



Evaluating the Genotoxic and Proximate Analysis of Ethanolic Extract of *Lecaniodiscus cupanioides* Planch. ex Benth.

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Abstract

Lecaniodiscus cupanioides has been playing vital roles in traditional therapies in Nigeria towards management of several ailments such as malaria and breast tumour. In the quest to investigating its genotoxicity, 30 mice weighing between 20 – 25g were placed in three groups of 10 mice each. Groups A and B, orally administered 100 and 400 mg kg⁻¹ of the extract respectively for 49 days, group C received distilled water as control. At expiration of treatment the mice were sacrificed via jugular puncture. Femurs were disarticulated to extract marrow in bone for genotoxic assay. Heavy metal and proximate analysis was investigated using Atomic Absorption Spectrophotometry. Bone marrow analysis revealed increased number of MN PCEs dose-dependently in treatments compared to control. A significant decrease in the number of Bud PCE was observed between treatment and control. Insignificant ($p>0.05$) dose-dependent increase in MN NCE and significant ($p<0.05$) dose related increase was observed in 100 mg kg⁻¹ treatment, also extract did not affect ratio of PCE /PCE +NCE. In proximate evaluation, carbohydrate was highest, followed by crude fiber, protein and ash respectively. From heavy metals evaluation, Zn was the highest, all metals observed were within permissible level. The insignificant result of PCE/ PCE+NCE or MNE PCEs against control may infer that the extract is not aneugenic, clastogenic, cytotoxic or genotoxic to the bone marrow. Availability of Cu, Cr, Mn, Ni, and Cd at permissible level suggest the plant possess some health potentials. The extract can be affirmed to be devoid of genotoxicity within period, doses and battery of exposure.

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Introduction

Medicinal plants have been utilized for numerous therapeutic purposes consequent upon abundant secondary metabolites bioavailability such as alkaloids, phenols and flavonoids. These therapeutic interest is tremendous such that about 85% of primary health medication are derived from natural resource globally [1]. Medicinal plants are lead sources for drug synthesis [2]. *Lecaniodiscus cupanioides* Planch, belonging to sapindaceae family, is a short tropical tree of about 6-12m high, commonly found in Asia and Africa. It is known as aaka or akika, okpu, kafi-nama-zaki and utantan in Yoruba, Igbo, Hausa and Edo respectively [3, 4]. It has been pharmacologically reported to be effective against myriads of health maladies such as measles, fever, some liver diseases and wounds [5]. In southwestern Nigeria it is claimed to manage

epilepsy and promote erection [4]. It also possess analgesic activities [6]. To our knowledge, this is the first study to determine the in vivo genotoxic or nongenotoxic effect of *L. cupanioides* in mice with the aim of establishing its safety. Thus, this study investigated its genotoxicity on Swiss mice.

Materials and Methods

Plant collection

Lecaniodiscus cupanioides leaves were sourced in December 2018 in Oyo state, Nigeria. The plant was authenticated at the forest research institute of Nigeria (FRIN), Ibadan courtesy Mr Odewo S.A. Voucher specimen was prepared and deposited with identification FHI111668 at the Herbarium of the Forestry Research Institute of Nigeria (FRIN).

Plant Extraction

Freshly collected or harvested leaves were cleaned, washed, shredded and air dried at 40°C for 21 days. It was pulverized into powdery texture. Powdered leave (1039g) sample was weighed and soaked in 2700mls ethanol for 48hours. Extract was filtered and filtrate was evaporated into paste using regulated hotplate at 40±1°C. The concentration was collected into a suitable container.

Experimental Animal

Thirty *Mus musculus* (Mice) weighing 20-25g were procured from BioVaccine Centre, NAFDAC, Yaba, Lagos. The animals were grouped into three cages of 10 mice each, and kept in animal house in zoological garden of the University of Lagos to acclimatize for 7 days. They were adequately supplied water and feeds ad-libitum. After acclimatization, groups; A and B were orally administered 100 and 400 mg kg⁻¹ of the extract respectively, while group C received distilled water. Treatment was administered daily for 49 days after which the mice were sacrificed via jugular puncture. The femur were harvested for bone marrow assays.

Micronucleus Assay

This assay was executed according to Alimba and Bakare [7]. The bone marrow cells from femur of mice were flushed with the FBS and centrifuged at 2000rpm 5minutes, the supernatant was removed. FBS was added and centrifuged again and supernatant was discarded. A drop of the cell suspension was placed onto a slide and using a pusher slide, a smear was created. The slides were air dried and fixed in methanol for 10minutes. The fixed slides were stained with 0.25% May-Grunwald stain and then rinsed after 15minutes with deionized water. After rinsing, it was stained using with Geimsa. The stained slides allowed to air dry for a day and then covered. The ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) was calculated based on the slides scored. Minimum of 1000 cells per mouse were examined for micronuclei in polychromatic erythrocytes (MnPCE). The differential staining of PCEs (bluish-purple), normochromatic erythrocytes (NCEs), pinkish-orange) and the relative size of the erythrocytes were indices for differentiating them. Nuclear abnormalities (NA) were also scored as cytotoxic parameters. Cells with two nuclei were considered as binucleated (BN), Notched nucleus (NT) contains vacuoles and appreciable depth into the nucleus that does not contain nuclear materials and budding nucleus (NBud). Decrease in PCE: NCE ratio was considered an indicator of bone marrow toxicity in-

duced by mutagens.

Determination of Heavy Metal

The atomic absorption spectrophotometer (AAS) was used to estimate the following metals: Mn, Zn, Cd, Cu, Cd, Ni, and Cr. As described by Ayaz et al. [8]. 2 g of sample was placed in a crucible and subjected to heating at 550°C in a muffle furnace for 6 h. The resultant ash was then placed in 5ml of concentrated nitric acid for digestion. It was then evaporated using hot plate. Small amount of distilled water was added to the digested residue, filtered and volume was made to 30 ml using distilled water. Atomic absorption spectrophotometer was then utilized in quantitative analysis of the solutions produced.

Determination of moisture content

Moisture content was estimated according to AOAC [9]. 5 g of the dried extract of *L. cupanioides* was subjected to 105 oC heating in a hot air oven for 2 h, dish was made to cool in desiccator, weighed and the moisture content evaluated by the following formula.

$$\text{Moisture (\%)} = \frac{(\text{Weight of sample} - \text{Dry weight})}{\text{Weight of the sample taken}} \times 100$$

Proximate analyses

This analysis was executed according to AOAC [9]. The Parameters investigated for included ash, protein, fat, and crude fiber. All of these were carried out using the methods described by AOAC (9).

Statistics

Statistical analyses were performed using Microsoft Excel. Data expressed in format of Mean ± SEM. The data was subjected to sample T-test, evaluating the statistical significance of the difference between two means of various parameters between the control and experimental group. The P value was found by means of Microsoft Excel.

Determination of pH

The pH influences the taste and stability of oral gels. The

Table 4: Proximate analysis of *L. cupanioides*

Results

Table1. Polychromatic erythrocytes (PCE) observed in bone marrow of mice administered with ethanolic *L. cupanioides* for 49 days.

Treatment	MnPCE	NT PCE	Normal PCE	BN PCE	BUD
Control	314.13±3.05	68±2.52	208.13±4.62	41±1.05	75.5±3.61
100mg/kg	351±10.41b	66.20±6.67	206.25±3.67	40±5.70	50.8±9.12a
400mg/kg	321.5±4.09	59.50±3.57	215.75±13.14	55±3.54c	56.25±8.09a

Data represent Mean±SEM, N=10

(a= P<0.05, b= P<0.01, c=P<0.001)

Table 1 shows dose dependent-increase in the number of MN PCEs in the low and high concentration. No statistical significant treatment related increase of MN PCEs in the low and high concentration respectively. There was also a significant decrease in the number of Bud PCE observed in the low and high concentration.

Table 2: Normochromatic erythrocytes (NCE) observed in bone marrow of mice administered with ethanolic *Lecaniodiscus cupanioides* for 49 days

Treatment	MnNCE	Normal NCE	NT NCE
Control	87.25±7.8	171.25±8.02	34.75±4.27
100mg/kg	95.80±4.96	136.80±14.34a	53.40±1.58a
400mg/kg	86±7.85	174±6.77	32±4.04

Data represented as Mean ± SEM, N=10 (a= P<0.05, b= P<0.01, c= P<0.001)

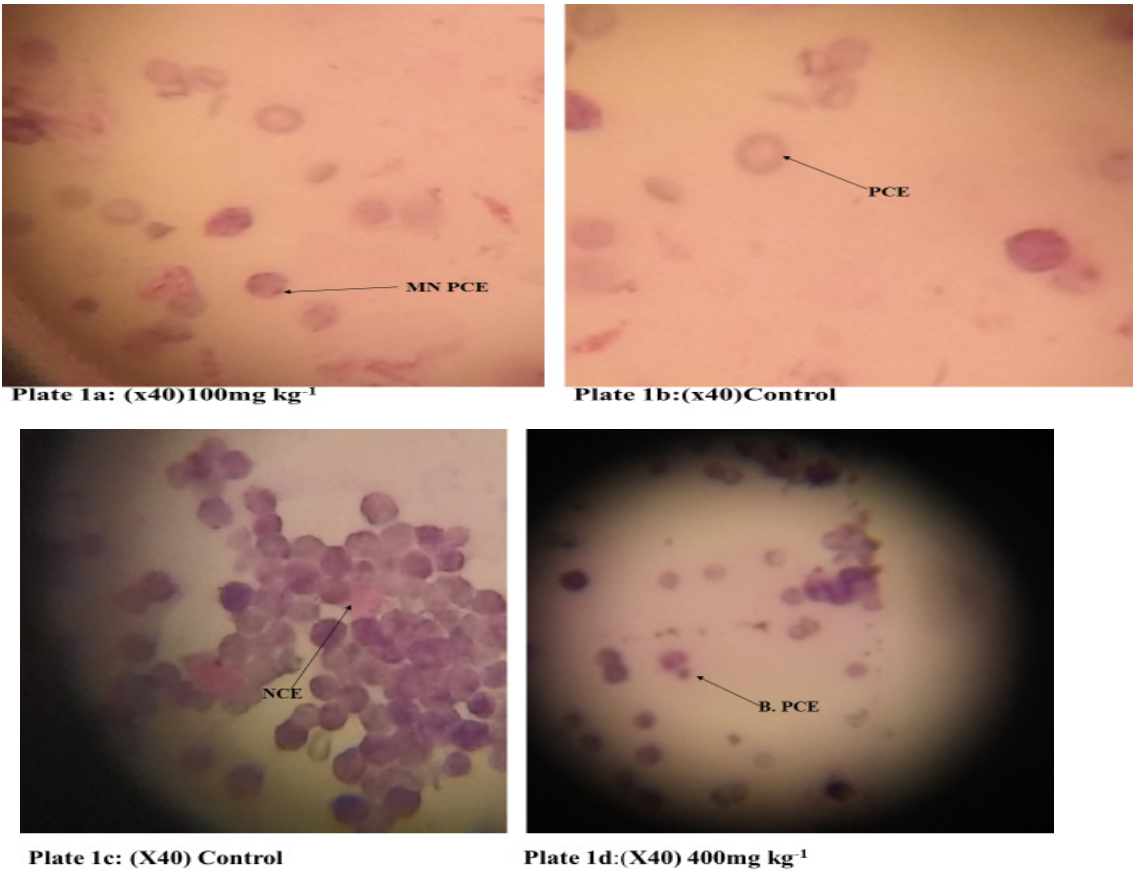
Table 2, shows a dose related increase in the frequency of MN NCEs and significant treatment-related decrease in the number of NCEs observed in the low concentration.

Table 3: PCE/ (PCE+NCE) ratio in bone marrow

Treatment	PCE	NCE	PCE/ (PCE + NCE)
Control	392.63±4.72	293.25±3.14	0.57±0.006
100mg/kg	363 ±17.5	286±9.57	0.56±0.02
400mg/kg	386.5±14.92	292±15.20	0.59±0.034

Data represented as Mean ± SEM, N=10 (a= P<0.05, b= P<0.01, c= P<0.001)

No significant difference in PCE/ (PCE+NCE) ratio representing the absence of bone marrow cytotoxicity was observed in the mice after exposure to ethanolic extract of *Lecaniodiscus cupanioides* when compared with the control. The extract did not affect the frequency of the PCE/ (PCE+NCE) ratio, an indicator of toxicity to the bone marrow cells.



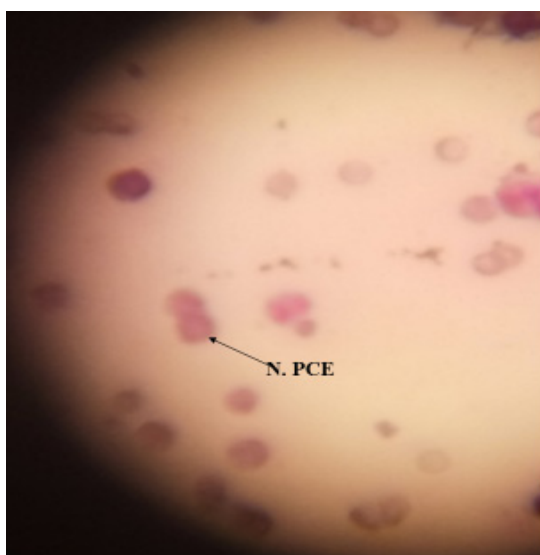
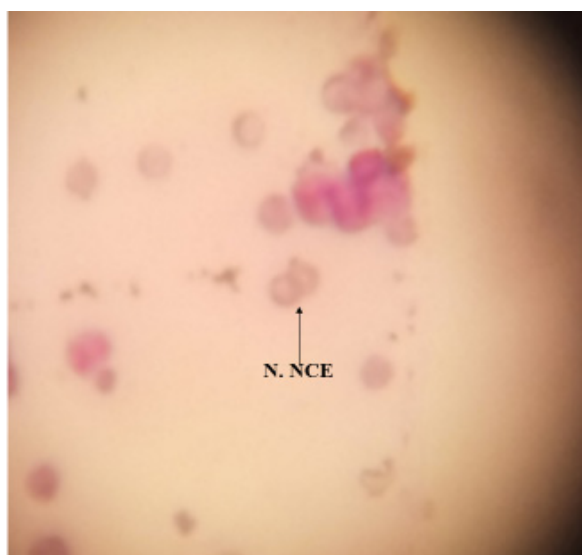
Plate 1e: (X40)100mg kg⁻¹

Plate 1f: (X40)Control



Plate 1g:(X40) 100mg

Plates 1A-G: showing various erythrocyte abnormalities. A; MN.PCE- micronucleated polychromatic erythrocytes (immature RBC possessing RNA and micronucleus). B; PCE- polychromatic erythrocytes (immature RBC containing RNA which matures into normochromatic erythrocytes lacking RNA). C; NCE- Normochromatic erythrocytes (mature RBC that lacks RNA). Other nuclear abnormalities observed include binucleated, bud and notched cells. D; B.PCE- binucleated PCE (cells with two nuclei). D; Notched PCE, contains vacuole and an appreciable depth into the nucleus without nuclear materials. F; Notched NCE, contains vacuole and an appreciable depth into the nucleus without nuclear materials. G; Bd. PCE- Bud PCE (Nucleus appears to be budding out of the cell).

Parameter	Level Detected
Moisture	0.88 %
Ash content	8.21%
Fat content	0.56%
Crude fibre	10.55%
Carbohydrate	70.27%
Crude protein	9.53%

Percentage value of proximate analysis revealed carbohydrate as the highest percentage followed by crud fibre and then crude protein and ash respectively. Fat is has the lowest percentage.

Table 5: Analysis of some heavy metals in *L. cupanioides*

Heavy metal	AAS value	Value in mg/kg
Cadmium (Cd)	0.009	0.09
Chromium (Cr)	0.011	0.11
Lead (Pb)	0.006	0.06
Zinc (Zn)	2.046	20.06
Copper (Cu)	0.056	0.55
Manganese (Mn)	0.044	0.43
Nickel (Ni)	0.014	0.14

In table 5; Zn was observed as the highest metal, followed by Cu and Mn respectively. The lowest was Cd. All metals were present at permissible level.

Discussion

Medicinal plants have been used for management of many health challenges as they are natural sources of numerous bio-active compounds (secondary metabolites) possessing vital bio-activities. These metabolites are chemical constituents and have been used in traditional folks as drugs [10]. Some of the advantages for medicinal plants patronage include cost effectiveness, broad spectrum activities, little or no side effects, curative and preventive activities [10-12]. In this study *L. cupanioides* was evaluated for its subchronic toxicity in mice.

Body weight is a pertinent criteria in evaluating toxic effects of treatments, drugs or toxic substances [13]. Variation in weights can be one of the first critical evidence of toxicity [14]. Evaluation of growth in animals utilizing weight index is a commonplace in toxicological investigations as it assist in interpreting compound-related effects [15]. In this investigation, weight of mice assessed weekly for 49days did not show any significant difference, implying that the extract does not seem to possess any threat to the physiognomy of the mice. The bone marrow Mn test is a popular in vivo assay for investigating genetical aberrations in animals like mice and rats. A Mn is round and has diameter of about 1/20th to 1/5th RBC. Bone marrow PCE to NCE ratio in prepared slides, is important in evaluating any aberrations in hematopoiesis consequent upon animal's exposure to treatments [16]. The choice of MN assay amongst array of genetic batteries was to investigate the potential risk for chromosome damage in bone marrow of mice exposed to different concentrations of *L. cupanioides*. MN has been described as a biomarker for chromosomal damage. In young and new erythrocytes. MN emerge mainly from fragments of chromosomes not incorporated within the daughter nuclei during mitosis of the erythropoietic blast cells [17]. The extract insignificant effect as observed against control in the PCE/ (PCE+NCE) suggest that it is not cytotoxic [18]. Significant increase in the frequencies of micronucleated PCE and NCE formation in mice confirms clastogenicity of test substance and that increased in frequency of NCE in exposed mice indicated that test substance constituents increased the rate of ageing of PCE, thereby decreasing their

normal life span and increasing their risk of genotoxicity [7, 19]. Since this study did not show any significant treatment related increase in the frequencies of MN PCEs and NCE it therefore predicts that *L. cupanioides* does not have clastogenic effects and does not increase the risk of genotoxicity. Various nuclear abnormalities scored to complement MN frequency in the toxicity assessment did not show significant increase against control, indicating absence of cytotoxic and genotoxic agents, as the simultaneous formation of nuclear abnormalities (NAs) and MN in erythrocytes treated with test substance is considered a better indicator of cytotoxicity and genotoxicity monitoring of the clastogenic effects of treatments [20]. However significant increase in binucleated erythrocytes formation suggests that constituent of test substance inhibited cytokinesis of the dividing cell, which is in line with Ozkan et al., [21]. There was no reported increase in the frequency of observed nuclear bud in the erythrocytes; as increase may suggest clastogenicity and aneugenicity to dividing cells in the bone marrow [22].

Heavy metals can be absorbed from soil contaminated with high level of it due to their non-biodegradability resulting in its bioavailability to humans. Heavy metals in high proportion portends high toxic risk to health. Pb, Cd, Cr, Cu, Ni, and Zn possess permissible limit of 10, 1, 1.5, 10, 1.5 and 50 ppm respectively [8]. Physico-chemical investigation on *L. cupanioides* revealed the presence of Cu, Cr, Mn, Ni, and Cd at permissible level, which implies that the extract may elicit myriads of health benefits to justify its traditional use. The extract may play an important role in a number of physiologic processes due to the presence of Manganese (Mn), which can also serve as activator of other enzymes [23]. Manganese is the preferred cofactor of enzymes called glycosyltransferases; these enzymes are required for the synthesis of proteoglycans that are needed for the formation of healthy cartilage and bone [24]. Manganese is required for the activation of prolidase, an enzyme that functions to provide the amino acid, proline, for collagen formation in human skin cells [25]. Availability of copper in the extract can also make *L. cupanioides* elicit many physiologic activities as copper is a critical functional component of several essential enzymes known as cu proenzymes [26]. The plant might also help in enhancing sensitivity of tissues to insulin and facilitating transportation of glucose into cells, due to the presence of chromium in its extract. Chromium has been proposed to be the cofactor for an oligopep-

tide called chromodulin which is a factor that may be able to potentiate the action of insulin [27]. Zinc, a nutritionally essential mineral needed for catalytic, structural, and regulatory functions in the body [28] also observed in *L. cupanioides* in conjunction with other element may be responsible for some of its medicinal values and managing of some health malaise [29]. Nickel also plays some role in body functions including enzyme functions [30]. Copper is a co-factor for metalloproteins and play a major role in several metabolic pathways [31]. Lead was found beyond permissible limit which may indicate that the plant may possess some toxicity [32].

Conclusion

L. cupanioides did not cause any genotoxic effect in the bone marrow cells within the exposure period and doses examined. Heavy metals were observed at permissible level, confirming its health potentials. However, further assessment and evaluation on the medicinal uses and safety especially when administered at higher doses and a longer period of time should be executed.

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Authors' contribution

The manuscript was written, and approved in collaboration with all authors.

Conflict of interests

All authors declare that there is no conflict of interest.

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