 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). For all statistical comparisons, $p < 0.05$ was regarded as significant probability level.

3. RESULTS AND DISCUSSION

3.1 Evaluation of Body Weight, Average Organ Weight and Relative Organ Weight of Study Animals

The mean values of body weight of study animals in Groups I – IV were given in Fig. 1. In comparison with the normal control Group I, mean values of body weight of study animals showed significant ($p < 0.05$) reduction in all treatment groups (II - IV).

The mean values of average organ weight and relative organ weight of study animals in Groups I – IV were given in Fig. 2. Similarly, In comparison with the normal control Group I, mean values of average organ weight and relative organ weight showed significant reduction ($p < 0.05$) in all treatment groups (II - IV).

3.2 Histopathological Results

Some histopathological changes were observed during microscopic examination of renal tissue sections of study animals. These observable renal histopathological features include inflammation, necrosis, glomerular congestion and tubular dilatation within renal parenchyma of study animals in Groups I – IV (Fig. 3). Evaluation of these renal histopathological features showed significant prominence within the renal parenchyma of treatment groups II – IV relative to normal control group I (Figs. 4 and 5).

Essentially, herbal medicinal preparations are mostly regarded as safe and non-toxic. However, some of their phytochemical constituents may exert toxic effects on internal body organs after prolonged use or at high dosages [12-14]. In phytotherapy, information on toxicological profile of phytochemicals is critical in determining the biosafety of the natural plant products [15-16]. Hence, the determination of toxicological profile of phytochemicals and their

potential deleterious effects on tissues constitute vital aspect of phytoscience.

Furthermore, studies have reported that changes in weight of body organs (including kidney) can be used as indicator of deleterious effect of xenobiotic (including some phytochemicals) exposure during toxicological studies [17-19]. The reduction in organ weight following exposure to cytotoxins is often a function of necrosis while increase in organ weight often relates with hypertrophy [20].

The results of this study showed that sub-chronic exposure to MECB resulted in significant reduction of body weight in general and renal tissue weight in particular (Figs. 1 and 2). In addition, the renal tissue is a very sensitive target organ of accidental or experimental exposure to cytotoxins leading to prominent renal histopathological changes [21,22]. According to the results of this study, sub-chronic exposure to MECB exerted significant pathological influences on renal parenchyma of study animals (Figs. 3-5).

The study by Akhigbemen et al. [23] reported similar findings following sub-acute exposure to *C. bicolor* in experimental rats. As earlier stated, *C. bicolor* contain toxic calcium oxalate which trigger toxicity or injury in internal body organs including kidney. According to the study by Cruzan et al. [24], exposure to a potential nephrotoxin-ethylene glycol leads to accumulation of calcium oxalate crystals in renal tissue which in turn results into degeneration of renal tubules. Therefore, the nephropathic effects of MECB on the renal parenchyma of study animals can be linked with oxalate crystals-induced toxicity.

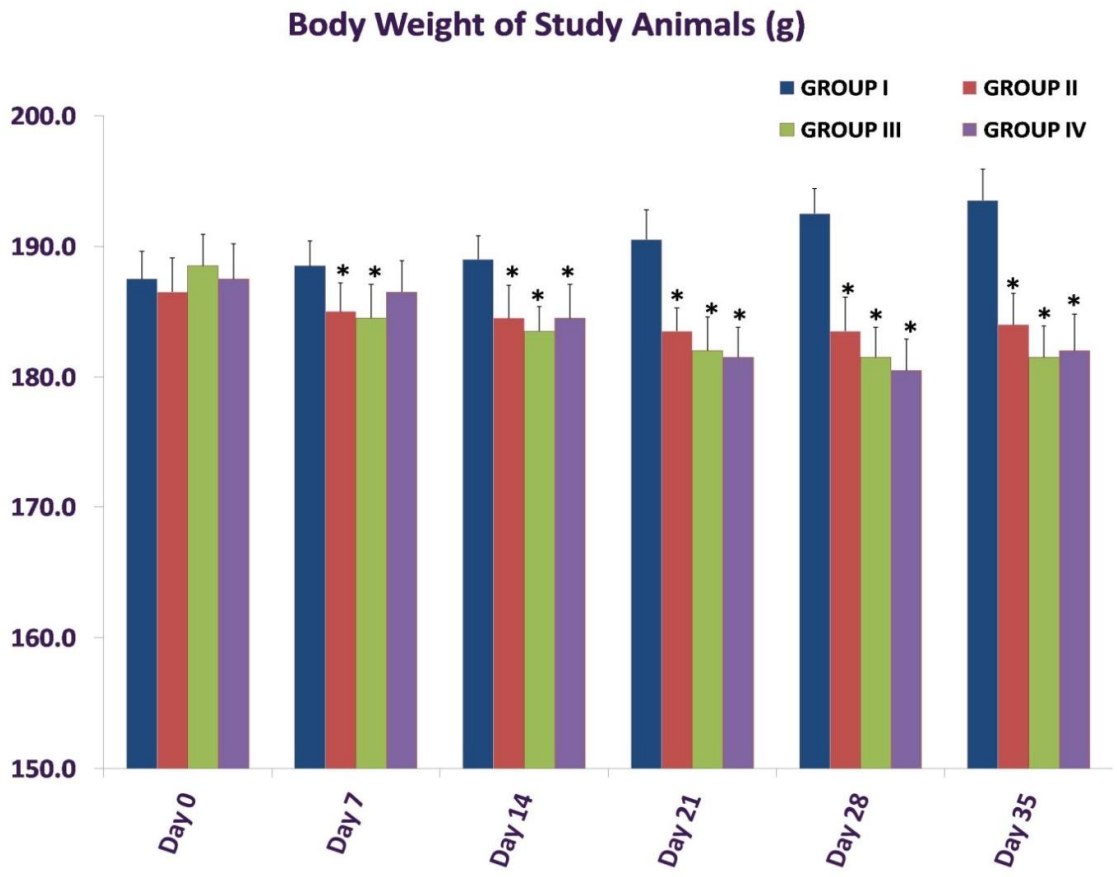

Fig. 1. Mean values of body weight of study animals in groups I – IV recorded during the study period (days 0, 7, 14, 21, 28 and 35)

(= significant difference from normal control at P < 0.05). Group I = Normal control group, Group II = 100 mg/kg MECB, Group III = 200 mg/kg, Group IV = 300 mg/kg*

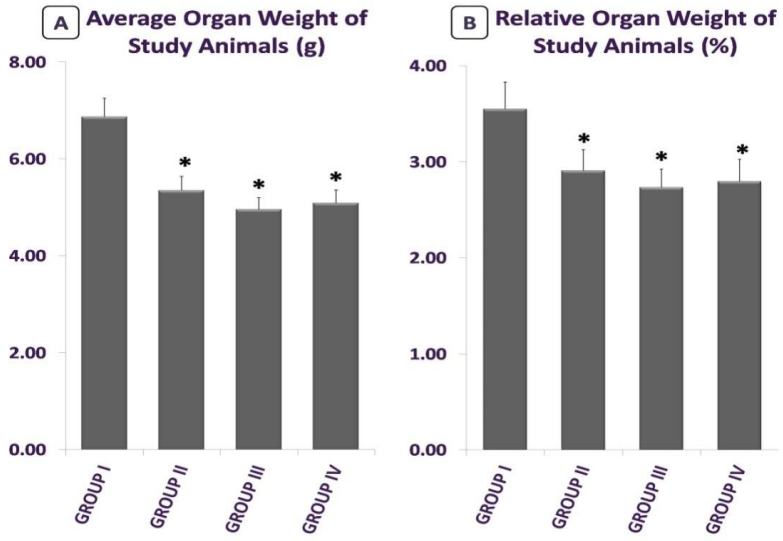

Fig. 2. Mean values of average organ weight [X 10^{-1}] (A) and relative organ weight (B) of study animals in groups I – IV

(= significant difference from normal control at P < 0.05). Group I = Normal control group, Group II = 100 mg/kg MECB, Group III = 200 mg/kg, Group IV = 300 mg/kg*

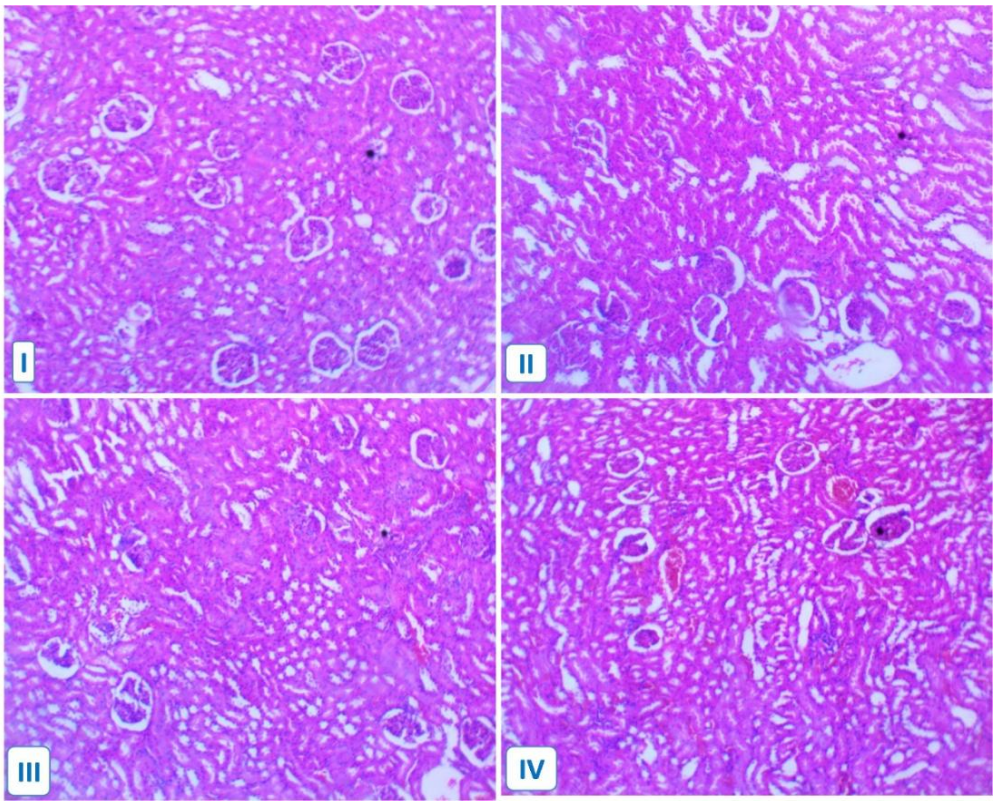

Fig. 3. Representative photomicrograph of kidney tissue showing prominent histopathological changes within the renal parenchyma of study animals in groups I – IV (H & E X100)

Group I = Normal control group, Group II = 100 mg/kg MECB, Group III = 200 mg/kg, Group IV = 300 mg/kg

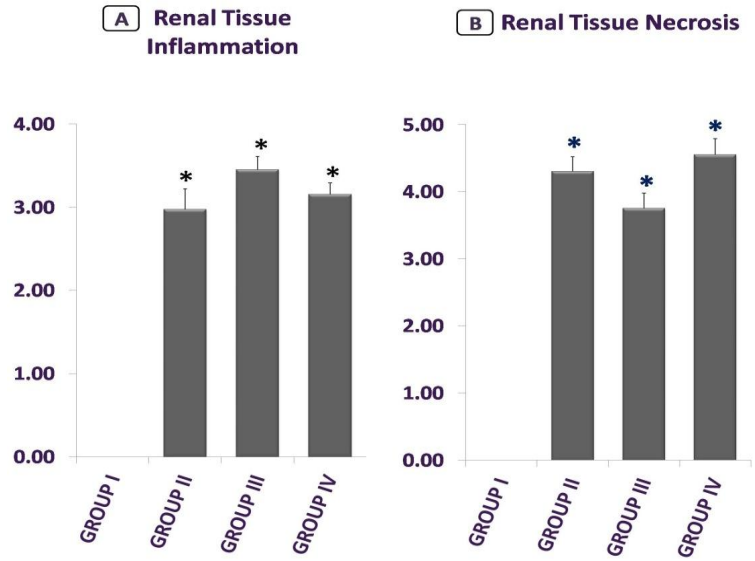

Fig. 4. Mean values of renal tissue inflammation (A) and renal tissue necrosis (B) scores within the renal parenchyma of study animals in groups I – IV

(= significant difference from normal control at P < 0.05). Group I = Normal control group, Group II = 100 mg/kg MECB, Group III = 200 mg/kg, Group IV = 300 mg/kg*

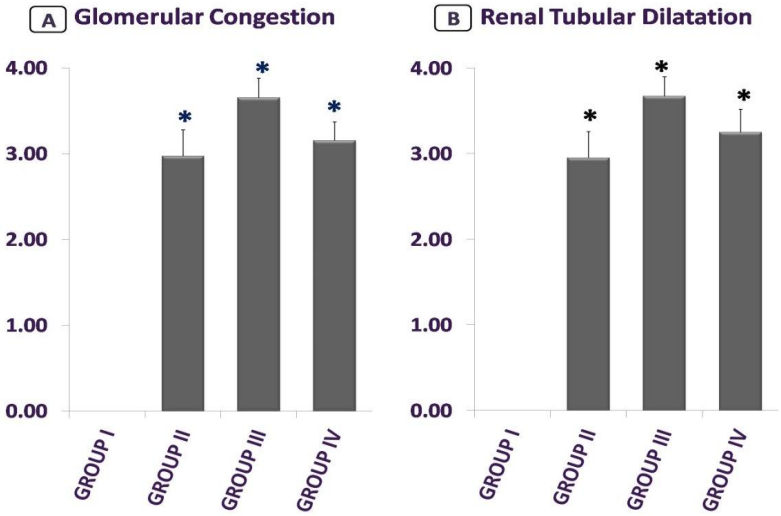

Fig. 5. Mean values of glomerular congestion (A) and renal tubular dilatation (B) scores within the renal parenchyma of study animals in groups I – IV

(= significant difference from normal control at P < 0.05). Group I = Normal control group, Group II = 100 mg/kg MECB, Group III = 200 mg/kg, Group IV = 300 mg/kg*

4. CONCLUSION AND RECOMMENDATION

4.1 Conclusion

The methanol extract of *C. bicolor* exhibited dose-independent nephropathic effect on renal parenchyma following a sub-chronic exposure in experimental Wistar rats. This resulted into prominent pathological changes within the renal histomorphology of the study animals.

4.2 Recommendation

Further studies are recommended on the safety profile of variable extracts of different parts of

C. bicolor as well as the cellular and molecular mechanisms of their therapeutic activity,

ETHICAL APPROVAL

This study was carried out after approval by 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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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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