Subacute toxicological profile of *Caladium bicolor* Aiton (*Araceae*)
methanolic leaf extract in rat

[Perfil toxicológico subagudo del extracto metanólico de hojas de *Caladium bicolor* Aiton (*Araceae*) en rata]

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**Abstract**

*Context:* *Caladium bicolor* is used in ethnomedicine for the treatment of convulsions without any scientific information of its safety profile following repeated administration.

*Aims:* To evaluate the sub-acute toxicological profile of the methanol leaf extract of *C. bicolor*.

*Methods:* Oral acute toxicity test was done using Lorke’s method. Oral sub-acute toxicity was done for 28 days, with hematological, biochemical and histological markers of toxicity evaluated.

*Results:* The results showed no significant change in the organ weights of rats. Hematological indices were comparable with control groups except for a slight increase in monocyte levels (p<0.05). There were no significant changes in alkaline phosphatase (ALP) and alanine aminotransferase (ALT) levels when compared to the control. However, there was a significant (p<0.05) decrease in aspartate aminotransferase (AST), levels in the rats treated with the extract. There were no significant differences in the values of serum lipids. Photomicrographs of the heart, lungs, brain, spleen, and liver showed no abnormalities, but in the kidneys, focal infiltrate of lymphocytes disrupting the renal tubules and interstitium were observed at 400 mg/kg/day.

*Conclusions:* The methanolic extract of *C. bicolor* appears safe; however, prolonged use might lead to renal failure.

**Keywords:** hematological; hepatotoxicity; organ weight; serum lipids.

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**Contexto:** *Caladium bicolor* se utiliza en etnomedicina para el tratamiento de convulsiones, sin ninguna información científica de su perfil de seguridad después de la administración repetida.

**Objetivos:** Evaluar el perfil toxicológico subagudo del extracto de hoja de metanol de *C. bicolor*.

**Métodos:** La prueba de toxicidad aguda oral se realizó con el método de Lorke. La toxicidad subaguda oral se realizó durante 28 días, y se evaluaron los marcadores hematológicos, bioquímicos e histológicos de toxicidad.

**Resultados:** Los resultados no mostraron cambios significativos en el peso de los órganos de las ratas. Los índices hematológicos fueron comparables con los grupos de control, excepto por un ligero aumento en los niveles de monocitos (p<0.05). No hubo cambios significativos en los niveles de fosfatasa alcalina (ALP) y alanina aminotransferasa (ALT) en comparación con el control. Sin embargo, hubo una disminución significativa (p<0.05) en los niveles de aspartato aminotransferasa (AST) en las ratas tratadas con el extracto. No hubo diferencias significativas en los valores de lípidos séricos. Fotomicrografías del corazón, cerebro, bazo, e hígado no mostraron anomalías, pero en los riñones se observó infiltrado focal de linfocitos que alteraron los túbulos renales y el intersticio a 400 mg/kg/día.

**Conclusiones:** El extracto metanólico de *C. bicolor* parece seguro; sin embargo, el uso prolongado podría provocar insuficiencia renal.

**Palabras Clave:** hematológico; hepatotoxicidad; lípidos en suero; peso del órgano.
INTRODUCTION

The World Health Organization has reported that 80% of the emerging world’s population relies on traditional medicine for therapy (WHO, 2008). In spite of this, safety is a fundamental principle in the provision of herbal medicines and herbal products for health care, and a vital component of quality control (WHO, 2004).

*Caladium bicolor* (CB) commonly known as “Angels wings”, “Heart of Jesus” and “Elephant ears” is an ornamental foliage plant grown from tubers. The aqueous extract has been shown to possess anti-diarrheal effect (Olanrewaju et al., 2015). In Nigeria, the leaves and rhizomes are used topically for boils, wounds, and ulcers (Odugbemi, 2006) as well as purgatives and management of convulsions. Despite these claims, there has been no study on the safety profile of this plant. In this study, the safety profile of the methanol extract was evaluated following 28 consecutive days of administration.

MATERIAL AND METHODS

Chemicals

Commercial kits for the assay of enzymes and lipids were manufactured by Randox Laboratories, (UK). Methanol for the extraction was manufactured by Sigma-Aldrich (Germany). Tween 80 (JAD, China) was used to reconstitute the extract before administration. All other chemicals and reagents were obtained from reputable manufacturers and solutions were prepared fresh each day.

Plant material and extraction

Fresh leaves of *Caladium bicolor* were collected from the horticultural garden of the University of Benin, Benin City (6° 20' 1.32" N, 5° 36' 0.53" E) in July 2015. It was identified and authenticated by Mallam Ibrahim Muazzam of the Department of Traditional Medicine and Medicinal Plant Research, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria where a voucher specimen with reference number NIPRD/H/6785 has been deposited. The plant material was dried under a shade to a constant weight and then ground to a fine powder. The powdered material (500 g) was macerated in 2 L of methanol for 72 h with intermittent shaking and stirring. The mixture was filtered, and the filtrate concentrated under reduced temperature and pressure and dried in an oven at 50°C. The dried extract was stored at 4°C until used.

Experimental animals

Experiments were performed using Wistar rats (222 g) of both sexes. The animals were obtained from the animal house, Department of Pharmacology and Toxicology, University of Benin, Benin City, Nigeria. They were kept in plastic cages and housed under natural lighting condition. They were fed with standard feed and water ad libitum. All experiments were carried out in accordance with the Institute for Laboratory Animal Research Guidelines for the Care and Use of Laboratory Animals (NRC, 2011). Ethical approval was obtained from the Faculty of Pharmacy Animal Ethics Committee (Reference number: EC/FP/017/03).

Oral acute toxicity

Oral median lethal dose (LD$_{50}$) of the whole extract (WE) was determined using the Lorke (1983) method. In the first phase, three groups of three mice each were administered 10, 100 and 1000 mg/kg. The second phase involved three groups of one mouse each administered 1600, 2900 and 5000 mg/kg. In both phases, the mice were observed for signs of writhing, diarrhea, tremor, and mortality within a period of 24 h.

Oral sub-acute (28-day) toxicity

Forty rats of both sexes were randomly allotted into eight groups comprising of five rats per group with male separated from female in cages (Bariweni et al., 2018).

Experimental groups A, B, C were administered pharmacological doses of 100, 200 and 400 mg/kg of the extract once daily for a period of 28 days. Group D served as control and received 0.2 mL of 5% tween 80. The rats were observed daily for...
signs of toxicity such as piloerection, lacrimation, diarrhea and mortality. On the 29th day, the animals were sacrificed under chloroform anesthesia, and blood samples were collected from the abdominal aorta into plain and EDTA-containing bottles. The heart, liver, lung, spleen, brain, and kidney were excised, placed on Whatman filter papers for 5 min before weighing.

**Hematological tests**

Blood samples in EDTA bottles were pipetted into a capillary tube, spun in a roller mixer for 2 - 3 min before values were read using the Human Automated Hematology System Analyzer (ERMA PCE 210, ERMA, Japan). Parameters analyzed were hemoglobin (Hb), hematocrit (HCT), white blood cell count (WBC) and differential count (granulocyte, lymphocyte, and monocyte), platelets (PLT) (Okwuofu et al., 2017).

**Biochemical analysis**

Blood samples in plain bottles were allowed to clot at room temperature for 4 h before centrifugation using a Hettich Centrifuge (Rototix 32A, Germany) at 4000 rpm for 10 min. Sera obtained were used for evaluation of biochemical parameters including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) was done using the enzyme kinetic method (Reitman and Frankel, 1957). Total bilirubin (TB) and direct bilirubin (DB) was determined using Jendrassik-Grof method (Spencer and Price, 1977).

**Histological analysis**

Tissues were sectioned at 4 μm thickness on a rotary microtome (Leica RM 2125, Germany), processed in gradients of alcohol and then stained with Ehrlich’s hematoxylin and eosin for microscopy (Bancroft and Gamble, 2002). Stained slides were viewed using an optical photomicroscope (Leica MC170 HD, Leica Biosystems, Germany) at ×100 magnification.

**Statistical analysis**

Data are expressed as mean ± SEM (standard error of mean) and analyzed using One Way Analysis of Variance (ANOVA) followed by Dunnett’s post hoc test (GraphPrism® 6, San Diego, USA). p<0.05 was considered statistically significant.

**RESULTS**

**Effects of C. bicolor extract on organ weight**

The weight of different organs in the treated rats were not significantly different from those of the control group (p<0.05) (Table 1).

**Effects of C. bicolor extract on hematological indices**

Hematological parameters (white blood cell, lymphocytes, monocytes, granulocytes, hemoglobin, hematocrit and platelets) in the treated rats were not significantly different from those of the control group except for a significant increase in monocyte (p<0.05) (Table 2).

**Effects of C. bicolor extract on biochemical indices**

There were no significant changes in ALP and ALT levels when compared to the control (Figs. 1 and 2). However, there was a significant decrease in AST levels (p<0.01, p<0.05 and p<0.001) in the rat that received 100, 200 and 400 mg/kg of the extract respectively (Fig. 3).

No significant differences were observed in the values of serum lipids (HDL, LDL, TC,) and bilirubins (TB and DB) in the test groups, when compared to the control in both male and female animals (Table 3).

**Effects of C. bicolor extract on histology of selected organ**

Sections of kidney, lungs, heart, brain, liver, and spleen of rats (Figs. 4, 5, 6, 7, 8 and 9, respectively) treated with doses of extract showed that there were no lesions or histological changes. In the kidney, focal infiltrate of lymphocytes indicating tubulointerstitial nephritis was observed in animals administered 400 mg/kg. In the lungs, interstitial pneumonitis was observed in both male and female animals in the control and test groups.
Table 1. Effect of sub-acute oral administration of C. bicolor methanol leaf extract on organ weight of rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Brain (g)</th>
<th>Liver (g)</th>
<th>Kidney (g)</th>
<th>Heart (g)</th>
<th>Spleen (g)</th>
<th>Lungs (g)</th>
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<tr>
<td>Control</td>
<td></td>
<td>1.44 ± 0.06</td>
<td>5.77 ± 0.68</td>
<td>0.53 ± 0.02</td>
<td>0.60 ± 0.05</td>
<td>0.67 ± 0.04</td>
<td>1.33 ± 0.07</td>
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<td>CBME 100</td>
<td>1.50 ± 0.03</td>
<td>6.31 ± 0.33</td>
<td>0.57 ± 0.03</td>
<td>0.55 ± 0.04</td>
<td>0.74 ± 0.05</td>
<td>1.4 ± 0.07</td>
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<tr>
<td>CBME 200</td>
<td>1.40 ± 0.05</td>
<td>5.80 ± 0.37</td>
<td>0.54 ± 0.02</td>
<td>0.57 ± 0.04</td>
<td>0.72 ± 0.03</td>
<td>1.24 ± 0.07</td>
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<tr>
<td>CBME 400</td>
<td>1.38 ± 0.06</td>
<td>5.7 ± 0.35</td>
<td>0.53 ± 0.02</td>
<td>0.60 ± 0.03</td>
<td>0.63 ± 0.04</td>
<td>1.26 ± 0.06</td>
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All values are expressed as mean ± SEM of n=10. Values are not significantly different from the control (0.2 mL of 5% tween 80). CBME: Caladium bicolor methanolic extract.

Table 2. Effect of 28-day oral administration C. bicolor methanol leaf extract on hematological indices

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>WBC ×10³/µL</th>
<th>LY (%)</th>
<th>MO (%)</th>
<th>GR (%)</th>
<th>Hb (g/dL)</th>
<th>HCT (%)</th>
<th>PLT (×10³/µL)</th>
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<tr>
<td>Control</td>
<td></td>
<td>15.1 ± 1.2</td>
<td>8.3 ± 1.0</td>
<td>1.3 ± 0.1</td>
<td>28.6 ± 1.6</td>
<td>15.5 ± 0.4</td>
<td>38.0 ± 0.6</td>
<td>489.9 ± 67.0</td>
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<td>CBME 100</td>
<td>15.4 ± 1.3</td>
<td>7.4 ± 0.9</td>
<td>0.2 ± 0.3</td>
<td>30.6 ± 3.0</td>
<td>14.5 ± 0.9</td>
<td>35.3 ± 2.1</td>
<td>397.3 ± 88.2</td>
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<tr>
<td>CBME 200</td>
<td>14.7 ± 1.1</td>
<td>7.0 ± 1.2</td>
<td>1.1 ± 0.2</td>
<td>30.0 ± 2.1</td>
<td>14.7 ± 0.3</td>
<td>30.8 ± 4.2</td>
<td>419.9 ± 98.6</td>
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<tr>
<td>CBME 400</td>
<td>14.9 ± 1.4</td>
<td>7.5 ± 0.9</td>
<td>4.2 ± 1.4*</td>
<td>30.3 ± 1.3</td>
<td>14.9 ± 0.9</td>
<td>36.2 ± 2.0</td>
<td>338.0 ± 25.3</td>
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</table>

All values are expressed as mean ± SEM of n=10. *P<0.05 versus Control (0.2 mL of 5% Tween 80). CBME: Caladium bicolor methanolic extract; WBC: white blood cells; LY: lymphocytes; MO: monocytes; GR: granulocytes; Hgb: hemoglobin; HCT: hematocrit; PLT: platelets.

Table 3. Effect of 28 days administration of C. bicolor methanol leaf extract on serum lipids in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>HDL (mg/dL)</th>
<th>LDL (mg/dL)</th>
<th>TC (mg/dL)</th>
<th>TB (mg/dL)</th>
<th>DB (mg/dL)</th>
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<tr>
<td>Control</td>
<td></td>
<td>44.20 ± 3.10</td>
<td>9.60 ± 1.00</td>
<td>71.80 ± 3.80</td>
<td>0.36 ± 0.00</td>
<td>0.15 ± 0.01</td>
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<tr>
<td>CBME 100</td>
<td>41.90 ± 2.10</td>
<td>12.20 ± 1.40</td>
<td>71.90 ± 2.50</td>
<td>0.35 ± 0.00</td>
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<tr>
<td>CBME 200</td>
<td>40.00 ± 2.40</td>
<td>9.70 ± 1.10</td>
<td>64.40 ± 3.20</td>
<td>0.39 ± 0.30</td>
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<tr>
<td>CBME 400</td>
<td>43.00 ± 2.00</td>
<td>11.20 ± 1.60</td>
<td>72.30 ± 2.90</td>
<td>0.35 ± 0.00</td>
<td>0.13 ± 0.01</td>
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</tbody>
</table>

All values are expressed as mean ± SEM of n=10. Values are not significantly different from the control (0.2 mL of 5% tween 80). CBME: Caladium bicolor methanolic extract; HDL: high-density lipoprotein; LDL: low-density lipoprotein; TC: total cholesterol; B: total bilirubin; DB: direct bilirubin.
Figure 1. Effect of sub-acute oral *C. bicolor* methanolic leaf extract on serum alkaline phosphatase (ALP) levels.

Figure 2. Effect of sub-acute oral *C. bicolor* methanolic leaf extract on serum alanine transaminase (ALT) levels.

Figure 3. Effect of sub-acute oral *C. bicolor* methanolic leaf extract on serum aspartate transaminase (AST) level.

All values are expressed as mean ± SEM of n=10. **P<0.01; *p<0.05; ***p<0.001 compared to the control (0.2 mL of 5% tween 80). WE: *C. bicolor* methanolic leaf extract.
Figure 4. Representative photomicrographs of the kidney of rats administered methanol leaf extract of *C. bicolor* orally for 28 days.

A: control (0.2 mL of 5% Tween 80), B: 100 mg/kg, C: 200 mg/kg, D: 400 mg/kg. Kidneys of rat administered 400 mg/kg of the extract revealed focal infiltrate of lymphocytes (stars) disrupting the renal tubules and interstitium. Other tubular and interstitial areas and the glomeruli (arrow) are essentially normal. H & E, x 100.
Figure 5. Representative photomicrographs of the lungs of rats administered methanol leaf extract of *C. bicolor* orally for 28 consecutive days.

A: control (0.2 mL of 5% tween 80), B: 100 mg/kg, C: 200 mg/kg, D: 400 mg/kg, respectively. There is mild interstitial pneumonitis manifested by inflamed and thickened interstitium or alveolar septa (arrows), compressing some alveolar spaces (stars) in the lungs of both treated and control animals. H & E, x 100.
Figure 6. Representative photomicrographs of the heart of rats administered methanol leaf extract of *C. bicolor* orally for 28 consecutive days. Micrographs are essentially normal in both control and treated animals.

A: control (0.2 mL of 5% tween 80), B: 100 mg/kg, C: 200 mg/kg, D: 400 mg/kg, respectively. H & E, x 100.
Figure 7. Representative photomicrographs of the brain of rats administered with methanol leaf extract of *C. bicolor* orally for 28 consecutive days.

Micrographs are essentially normal **A**: control (0.2 mL of 5% tween 80), **B**: 100 mg/kg, **C**: 200 mg/kg, **D**: 400 mg/kg, respectively. H & E, x 100.
Figure 8. Representative photomicrographs of the liver of rats administered with methanol leaf extract of *C. bicolor* orally for consecutive 28 days.

Micrographs are essentially normal. **A:** control (0.2 mL of 5% tween 80), **B:** 100 mg/kg, **C:** 200 mg/kg, **D:** 400 mg/kg, respectively. H & E, x 100.
Figure 9. Representative photomicrographs of the spleen of rats administered with methanol leaf extract of *C. bicolor* orally for 28 consecutive days.

Micrographs are essentially normal. A: control (0.2 mL of 5% tween 80), B: 100 mg/kg, C: 200 mg/kg, D: 400 mg/kg, respectively. H & E, x 100.
DISCUSSION

The extract appears to be relatively safe in rodents. Organ weights are widely accepted in the evaluation of toxicities (Black, 2002; Bucci, 2002). Elevated heart weight may be the only proof of myocardial hypertrophy that is often macroscopically and microscopically complex to recognize (Thiedemann, 1991; Greaves, 2000). Changes in kidney weight may reveal renal toxicity manifesting as tubular hypertrophy or chronic progressive nephropathy (Greaves, 2000). Alterations in liver weight may suggest treatment-related changes including hepatocellular hypertrophy (Greaves, 2000). Summarily, an increase in organ weight suggests the occurrence of hypertrophy while a decrease suggests necrosis of the target organ (Teo et al., 2002). After the 28-day repeated exposure test, there was no mortality nor significant changes in the weight of the organs in the rats.

Hematological evaluations are useful in the diagnosis of disease states hence, their relevance in toxicological studies (Togun et al., 2007). Blood acts as a pathological reflector of the status of animals exposed to a toxicant or other condition (Olafede, 2010). White blood cells by phagocytosis defend the body against foreign invasion by microorganism or xenobiotics. The cells also produce and distribute antibodies in immune response. In this study, hematological indices were normal in both the test and control groups except for an increase in monocyte levels, which occurred at the dose of 400 mg/kg/day. Monocytes produced in the bone marrow diffuse into the blood, where they account for about 1 to 10% of the circulating white blood cells. After a few hours, this white blood cell differential migrates to tissues (such as spleen, liver, lungs, and bone marrow tissue), where they become established as macrophages. It is not known why monocyte level was elevated in the animals that received 400 mg/kg/day of the whole extract but could due to their mobilization against constituent(s) of the extract.

Liver chemistry tests are frequently used to assess symptomatic and asymptomatic liver diseases. The aminotransferases (e.g., ALT and AST) describe hepatocellular integrity; alkaline phosphatase (ALP) describes the relationship of the liver with the biliary tract while albumin and protein levels describe hepatic functionality (Boyde and Latner, 1961; Adeoye and Oyedapo, 2004). The highest levels of AST are present in liver, cardiac tissue, and skeletal muscle, with smaller amounts present in the kidneys, pancreas, and erythrocytes. ALT is predominantly present in the liver. Hence, it is said to be liver-specific (Esani, 2014). The highest concentrations of ALP are found in bone, liver, spleen, intestine, placenta, and kidneys. In this study, ALT and ALP levels were not significantly altered by the treatment but the significant decrease in AST level observed may indicate organoprotective non-specific effect due to the presence of substances such as flavonoids.

The belief that low-density lipoprotein (LDL) cholesterol causes atherosclerosis and subsequent heart disease is a fundamental of modern medicine (Colpo, 2005). The primary function of HDL is to mobilize excess cholesterol to the liver metabolized into bile salts. This cholesterol removal from the tissues underlies the inverse relationship between the plasma concentration of HDLs and the incidence of heart diseases (Navin et al., 2008). In the present study, there were no significant differences in values of the serum lipids (HDL, LDL, TC) and bilirubin (TB and DB) in the test groups, when compared to the control.

Histomorphological changes in target organs are an indication of toxicity of a chemical or biological substance (Ping et al., 2012). Histological examination of the heart, brain, liver and spleen showed no abnormality nor structural change in the cells of the organs in both the treated groups and the control group. However, in the kidneys, focal infiltrate of lymphocytes was seen at a dose of 400 mg/kg/day indicating tubulointerstitial nephritis. Lymphocytes mediate immune response to the foreign substance. In the lungs structural changes occurred in both the treated and control groups. These changes, which manifested as inflamed and thickened interstitium of alveolar septa could be as a result of environmental factors leading to inflammation, oxidative stress, or viral infections (Clement et al., 2010).
CONCLUSIONS

Repeated administration of pharmacological doses of methanol leaf extract of *C. bicolor* is toxic to the kidney but appears to have no adverse effect on the heart, lungs, spleen, liver, and brain. It has no harmful effect on circulating red and white blood cells, as well as on serum lipid levels.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

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REFERENCES


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<th>Okwuofu EO</th>
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