PRELIMINARY INVESTIGATION ON THE GENOTOXIC REMEDIATING POTENTIAL OF ETHANOLIC EXTRACT OF *FICUS PLATYPHYLLA* BARK ON NITROSOMETHYLUREA (NMU) INDUCED *MUS MUSCULUS*

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Abstract: This study investigated the mitigating effect of ethanolic extract of *F. platyphylla* bark on genotoxicity of Nitrosomethylurea (NMU) on erythropoiesis in mice. 25 albino mice of 19-28g, divided into 5 cages of 5 mice, were pre-treated with 50mg kg^{-1} single dose NMU intraperitoneally. Groups A, B and C were orally administered 400, 800 and 1600mgkg^{-1} doses for 10 weeks, while C and D received 3mg kg^{-1} cisplatin and normal saline respectively. At expiration of the experiment all mice were sacrificed, femur bones isolated and aspirated for micronucleus assay. Results revealed gradual but insignificant body weight increase in treated mice compared to control. Micronucleus assay showed significant dose-dependent decrease in MnPCE and BlebPCE with the highest frequency in negative control. Insignificant dose-dependent decrease was observed in Normal polychromatic erythrocytes (PCE). PCE/ (PCE+NCE{normochromatic erythrocytes}) significantly increased with 1600mgkg^{-1} dose. The gradual, but insignificant increase in weight of treated mice suggest preliminary mitigating potential of the extract. Increased MnPCE and BlebNCE in control established extent of genetic damage on erythropoiesis by NMU, as MnPCE and BlebNCE decreased dose-dependently, inferring the remediating potential of the plant. *F. platiphylla* may be adduced to have reduced clastogenic and cytotoxic effects of NMU in erythropoiesis in this study. This is the first study to investigate the mitigating potential of *F. platyphulla* on genotoxicity of NMU on bone marrow of mice.

Keywords: *F. platyphylla*, NMU, genotoxicity, micronucleus assay, erythropoiesis

Introduction

Ficus platyphylla Del. Holl. Belong to the family Moraceae, it is commonly found in savannah area of West Africa coast (Becker *et al*., 2021). Various preparations of the plant have been implored traditionally in the management health malaise such as inflammation, insomnia, epilepsy, pain, psychosis and depression (Becker *et al*., 2021). Chindo *et al*., (2016) also reported the anticonvulsant potential of the plant. It has also been described as neuroleptic-like (Sutter *et al*., 2019 ; Zhang *et al*., 2018), analgesic (Chindo *et al*., 2016) and hypothermic (Sutter *et al*., 2019). N-Nitroso-N-methylurea (NMU) used to induce breast tumour in this study is a potent carcinogen, mutagen and teratogen (Tsubura *et al*., 2011). NMU has been described as an alkylating agent which react with nucleophillic nitrogen, oxygen atoms in bases and DNA phosphate groups thereby creating mutagenic lesions (Verma *et al*., 2012) hence playing a vital role in cancer initiation (Verma *et al*., 2012). Continuous exposure to NMU can increase formation, repair and persistence of DNA adducts and consequently to inflammation and increased levels of inflammatory cytokines creating a microenvironment convenient and conducive for the survival and development of cancer cells (Verma *et al*., 2012). Generally, MNU-induced mammary carcinomas have been described to be aggressive and locally invasive (Roomi *et al*., 2005). However, some authors have reported the ability of NMU to cause metastasis at distance sites (Gullino *et al*., 1975; McCormick *et al*., 1981). This study investigated the possible toxic effect of NMU in bone marrow of mice with a view to possibly mitigating it by exploring the medicinal potential of *Ficus platyphylla*.

Materials and Methods

Ethical Approval

The ethical approval reference no CMUL/HREC/0887/19 was gotten for the project.

Plant Collection and Identification

Bark of *Ficus platyphylla* were collected in the month of March, 2022, from traditional merchants in Bariga market, Lagos. The leaves were identified and authenticated at the department of Botany, University of Lagos, with voucher number LUT 8746 of the plants deposited in the herbarium.

Plant Preparation and Extraction

The barks of *Ficus platyphylla* were cut in bits and air dried, plant samples were then dehydrated at 35℃ in a food dehydrator with air circulation for 60minutes; for total removal of any present water molecules. The dehydrated bark of *Ficus platyphylla* was granulated to a fine powdered form. The bark extracts were obtained by using crude extraction protocol, using a ratio of 1:3 solutes to solvent. The preparation was soaked for 72hours after which it was filtered using a funnel and cotton wool. The filtrate was subjected to heating at 40 ± 1^0C using a regulated hot plate. Extract derived was weighed and reconstituted for bioactivity.

Experimental Animals

Twenty-five female albino mice obtained from the National Agency for Food and Drug Administration and Control, Lagos, Nigeria (NAFDAC) of average weight of 19-28g were used for the study and were acclimatized for two weeks in animal house of the Department of Cell Biology and Genetics, University of Lagos. The mice were thereafter divided into five groups of five mice each for the experiment.

Genotoxic Induction and Treatment

50mg kg^{-1} of NMU was prepared for administration to the mice. After acclimatization of the mice, the NMU was administered intraperitonealy in the ventral midline of the animal (between the third and fourth pair of the mammary gland) as described by Rajmani *et al*., (2011). Mice were allowed to stay 7 days before the commencement of the treatment. The twenty-five albino mice were weighed and grouped into five groups of five animals each. Three groups (A, B, C) received treatments while two (D, E) served as control (positive and negative). All the mice were induced with the prepared NMU, the positive control group was treated with 3mg kg^{-1} Cisplatin. Negative control group was administered normal saline intra-peritoneally, same time and duration as positive control. The treatments groups A, B, C were orally administered 400mg kg^{-1}, 800mg kg^{-1} and 1600mg kg^{-1} respectively for 10 weeks. All mice were weighed weekly and inspected for any irregularities. The mice were sacrificed via jugular puncture at the expiration of treatments.

Micronucleus Assay

This was executed according to Oloyede *et al.*, (2020). Briefly, bone marrow cells within femur of mice were flushed using FBS and centrifuged at 2000rpm for 5minutes, the supernatant was decanted. FBS was added and centrifuged again and supernatant was discarded. A drop of the cell suspension was placed on the slide and a smear was made using a pusher slide. The slides were air dried and fixed in methanol for 10minutes. The fixed slides were stained with 0.25% May-Grunwald stain and rinsed after 15minutes with deionized water. It was then stained with Geimsa. The stained slides were 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 induced by mutagens

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.

Results

Table 1 shows variation in the body weight of mice during the duration of extract administration. Though not significantly different when compared to the negative control. There were insignificant increase in the body weight of the treated mice compared to the control.

Table 1: Weight of the Mice during Treatment with *F. Platyphylla* for 10 weeks

Week	Control	Cisplatin	400mg kg^{-1}	800 mg kg^{-1}	1600 mg kg^{-1}
1	20.38±0.37	21.22±0.32	22.42±0.09	23.60±0.22	25.92±0.69
2	20.72±0.58	23.04±0.32	24.44±0.48	25.90±0.88	25.80±1.12
3	21.57±0.77	24.45±0.29	25.42±1.72	25.90±0.85	26.40±1.31
4	22.72±0.57	25.40±0.40	27.12±1.58	26.76±0.81	28.12±1.26
5	23.65±0.75	26.45±0.35	27.26±0.96	27.66±0.63	28.40±0.45
6	23.87±0.79	26.62±0.34	27.46±0.94	27.80±0.62	28.56±0.42
7	24.05±0.80	23.95±1.35	24.40±0.98	24.96±1.62	25.46±0.66
8	24.15±0.73	23.63±1.25	26.83±0.82	27.65±2.45	27.10±2.30
9	24.35±0.74	23.05±1.55	27.13±0.86	27.80±2.60	27.30±2.30
10	24.82±0.84	23.70±1.40	27.56±0.78	28.25±2.55	27.90±2.30

Data presented as Mean ± SEM (N=5) (a=P<0.05, b=P<0.01, c=P<0.001)

Table 2; shows bone marrow analysis revealing significant dose-dependent decrease in MnPCE and Bleb PCE with the highest frequency observed in negative control. Insignificant dose-dependent decrease was also observed in Normal PCE against the negative control. This shows that with increase in treatment concentration, there was decrease in abnormalities in bone marrow indices.

Table 2: Polychromatic Erythrocytes (PCE) observed in Bone Marrow of mice administered Ethanolic *F. Platyphylla* for 10 weeks.

Treatment	MnPCE	Normal PCE	Bleb PCE
Control	4.50±1.50	1985.50±2.50	12.50±2.50
Cisplatin	4.50±1.50	1988.50±1.50	4.00±1.00
400 mg kg^{-1}	3.00±1.00	1976.50 ± 2.50^{b}	8.50 ± 3.50^{a}
800 mg kg^{-1}	2.50 ± 2.50^{b}	1961.00 ± 14.00^{c}	7.50 ± 0.50^{b}
1600 mg kg^{-1}	1.50 ± 1.50^{c}	1961.00 ± 16.00^{c}	4.00 ± 1.00^{c}

Data represent Mean±SEM, N=5 (a=P<0.05, b=P<0.01, c=P<0.001)

Table 3; shows dose-dependent decrease in Normal NCE and Bleb NCE, but no significant difference was observed in MnNCE. Dose-dependent increase in Normal NCE was observed with normal saline treatment, recording the highest while the lowest was observed in the 1600 mg kg^{-1} dose. The highest frequency of bleb NCE was also observed in the saline group (negative control), whereas dose-dependent decrease was observed from the lowest dose to the highest dose.

Table 3: Normochromatic Erythrocytes (Nce) Observed in Bone Marrow of Mice Administered with Ethanolic *F. Platyphylla* for 10 weeks Treatment

Treatment	MnNCE	Normal NCE	Bleb NCE
N.Saline	0.50±0.50	39.00±16.00	24.00±2.00
Cisplatin	0.00	11.50±1.50^{c}	9.50±2.50
400 mg kg^{-1}	0.00	39.00±14.00	18.50±5.50
800 mg kg^{-1}	**0.00**	30.00±8.00^{a}	15.00±5.00
1600 mg kg^{-1}	**0.00**	14.50±2.50^{c}	12.00±2.00

Data represented as Mean±SEM, N=5 (a=P<0.05, b=P<0.01, c=P<0.001)

Table 4: PCE/(PCE+NCE) ratio in the Bone Marrow of Mice Administered with Ethanolic *F. Platyphylla* for 10 weeks Treatment

Showing significant difference (p<0.05) in PCE/ (PCE+NCE) with the 1600 mg kg^{-1} dose and standard drug. While other doses reveal the same value with -ve control group.

Treatment	PCE	NCE	PCE/(PCE+NCE)
N.Saline	1985.50±2.50	39.00±16.00	0.98±0.05
Cisplatin	1988.50±1.50	11.50±1.50	0.99±0.05
400 mg kg^{-1}	1976.50±2.50	39.00±14.00	0.98±0.05
800 mg kg^{-1}	1961.00±14.00	30.00±8.00	0.98±0.05
1600 mg kg^{-1}	1961.00±16.00	14.50±2.50	0.99±0.05^{b}

Data represented as Mean ± SEM, N=5 (a=P<0.05, b=P<0.01, c=P<0.001

Micrographs showing Erythropoietic abnormalities

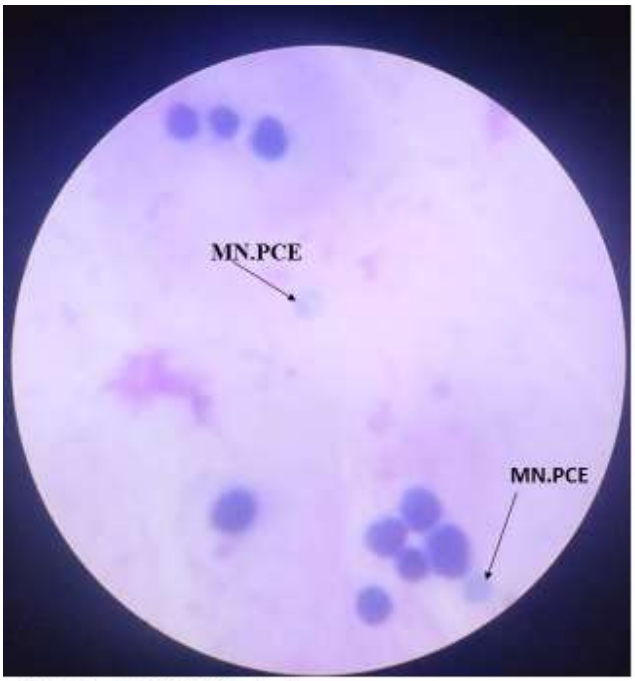

Plate 1a: (X100) 400mg kg^{-1}

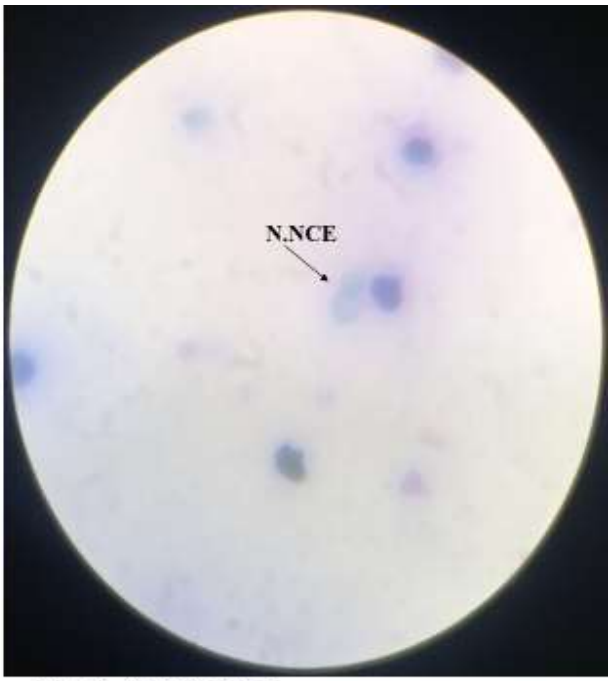

Plate 1b: (X100). N.Saline

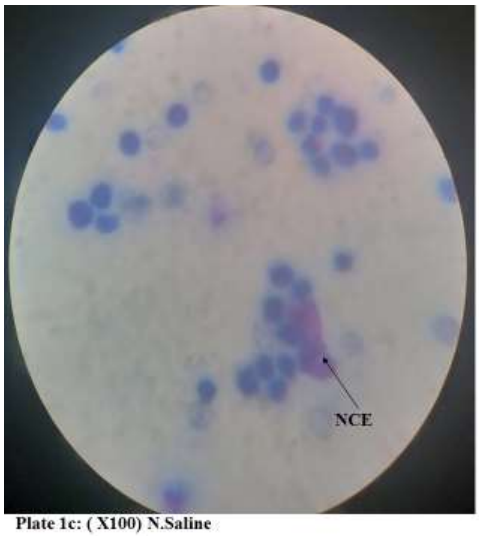

Plate 1c: (X100) N.Saline

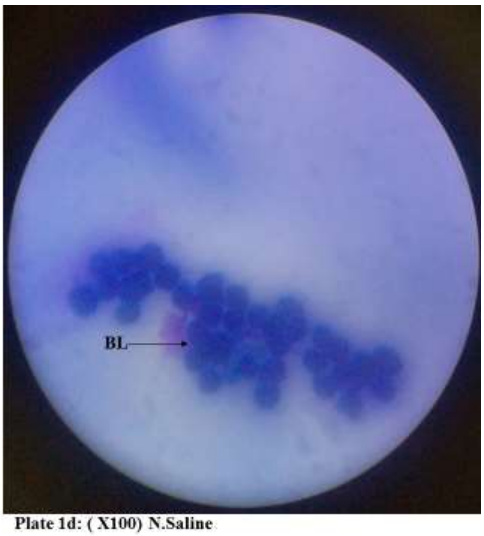

Plate 1d: (X100) N.Saline

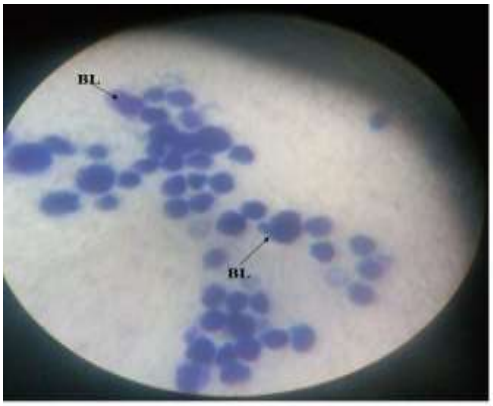

Plate 1e: (X100) 1600mg kg^{-1}

Plate 1A–E shows different abnormalities in erythrocyte from bone marrow. **A**: MN PCE: Micronucleated Polychromatic Erythrocytes which are PCEs immature red blood cells, with RNA and micronucleus in them. **B**: N.NCE-Notched Normochomatic erythrocytes contain a vacuole and a depth into the nucleus lacking nuclear content .**C**: NCE-Normochromatic erythrocytes which is mature red blood cell that lacks RNA. **D&E**: Bleb PCE where the cells appear to have a protrusion on its surface.

Discussion

Body weight has been used as a routine index for toxicologic investigation, as it aids in compound-related effects (Oloyede *et al*., 2020). Body weight index is vital in probing effects of toxic substances or drugs (Oloyede *et al*., 2020). It is therefore a common place in toxicologic studies to evaluate animal growth using weight index, especially in interpreting compound-related effects (Oloyede *et al*., 2020). In this study the variation in body weight showing gradual, but insignificant increase in mice treated with *F. platyphylla* compared to control may be a preliminary sign of mitigating potential of the extract. Micronucleus (MN) is alluded as a biomarker for chromosomal damage. It is generally accepted as in vivo assay for investigating genetical aberrations in rodents such as mice and rats in nascent erythrocytes (Oloyede *et al*., 2020). MN are mainly produced from chromosomes fragments not incorporated within the daughter nuclei during mitosis of the erythropoietic blast cells (Habibi *et al*., 2015). The bone marrow assay is one of the most popular invivo assay used in investigating genetic aberrations in animals. This assay explores erythropoietic principle to detect aberrations like clastogenicity, aneugenicity and spindle poisoning potential of a substance (Krishna and Hayashi, 2000). Therefore, the frequency of micronucleus in PCE is an indication of extent of genetic damage inflicted on the erythropoietic system of organisms (Masfria *et al*., 2021) Genetic toxicity assays have been used in the identification of gene mutations, chromosome changes and alterations in the DNA sequencing (Parasuraman, 2011). Ratio of PCE/NCE is also accepted as an indicator for toxicity against the cellular integrity of the bone marrow (Rina *et al*., 2014). When healthy proliferation of bone marrow cells is halted or affected by a toxic agent, the ratio of PCE/NCE may decrease (Rina *et al*., 2014). Observation in this study revealed the cytotoxic effect of NMU in consonance with the study of Rina *et al*., (2014) and its possible mitigation by *F. platyphylla* through the PCE/NCE relationship. The increased MnPCE and BlebNCE in the group administered saline and NMU may be a reflection of extent of genetic damage inflicted on the erythropoietic system of the bone marrow by the NMU as other groups administered NMU+ F. *platyphylla* revealed dose-dependent decrease in MnPCE and BlebNCE, this may indicate the ability of the plant to remediate the toxic

influence of NMU. *F. platiphylla* may be adduced to have reduced clastogenic and cytotoxic effects of NMU in mice bone marrow cells especially at high dose (1600 mg kg^{-1}) used in this study.

Conclusion

This is the first study to investigate the potential of *F. platyphulla* to mitigate genotoxic effects of NMU on bone marrow of mice. *F. platyphyla* was able to mitigate the genotoxic effect of NMU dose-dependently. However a significant activity was achieved with the highest dose used in this study. Investigation is still ongoing on the antitumour activity of the extract.

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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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