EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES ZOOLOGY

B

ISSN
2090-0759

WWW.EAJBS.EG.NET

Vol. 16 No. 2 (2024)

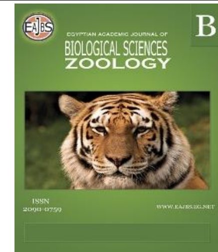
.

www.eajbs.eg.net

Citation: *Egypt. Acad. J. Biolog. Sci. (B. Zoology) Vol. 16(2) pp:209-231 (2024)*
DOI: 10.21608/EAJBSZ.2024.400568

Egypt. Acad. J. Biolog. Sci., 16(2):209-231(2024)

Egyptian Academic Journal of Biological Sciences
B. Zoology
ISSN: 2090 – 0759
Res. Ethics No.: 03-2024-0009
http://eajbsz.journals.ek b.eg/



Assessment of Bark of *Ficus platyphylla* on NMU-Induced Broad-spectrum Toxicity in *Mus musculus* (Albino mice)

Oloyede, M. Adeola*; Ottu, Bodunrin;Agbonifo, O. Worship;Adebesin Oyindamola;Osho, S. Omolara;Alli, I. Owomide; Oluwakemi, Oyedele and Oseleye, Oluwatobi
Department of Cell Biology and Genetics, University of Lagos, Akoka, Lagos-Nigeria
***E-mail: moloyede@unilag.edu.ng**

ARTICLE INFO
Article History
Received:4/11/2024
Accepted:27/12/2024
Available:29/12/2024

Keywords:
Ficus platyphylla, NMU toxicity, antioxidant defenses, liver enzymes, cytotoxicity mitigation.

ABSTRACT

Ficus platyphylla is a medicinal plant widely used in West African folk medicine for the treatment of inflammatory conditions, fevers and bacterial infection. This study evaluated the protective effects of *Ficus platyphylla* extract against N-nitroso-N-methylurea (NMU)-induced toxicity in mice. 25 female mice between 19g and 25g were divided into five groups (A-E) based on body weight. All mice were initially pretreated with NMU, and then subjected to treatments of 200 mg kg^{-1} and 100 mg kg^{-1} of the extract, cisplatin, NMU only, and distilled water respectively over twelve weeks. Upon completion of the treatment, animals were euthanized via cervical dislocation. Their liver, kidney, breast, heart, and femur were harvested for analysis. Results showed that the 200 mg kg^{-1} plant extract treatment exhibited significant increased body weight and showed significant decreased AST, ALT, and ALP. Antioxidants analysis revealed significantly increased SOD for 100 mg kg^{-1}. Haematological analysis showed decreased monocytes at 100 mg kg^{-1}. Breast tissue assessment revealed significantly increased GSH and GPx at 200 mg kg^{-1}. Histology showed that higher doses preserved structural integrity in breast and liver tissues despite NMU-induced challenges. Bone marrow analysis showed a significant increase in polymorphic nuclei (PM) at 200 mg kg^{-1}. At 100 mg kg^{-1} there was significant increase in counts of segmented nuclei (SM), kidney-shaped nuclei (KN), and blebbed nuclei (BLN) compared to Control. The significant decrease in AST, ALT, and ALP generally implies improved liver health or reduced liver damage. Significantly increased GPx and GSH may suggest lipid peroxidation inhibition, thereby reducing oxidative stress risks, and improving overall health. Decreased monocytes suggest a modulation of immune response. Lesser PM frequency in extract groups compared with NMU group, suggest decrease in genetic imbalance. Decreased SM, KN, and BLN counts in mice treated with the extract in comparison with NMU group may depict an improvement in tubulin function, reduction of mitotic errors, and decreased structural chromosomal abnormalities, suggesting that extract may possess components that counteract cytotoxicity. This study inferred that the extract effectively offered protection against NMU induced toxicity.

Citation: *Egypt. Acad. J. Biolog. Sci. (B. Zoology) Vol. 16(2) pp:209-231 (2024)*
DOI: 10.21608/EAJBSZ.2024.400568

INTRODUCTION

Ficus platyphylla is a deciduous fig tree belonging to the Moreaceae family (Chindo *et al*., 2014). It is native to tropical Africa and has been used in traditional medicine for various ailments (Adamu *et al*., 2022). Locally known as "Epo obo" in Yoruba and "Gamji" in Hausa, this tree is traditionally believed by Nigerians to be effective in treating colds, flu, diabetes, psychosis, inflammation, epilepsy, depression, and gonorrhea (Abubakar and Haque, 2020). Decoction of the bark is used to treat leprosy and stomach aches, while a decoction of the leafy branch tips is believed to serve as an antidote for arrow poisoning (Sheidu *et al.,* 2020). The ethanolic extract of the leaves has been reported to possess analgesic and anti-inflammatory activities (Onuh *et al.,* 2022).

The herb has been used locally to treat trypanosomiasis, convulsive disorder, and mental disease and effectively control tuberculosis and cough in the northeastern region of Nigeria, where tuberculosis is endemic (Adisa *et al*., 2022). Preparations of this plant with other herbs have also been employed in traditional medicine in Northern Nigeria to enhance patient outcomes (Chindo *et al*., 2015; Sutter *et al*., 2019). Occasionally, powdered extracts from the stem or root bark are ingested or inhaled from burning charcoal to boost fertility (Ndatsu and Abdullahi, 2019).

Ficus platyphylla has medicinal potential due to its phytochemical and biological activity (Hassan *et al*., 2022). Studies have detected the presence of various phytochemicals such as flavonoids, tannins, alkaloids, saponins, glycosides, phenols, and anthraquinones (Hassan *et al.,* 2022). This study aimed to explore the potential mitigating effects of *Ficus platyphylla* on N-Methyl-N-Nitrosourea (NMU)-induced toxicity.

MATERIALS AND METHODS

Plant Collection and Identification:

The stem bark of *Ficus platyphylla* was sourced from Bariga Market, a popular market in Lagos State, southwest Nigeria, and the plant was identified at the Herbarium of the Department of Botany, University of Lagos, with voucher number LUT 8746 of the plants deposited in the herbarium.

Plant Preparation and Extraction:

The bark extracts were obtained by using crude extraction protocol, using a ratio of 1:3 solutes to solvent. 2.9 kg of *Ficus platyphylla* was soaked in 5 liters of ethanol at a concentration of 90% for 48 hours. The solution was later filtered using a funnel and cotton wool. The filtrate was evenly distributed into ten beakers. These were then left to evaporate in an oven set at a temperature of 40±1°C for 5 days, until they were completely dry. The extracts obtained from the ethanol filtrate were weighed and dissolved in distilled water for further use.

Experimental Animals:

Twenty-five (25) female albino mice weighing between 19 g and 25 g were sourced from the National Institute of Medical Research (NIMR), Yaba, Lagos State. They were left to acclimatize for two (2) weeks. Afterward, the mice were weighed and placed into five (5) groups of five mice each, based on their body weight. They were pre-treated with NMU and kept in plastic cages under standard temperature and humidity, fed standard rat chow, and had access to clean drinking water.

The groupings and treatments are as follows:

Group A - 200mg kg^{-1} of *F. platyphylla* extract

Group B – 100 mg kg^{-1} of *F. platyphylla* extract

Group C - Cisplatin
Group D - NMU Only
Group E (Control) - Distilled water

Animals were orally administered treatments daily for 12 weeks and weighed weekly. At the expiration of the experiment, mice were sacrificed via cervical dislocation. The internal organs (breast tissue, livers and kidneys) and femur were harvested for biochemical, antioxidants, histologic and bone marrow assays.

Biochemical Analysis:

The kidney, liver, and breast tissue samples were initially washed with a phosphate-buffered saline (PBS) solution to remove any residual blood cells and clots. Subsequently, analysis of aspartate transaminase (AST) and alanine transaminase (ALT) was conducted using the Randox Laboratories Kit protocols. The quantitative determination of Alkaline Phosphatase (ALP) in serum was conducted using a colorimetric method with the Randox kit. The bromocresol green (BCG) and bromocresol purple (BCP) method was used to determine the serum albumin levels. Additionally, protein levels were quantified using the Biuret method. The determination of Blood Urea (BU), measured as serum urea, was conducted following the procedures outlined in the Randox Laboratories Kit. The determination of serum creatinine was conducted using the Jaffe method of deproteinization provided by the Human Diagnostic Laboratories Kit.

Haematology Analysis:

In determining Packed Cell Volume (PCV), blood samples collected in EDTA tubes were transferred to anticoagulant-filled capillary tubes, sealed with soft wax, and centrifuged at 13,000 rpm for 5 minutes. The supernatant was discarded, and PCV percentages were assessed using a haematocrit reader. For the Total White Blood Cell Count, 0.38 ml of diluting fluid was added to well-labeled test tubes, followed by 20 µl of anti-coagulated whole blood. After preparing the counting chamber and ensuring proper mixing, the diluted sample was loaded into the chamber grid. Cells in the four large corner squares were counted under a ×10 microscope Objective, with total WBC calculated by multiplying the count by 50. The Differential White Blood Cell Count involved making a blood smear on a labeled glass slide, fixed with ethanol, stained using the Leishman technique, and differentiated with distilled water. The absolute numbers of each WBC type were then calculated by examining the slide under a x100 objective lens.

Antioxidant Analysis:

Estimation of catalase (CAT), superoxide dismutase (SOD), malondialdehyde analyses (MDA), pro-oxidant (PRO), glutathione, (GSH), and glutathione perioxidase (GPx) activities were carried out using the supernatant of the liver sample. The tests were carried out using the respective kits and following manufacturer's specification.

Histological Analysis:

The tissues were preserved through fixation using 10% buffered formalin. This was followed by grossing, and tissue processing involving a series of reagents that replaced the aqueous environment with a hydrophobic one. Dehydration was accomplished using alcohol in increasing grades of 70%, 80%, 95%, and absolute. This was succeeded by clearing in xylene, which facilitated tissue infiltration with paraffin wax. Embedding was executed to facilitate specimen orientation and secure the specimen within a wax block. Sectioning was conducted using a microtome to produce micron-thick slices, which were floated on a water bath and transferred to microscope slides. These slides were dried on a hot plate to remove moisture and ensure the tissue adhered to the slides.

For staining, the wax was removed from the slides with xylene and the slides were rehydrated through descending alcohol grades before being submerged in water. Staining was then carried out with hematoxylin for 10 minutes, followed by a brief differentiation in 1% acid alcohol, rinsing, and counter-staining with 1% eosin for 3 minutes. After a final rinse,

the slides were dehydrated in ascending alcohol grades, cleared in xylene, mounted in DPX, and covered with a slip to finalize the preparation.

Photomicrography:

After the stained slides were prepared, they were examined using a light microscope. A low magnification objective (x10) and a high magnification objective (x40) were used to observe the breast tissues on the slides. Images seen were captured through the eyepiece of the light microscope.

Bone Marrow Assay:

This was done according to Oloyede *et al* (2020a), briefly, the epiphyses of the bones were cut off, and bone marrow from each animal flushed out with 1ml of fetal bovine serum (FBS) into separate 1.5ml Eppendorf tubes. The solution was gently mixed using a micropipette to disperse the cells uniformly. This was then centrifuged at 1000 rpm for 10 minutes. After discarding the supernatant, the pellet was re-suspended in another 1ml of FBS in the microcentrifuge tube. This was thoroughly mixed, using a micropipette, then centrifuged at the same rate.

Once the supernatant was removed, 0.5ml of FBS was added to the pellet and thoroughly mixed. Subsequently, drops of the viscous suspension were placed onto a clean, grease-free slide, and a smear was prepared and allowed to air-dry. The air-dried slides were then fixed in 70% methanol for 3 minutes.

For staining, the slides were first immersed in 0.4% May Grunwald stain I (MG I) for 3 minutes. Then, they were transferred to another jar containing MG II- a 1:1 mixture of MG I and distilled water, and stained for another 3 minutes. Once the staining was complete, the slides were thoroughly rinsed in distilled water and then left to air dry. Next, they were counter-stained with 5% Geimsa stain for 5 minutes, rinsed, and allowed to dry fully. Lastly, the slides were dipped in xylene, and 2-3 drops of DPX mountant were added before placing a coverslip.

The stained slides were examined using a light microscope. A low magnification was used to initially locate areas with a uniform distribution of cells. Once these areas were identified, the magnification was increased to the 100x oil immersion objective to count the cells.

Each slide was thoroughly scanned, ensuring that cells did not overlap and were within the focal plane. Distinct cell types were identified and counted based on their unique morphology and staining characteristics. This counting procedure was conducted across several fields to ensure precision. The average count from multiple fields was noted.

Statistical Analysis:

The statistical analysis was carried out using Microsoft Excel 365. A sample T-test was carried out to assess the statistical significance of differences in various parameters between the control such that the mean of each treatment was compared against the control one at a time. and experimental groups at p levels of significance ($p<0.05$).

RESULTS

Effect of *Ficus platyphylla* Ethanolic Extract on Body Weight of *Mus musculus* induced with NMU:

Table 1, below showed the results of the body weights across 12 weeks.The 200 mg kg^{-1} treatment showed a significant increase in weight from onset of the treatment, till end of the experimental period. 100 mg kg^{-1} treatment had a significant increase in weight up until week 7. The weight peaked and then showed a decline by the end of 12 weeks, ending below the starting weight. Mice treated with Cisplatin, showed fluctuations in weight over the treatment period. However, they ultimately displayed an increase in weight. The NMU only treatment showed a decline in body weight towards the end of the experiment, with the final

weight slightly lower than the starting weight. The control group experienced a gradual and consistent increase in weight throughout the study period.

Table 1: Effects of Ethanolic Extract of *F. platyphylla* on the weight of *Mus musculus* over a 12-week period.

WEEK	200 mg kg^{-1}	100 mg kg^{-1}	Cisplatin	NMU Only	Control
1	26.96 ± 0.61^{b}	25.48 ± 0.74^{a}	25.54 ± 0.56^{b}	25.08 ± 0.61^{a}	22.48 ± 0.48
2	27.10 ± 0.58^{b}	26.06 ± 0.98^{b}	25.76 ± 0.59^{a}	25.32 ± 0.61^{a}	22.78 ± 0.44
3	29.4 ± 1.03^{a}	28.04 ± 1.09^{b}	26.72 ± 0.65^{b}	26.84 ± 0.42^{a}	24.52 ± 0.71
4	28.66 ± 1.14^{b}	26.66 ± 1.16	27.34 ± 0.63^{b}	26.58 ± 0.99^{a}	24.32 ± 0.36
5	29.08 ± 1.14^{a}	27.24 ± 1.05^{a}	28.00 ± 0.75^{a}	28.02 ± 0.79^{b}	24.88 ± .0.44
6	29.48 ± 1.08^{a}	27.78 ± 0.85^{b}	28.54 ± 0.74^{a}	28.44 ± 0.83^{a}	25.54 ± 0.42
7	29.96 ± 1.15^{a}	28.50 ± 1.01^{a}	29.00 ± 0.70^{a}	28.50 ± 0.85^{a}	25.74 ± 0.50
8	28.34 ± 1.40^{a}	27.92 ± 1.05	27.86 ± 0.64	27.46 ± 0.49	26.02 ± 0.39
9	29.86 ± 0.86^{b}	27.68 ± 1.46	28.54 ± 0.77^{a}	27.98 ± 0.50^{a}	26.14 ± 0.35
10	29.98 ± 0.94^{a}	23.06 ± 5.84	28.62 ± 0.79^{a}	22.68 ± 5.69	26.32 ± 0.34
11	30.00 ± 1.05^{a}	23.66 ± 5.96	27.96 ± 0.84	21.86 ± 5.76	26.66 ± 0.38
12	31.60 ± 0.38^{c}	23.22 ± 5.89	28.78 ± 0.79^{a}	22.58 ± 5.67	26.52 ± 0.37

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

Effect of *Ficus platyphylla* Ethanolic Extract on the Haematologic Parameters of *Mus musculus* induced with NMU:

Table 2, showed the haematologic parameters observed in the wistar rats treated with NMU. Significant decreases were observed in the MON $\times 10^{9}$/L and MONO% values for the 100mg kg^{-1} and cisplatin treatments compared to control. Cisplatin treatment additionally showed significantly elevated IG levels. Other comparisons showed no statistical significance.

Effect of *Ficus platyphylla* Ethanolic Extract on the Antioxidant properties in the Kidney of *Mus musculus* induced with NMU:

Table 3 and Figer 1, indicated the various antioxidants observed in the kidney. The 200 mg kg^{-1} treatment showed a significant increase in PRO while there was no was no statistical significance in the CAT, SOD, MDA, GSH, GPx values. The 100 mg kg^{-1} treatment showed a significant decrease in the PRO, CAT, SOD and GSH values. In the Cisplatin treatment, there was a significant decrease in the PRO and GPx values, while no statistical significance was observed for CAT, SOD, MDA, and GSH values.

Table 2: Effects of Ethanolic Extract of *F. platyphylla* on the Haematologic Parameters of *Mus musculus* induced with NMU.

Parameters	200mg kg^{-1}	100mg kg^{-1}	Cisplatin	NMU Only	Control
WBC $\times 10^9$/L	3.50 ± 0.29	3.36 ± 0.66	3.63 ± 0.52	4.63 ± 0.29	3.79 ± 0.63
RBC $\times 10^{12}$/L	9.39 ± 0.44	8.96 ± 0.15	7.89 ± 0.23	9.28 ± 0.23	191.45 ± 182.89
HGB g/L	131.20 ± 6.08	129.75 ± 2.32	130.00 ± 12.20	128.50 ± 2.90	124.20 ± 5.83
HCT g/L	0.48 ± 0.02	0.47 ± 0.01	0.42 ± 0.02	0.46 ± 0.01	0.46 ± 0.02
MCV FL	51.42 ± 1.24	52.40 ± 1.31	53.96 ± 0.85	50.08 ± 1.72	52.54 ± 1.54
MCH Pg	14.00 ± 0.19	14.50 ± 0.23	14.96 ± 0.16	13.88 ± 0.49	14.30 ± 0.32
MCHC g/L	272.40 ± 4.53	277.00 ± 9.39	277.40 ± 4.61	277.25 ± 3.66	272.60 ± 6.45
PLT $\times 10^9$/L	543.00 ± 44.46	360.50 ± 53.65	571.40 ± 83.31	687.50 ± 170.67	499.40 ± 89.97
RDW-SD FL	38.80 ± 1.73	43.28 ± 4.16	38.00 ± 1.61	40.00 ± 1.55	39.92 ± 2.08
RDW-CV %	25.54 ± 0.43	26.48 ± 0.99	23.00 ± 0.82	25.88 ± 0.69	25.16 ± 0.85
PDW FL	10.36 ± 0.34	9.85 ± 0.06	9.78 ± 0.47	10.53 ± 0.27	9.76 ± 0.34
MPV FL	8.76 ± 0.24	8.40 ± 0.27	8.34 ± 0.22	8.58 ± 0.16	8.36 ± 0.19
P-LCR %	12.68 ± 0.41	12.70 ± 0.84	12.64 ± 1.13	12.25 ± 0.43	12.58 ± 0.96
PCT %	0.37 ± 0.05	0.31 ± 0.03	0.43 ± 0.06	0.34 ± 0.01	0.40 ± 0.05
NEU $\times 10^9$/L	0.71 ± 0.06	0.71 ± 0.11	0.95 ± 0.25	0.73 ± 0.09	0.88 ± 0.10
LYMP $\times 10^9$/L	1.80 ± 0.18	2.02 ± 0.39	2.03 ± 0.24	2.89 ± 0.24	2.02 ± 0.41
MON $\times 10^9$/L	0.21 ± 0.07	0.16 ± 0.08[a]	0.10 ± 0.04[b]	0.24 ± 0.04	0.27 ± 0.05
EOS $\times 10^9$/L	0.06 ± 0.03	0.04 ± 0.03	0.02 ± 0.00	0.02 ± 0.00	0.08 ± 0.04
BAS $\times 10^9$/L	0.71 ± 0.13	0.44± 0.12	0.53 ± 0.08	0.75 ± 0.11	0.55 ± 0.13
NEUT %	20.48 ± 1.25	22.10 ± 2.34	24.46 ± 3.27	16.23 ± 2.71	24.20 ± 2.59
LYMPH %	51.64 ± 4.44	60.00 ± 2.58	56.98 ± 2.32	62.28 ± 1.45	52.30 ± 4.58
MONO %	6.20 ± 2.28	4.08 ± 1.26[a]	2.90 ± 0.92[a]	5.15 ± 0.69	7.12 ± 0.98
EO %	1.62 ± 0.71	1.15 ± 0.68	0.50 ± 0.08	0.33 ± 0.08	1.72 ± 0.80
BAS %	20.06 ± 2.54	12.68 ± 1.38	15.16 ± 2.02	16.03 ± 1.643	14.66 ± 2.54
IG $\times 10^9$/L	0.03 ± 0.01	0.08 ± 0.03	0.06 ± 0.03	0.05 ± 0.02	0.03 ± 0.029
IG %	0.78 ± 0.29	2.55 ± 0.92	1.54 ± 0.80[a]	1.10 ± 0.46	0.72 ± 0.39

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

Table 3: Antioxidant properties observed in the Kidney of *Mus musculus* treated with *Ficus platyphylla* Ethanolic Extract.

Treatment	PRO g/L	CAT min/mg	SOD min/mg	MDA nmol/ml	GSH µmol/ml	GPx µmol/ml
200mg kg^{-1}	6.71 ± 0.29[a]	3.21 ± 0.26	1613.68 ± 221.45	2.52 ± 0.32	1.24 ± 0.03	232.82 ± 5.67
100mg kg^{-1}	7.21± 0.37[a]	2.61 ± 0.10[c]	1377.78 ± 77.01[c]	2.13 ± 0.05	0.99 ± 0.03[c]	232.65 ± 5.10
Cisplatin	5.90± 3.07[a]	3.07 ± 0.25	1730.63± 148.16	2.13 ± 0.03	1.13 ± 0.04	225.24 ± 3.19[b]
NMU only	7.22 ± 0.39	4.08 ± 0.59	1718.88± 117.76	2.19 ± 0.03	1.28 ± 0.02[b]	231.24 ± 1.95
Control	8.84 ± 0.50	3.23 ± 0.12	1481.15 ± 62.26	2.16 ± 0.07	1.19 ± 0.03	251.08 ± 3.82

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

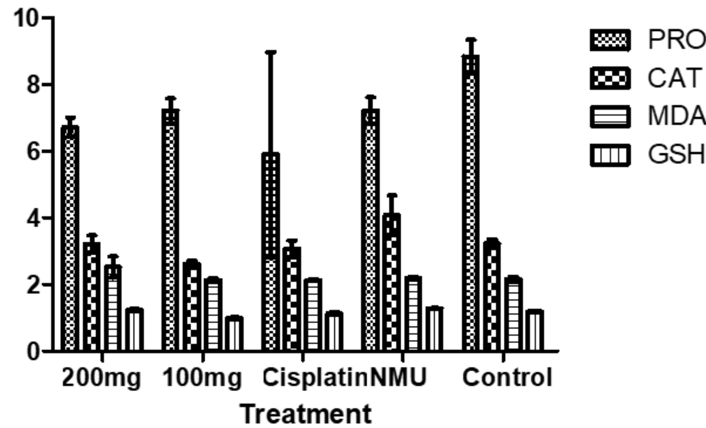


Fig.1: Effect *Ficus platyphylla* Ethanolic Extract on the Antioxidant Properties in the Liver of *Mus musculus* induced with NMU.

There was a significant increase in the MDA values, and a significant decrease in SOD values in the 200mg kg^{-1} treatment (Table **4**). For the 100mg kg^{-1} treatment, there was a significant increase in the SOD value and a significant decrease in the values for CAT, GSH, and GPx. treatment.

Table 4: Antioxidant Properties observed in the Liver of *Mus musculus* treated with *F. platyphylla* ethanol extract for 12 weeks.

Treatment	PRO g/L	CAT min/mg	SOD min/mg	MDA nmol/ml	GSH µmol/ml	GPx µmol/ml
200mg kg^{-1}	10.04 ± 1.02	2.91±0.39	1048.17 ± 86.48[b]	2.41 ± 0.15[a]	1.31±0.02	325.28 ± 9.52
100mg kg^{-1}	7.83 ± 0.66	1.86±0.19[b]	1348.08 ± 49.96[a]	2.08 ± 0.04	1.16 ± 0.02[b]	277.14 ± 8.44[b]
Cisplatin	9.66 ± 0.56	3.06 ± 0.28	1113.85 ± 64.37	2.65 ± 0.62	1.31 ± 0.01	316.36 ± 17.11
NMU only	8.86 ± 0.36[a]	2.67 ± 0.13[a]	1376.43 ± 40.59	2.95 ± 0.91	1.31 ± 0.03	274.14 ± 3.23[c]
Control	10.35 ± 0.61	3.20 ± 0.09	1263.49 ± 110.03	2.04 ± 0.06	1.34 ± 0.03	338.84 ± 5.48

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

Effect *Ficus platyphylla* Ethanolic Extract on the Antioxidant properties in the Breast of *Mus musculus* induced with NMU:

Table 5, showed the antioxidant properties in the breast tissue of treated mice. There was a significant increase in the GSH and GPx in 200mg kg^{-1} treatment. The 100mg kg^{-1} treatment showed a significant decrease in the CAT, SOD, and GPx, and a significant increase in the GSH compared to control. There was a significant decrease in SOD and a significant increase in GSH in Cisplatin. Significant increase in GSH was observed in NMU.

Table 5: Antioxidant properties observed in the Breast of *Mus musculus* treated with *F. platphylla* ethanol extract for 12 weeks.

Treatment	PRO g/L	CAT min/mg	SOD min/mg	MDA nmol/ml	GSH µmol/ml	GPx µmol/ml
200 mg kg^{-1}	11.01 ± 0.61	3.21 ± 0.52	862.02 ± 47.13	2.05 ± 0.04	0.79 ± 0.02[a]	237.74 ± 7.55[a]
100 mg kg^{-1}	13.29 ± 1.26	1.65 ± 0.23[a]	761.86 ± 53.34[a]	2.06 ± 0.08	1.01 ± 0.16[a]	183.18 ± 6.56[b]
Cisplatin	13.36 ± 0.54	3.58 ± 0.71	813.22 ± 39.32[a]	2.06 ± 0.04	0.96 ± 0.03[c]	211.63 ± 2.00
NMU only	12.23 ± 0.37	1.80 ± 0.11	879.66 ± 30.94	2.10 ± 0.05	1.02 ± 0.02[b]	205.9 ± 2.33
Control	11.87 ± 0.69	2.09 ± 0.07	910.68 ± 30.76	2.16 ± 0.07	0.69 ± 0.04	216.10 ± 3.83

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

Effect of the Ethanolic Extract of *Ficus platyphylla* on biochemical parameters of *Mus musculus* Kidney induced with NMU

The data presented in Table 6, showed the biochemical parameters of the impact of the ethanolic extract derived from *Ficus platyphylla* on *Mus musculus* kidney. Mice treated with 200 mg kg^{-1} showed a significant decrease in serum albumin compared to control. There was also significant decrease in serum albumin of 100 mg kg^{-1} treatment compared to control. However, ALP was significantly higher than control. Cisplatin as well as the NMU groups had significantly higher ALP levels, and significantly lower albumin compared to control.

Table 6: Biochemical parameters observed in the Kidney of *Mus musculus* treated with *F. platphylla* ethanol extract for 12 weeks.

Treatment	AST U/L	ALT U/L	ALP U/L	UREA mmol/ml	CRT mg/dl	ALB mg/dl
200 mg kg^{-1}	183.05 ± 13.36	158.30 ± 21.82	151.11 ± 9.09	1.66 ± 0.16	0.14 ± 0.01	2.16 ± 0.05[a]
100 mg kg^{-1}	111.12 ± 8.39	69.38 ± 6.84	48.23 ± 2.91[a]	1.40 ± 0.18	0.10 ± 0.03	2.38 ± 0.05[a]
Cisplatin	128.54 ± 7.78	89.88 ± 1.80	272.27 ± 17.88[c]	2.95 ± 0.44	0.09 ± 0.02	2.23 ± 0.13[a]
NMU only	118.76 ± 2.76	68.50 ± 8.55	235.13 ± 20.92[b]	1.46 ± 0.15	0.11 ± 0.02	2.30 ± 0.07[a]
Control	151.87 ± 17.07	466.63 ± 34.72	36.76 ± 1.63	2.27 ± 0.44	0.16 ± 0.02	3.13 ± 0.25

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

Effect of the Ethanolic Extract of *Ficus platyphylla* on biochemical parameters of *Mus musculus* Liver induced with NMU:

The 200 mg kg^{-1} treatment demonstrated significant increase in ALP compared to Control. ALP and urea in 100 mg kg^{-1} , and cisplatin groups were significantly higher than Control. The NMU group showed significant increase in AST and ALP compared to Control.

Table 7: Biochemical parameters observed in the liver of *Mus musculus* treated with *F. platphylla* ethanol extract for 12 weeks.

Treatment	AST U/L	ALT U/L	ALP U/L	UREA mmol/ml	CRT mg/dl	ALB mg/dl
200mg kg^{-1}	195.83 ± 15.52	194.65 ± 34.92	65.98 ±4.46[b]	3.80 ± 0.66	0.17 ± 0.03	77.65±74.78
100mg kg^{-1}	158.47 ±8.93	76.95 ± 1.47	66.65 ± 3.92[b]	5.51 ± 0.79[b]	0.19 ± 0.01	2.55 ± 0.13
Cisplatin	133.97 ± 10.68	70.18 ± 20.30	69.25 ± 1.70[c]	4.19 ± 0.31[a]	0.19 ± 0.01	2.73 ± 0.09
NMU only	250.95 ± 12.54[a]	41.70 ± 1.77	62.89 ± 5.49[a]	3.54 ± 0.20	0.14 ± 0.02	2.58 ± 0.17
Control	151.87 ± 17.07	466.63 ± 34.73	36.76 ± 1.63	2.27 ± 0.44	0.16 ± 0.02	3.13 ± 0.25

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

Effect of the Ethanolic Extract of *Ficus platyphylla* on biochemical parameters of *Mus musculus* Breast induced with NMU:

The data presented in Table 8, provided a comprehensive analysis of several biochemical parameters in the breast tissue across distinct experimental groups. There were significant increases in the ALT and creatinine values for 200mg kg^{-1}, however, AST and ALP significantly decreased compared to Control. Mice treated with 100mg kg^{-1} showed significant decrease in AST, ALT, and ALP compared to control, whereas significant increase in creatinine was observed. Cisplatin showed significantly reduced AST and urea compared to Control. For NMU treatment, there was a significant decrease in AST levels when compared to the Control.

Table 8: Biochemical parameters observed in the breast of *Mus musculus* treated with *F. platphylla* ethanol extract for 12 weeks.

Treatment	AST U/L	ALT U/L	ALP U/L	UREA mmol/ml	CRT mg/dl	ALB mg/dl
200 mg kg^{-1}	66.10 ± 7.76[a]	168.02 ± 3.25[b]	12.83 ± 1.52[a]	0.76 ± 0.14	0.16 ± 0.01[a]	2.18 ± 0.11
100 mg kg^{-1}	27.61 ± 3.68[c]	47.44 ± 9.34[a]	27.82 ± 3.81[b]	1.01 ± 0.15	0.13 ± 0.04[a]	2.15 ± 0.07
Cisplatin	37.35 ± 7.59[b]	120.64 ± 18.50	38.64 ± 5.65	0.54 ± 0.08[a]	0.08 ± 0.02	2.20 ± 0.04
NMU only	74.42 ± 6.45[b]	140.42 ± 20.07	39.59 ± 1.76	0.90 ± 0.223	0.11 ± 0.05	2.88 ± 0.35
Control	134.75 ± 10.71	98.20 ± 12.65	45.45 ± 3.65	0.86 ± 0.16	0.08 ± 0.02	2.50 ± 0.28

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

Effect of the Ethanolic Extract of *Ficus platyphylla* on the Breast Histology of *Mus musculus* induced with NMU:

The sections of breast tissue examined across various groups displayed a range of histopathological features (Plate 1a,b,c &d). The 200 mg kg^{-1} treatment revealed atrophic keratinized stratified squamous epithelium overlying loose fibrous collagenous stroma containing skin adnexal structures, with fibrofatty tissue housing several variably sized breast ducts and mild inflammatory infiltration, yet preserving ductal architecture. Additionally, bundles of skeletal muscle, nerve fibers, fibroblasts, and inflammatory cells are noted. Mice treated with 100 mg kg^{-1} of the extract exhibit similar epithelium over fibrous tissue with marked inflammatory infiltration leading to destruction of ductal lining epithelium, consistent with mastitis, alongside infiltrated fibrofatty connective tissue, vascular channels, and nerve bundles. Cisplatin treatment shows no identifiable breast ducts within the fibrofatty tissue but includes vascular channels, fibrocytes, and inflammatory cells. In NMU only treatment, atrophic breast ducts within fibrofatty tissue are heavily infiltrated by inflammatory cells that disrupt their architecture and extend into the surrounding stroma, indicative of mastitis. The Control group features atrophic epithelium over loose collagenous tissue with skin adnexal structures, dilated and unremarkable breast ducts free from inflammatory infiltration, alongside blood vessels, a few inflammatory cells, and spindle cells.

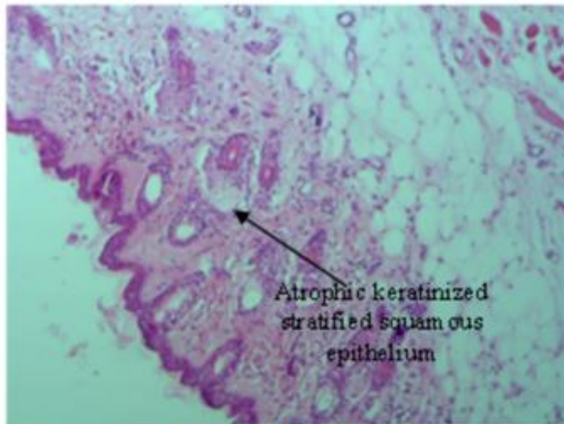


Plate 1A: (x40) 200mg kg^{-1}

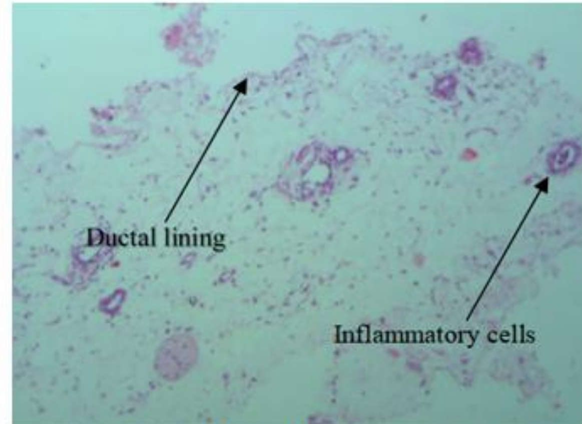


Plate 1B: (x40) 100mg kg^{-1}

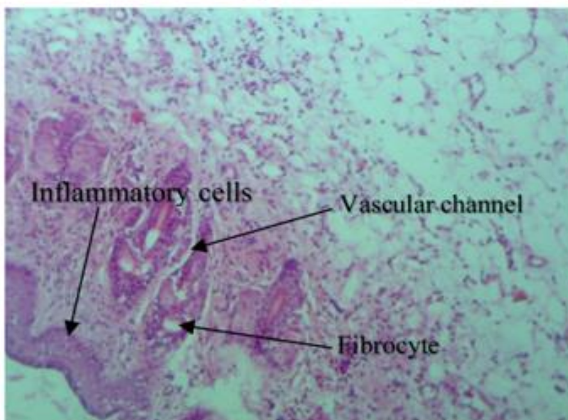


Plate 1C: (x40) Cisplatin

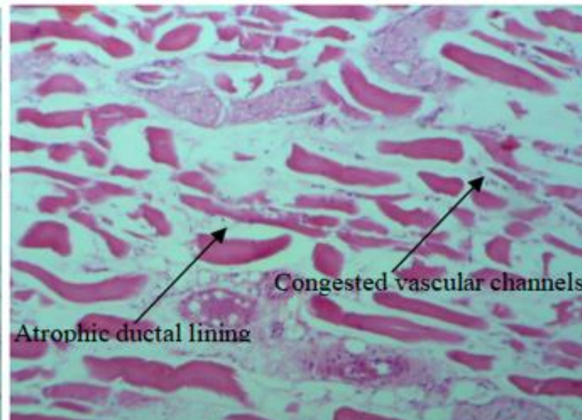


Plate 1D: (x40) NMU Only

Effect of the Ethanolic Extract of *Ficus platyphylla* on the Liver Histology of *Mus musculus* induced with NMU:

In the histological examination of different liver sections, various pathological changes were observed across five groups (Plate 2a, b, c &d). The liver section of mice treated with 200 mg kg^{-1} of the extract highlighted hepatic cells radiating from constricted and congested central veins, with sinusoidal spaces showing endothelial lining, lymphocytes, and yellow pigment, alongside congested interlobular vessels and destruction of portal bile ducts by infiltrating inflammatory cells. The 100 mg kg^{-1} treatment displayed increased lymphocyte aggregates in loose and tight clusters, destruction of bile ducts and vascular channels, expanded sinusoid spaces filled with lymphocytes and brown pigment, and generalized vascular congestion. Liver section for the cisplatin treatment revealed distorted and congested central veins with inflammatory cells aggregating, largely unremarkable hepatic cells except for areas with lymphocyte-induced architectural distortion, and periductal inflammatory infiltrates distorting the epithelial lining. The NMU group showed severe destruction of hepatic cells and sinusoidal spaces expanded with lymphocytic infiltrates, alongside the destruction and infiltration of portal veins and bile ducts by inflammatory cells. In contrast, the Control presented largely unremarkable hepatic structures with only mild issues like dilated and congested interlobular vessels with sparse inflammatory cells, indicating minimal pathological changes.

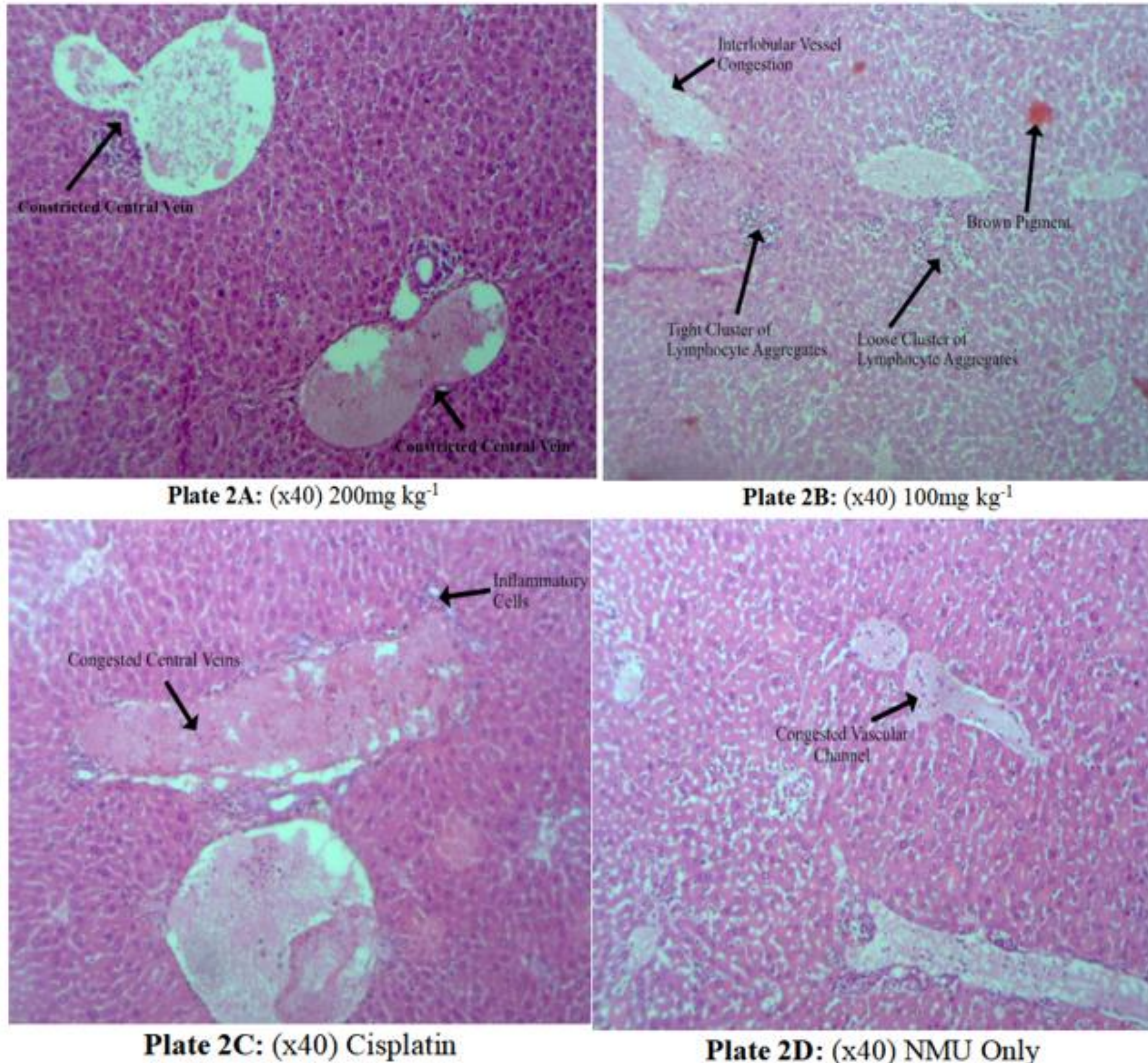


Plate 2A: (x40) 200mg kg^{-1} **Plate 2B:** (x40) 100mg kg^{-1}

Plate 2C: (x40) Cisplatin **Plate 2D:** (x40) NMU Only

Effect of the Ethanolic Extract of *Ficus platyphylla* on the Heart Histology of *Mus musculus* induced with NMU:

Sections of heart tissue from various groups showed a range of pathological features. (Plate 3a, b, c &d). The 200 mg kg^{-1} exhibited both transverse and longitudinal sections of muscular bundles with decreased nuclei to cytoplasmic ratio and interspacing, large vessels with walls infiltrated and distorted by inflammatory cells and congested with red blood cells, mild degeneration and distortion of muscular fibers and peripheral infiltration by lymphocytes in fibrofatty connective tissue, indicating systemic injury. 100 mg kg^{-1} showed muscular bundles with congestion and mild inflammatory infiltration in the vascular walls, intermuscular spacing, muscular degeneration, and vascular congestion, suggestive of mild systemic injury. Cisplatin group showed congested vascular channels, slit-like intermuscular spaces, vacuolation, muscular degeneration, and severe infiltration of fibrofatty tissue by inflammatory cells, indicating systemic injury. The NMUgroup displayed extravasation of red blood cells, mild inflammatory cell presence, fragmentation, and vascular wall thickening with congested lumens, consistent with mild cardiac injury. Finally, the Control group revealed muscular bundles with interspacing, unremarkable nuclei, and congested vascular channels, with large vessels showing empty lumens.

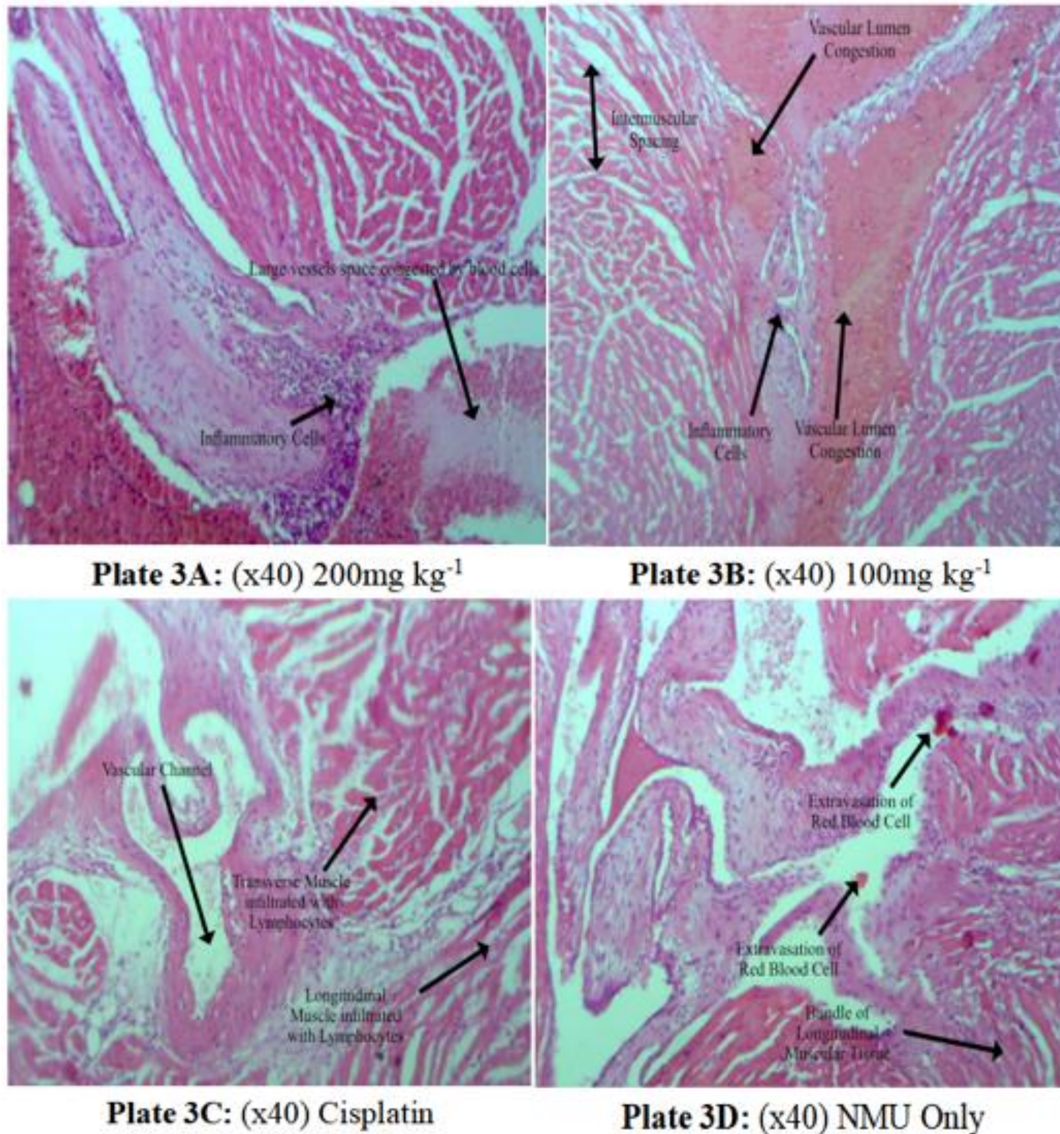


Plate 3A: (x40) 200mg kg^{-1} **Plate 3B:** (x40) 100mg kg^{-1}

Plate 3C: (x40) Cisplatin **Plate 3D:** (x40) NMU Only

Effect of the Ethanolic Extract of *Ficus platyphylla* on the Kidney Histology of *Mus musculus* induced with NMU:

The kidney sections from different groups showed a range of pathological changes (Plate 4a, b, c &d).. 200 mg kg^{-1} treatment features variable-sized glomeruli with congested supraglomeruli spaces filled with loose eosinophilic material, degenerating glomeruli, and congested vessels in both the cortex and medulla, alongside focal areas of hemorrhage, thin connective tissue stroma cell activation, and renal tubules lumen congestion. 100 mg kg^{-1} treatment group displayed distorted renal architecture, supraglomeruli space expansion, increased red blood cells within glomeruli, renal tubule destruction, inflammatory cell infiltrates around the renal corpuscle and vascular channels, interstitial connective tissue reaction, and capillary congestion predominantly in the medulla. The Cisplatin group presented increased inflammatory cells, particularly around vascular channels, renal tubule destruction, glomeruli degeneration, congestion with red blood cells, lymphocyte aggregates, moderate inflammatory infiltration in the cortex distorting the architecture, dilated renal tubules in the medulla, and vascular muscular wall thickening with red cells extravasation. The NMU group exhibited a distorted cortex with increased lymphoid aggregates, glomeruli degeneration, distorted proximal and distal convoluted tubules, peripheral inflammatory infiltrates, muscular wall infiltration by inflammatory cells, haemorrhage, and a reactive stroma around dilated renal tubules in the medulla. In contrast, the Control group showed

unremarkable renal architecture with normal-appearing cortex and medulla, renal corpuscles, tubules, and slit-like spaces in the medulla, indicating preserved renal structure.

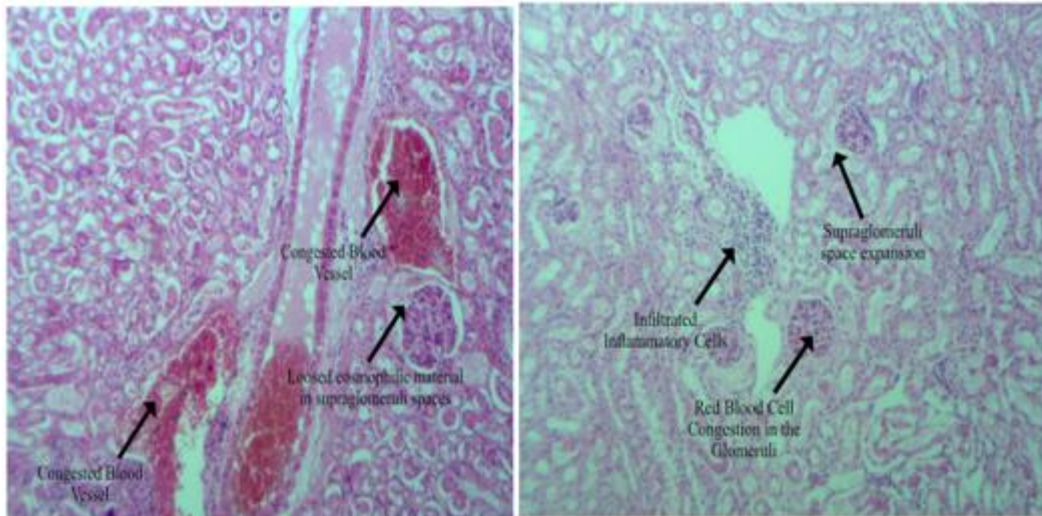


Plate 4A: (x40) 200mg kg^{-1} **Plate 4B:** (x40) 100mg kg^{-1}

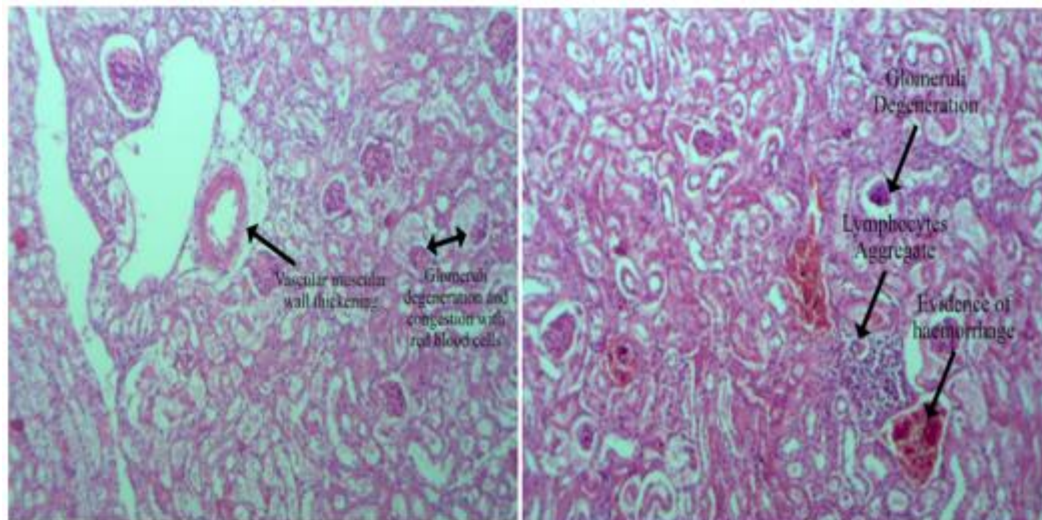


Plate 4C: (x40) Cisplatin **Plate 4D:** (x40) NMU Only

Effect of the Ethanolic Extract of *Ficus platyphylla* on the Kidney Histology of *Mus musculus* induced with NMU

The lung sections across all groups exhibited a range of pathological changes indicative of inflammation and tissue remodelling (Plate 5a, b, c &d). The 200 mg kg^{-1} treatment showed congestion in the alveoli sacs with red blood cells and inflammatory cells, reactive bronchioles epithelium with condensed chromatin, nucleoli, and vascular muscular wall thickening invaded by inflammatory cells within a loose connective tissue stroma, along with macrophages, congested capillaries, reactive alveoli lining, and brown pigment. 100 mg kg^{-1} revealed distorted bronchioles and vascular channels infiltrated by inflammatory cells, with constricted, dilated, and distorted alveoli sacs amidst congested capillaries and heart failure cells within the interstitial stroma. Cisplatin exhibited constricted alveoli sacs with alveoli wall destruction, increased tissue haemorrhage, capillaries congestion, bronchioles epithelium and architecture destruction by inflammatory cells, vascular channel dilation and congestion with red blood cells and eosinophilic material, increased lymphoid aggregates, macrophages, and dark pigment. Sections from mice administered NMU only highlight dilated and congested vascular channels, distorted muscular walls, constricted and

compressed alveoli sacs, bronchioles dilation and constriction with inflammatory infiltration, and capillaries congestion with reactive cells within vacuolated respiratory tissue. Control showed bronchioles, alveoli sacs, and congested vascular channels slightly infiltrated by lymphocytes, along with capillaries, red blood cells, and alveoli sacs lining epithelium in the interstitial connective tissue.

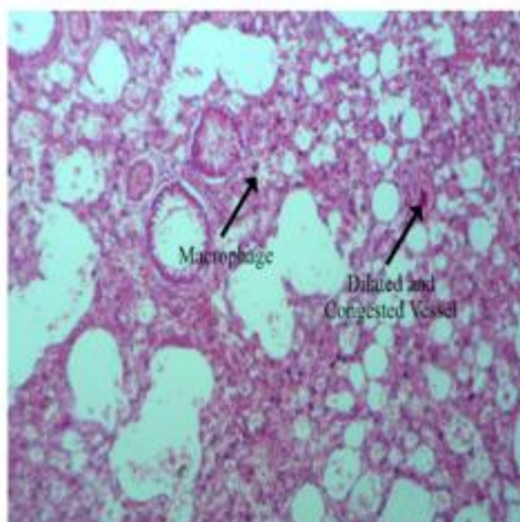


Plate 5A: (x40) 200mg kg^{-1}

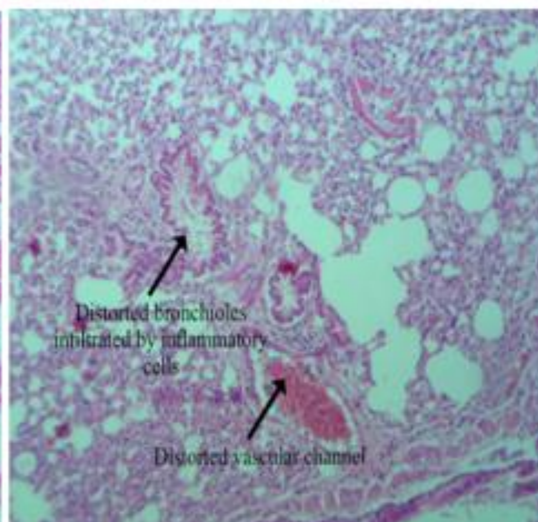


Plate 5B: (x40) 100mg kg^{-1}

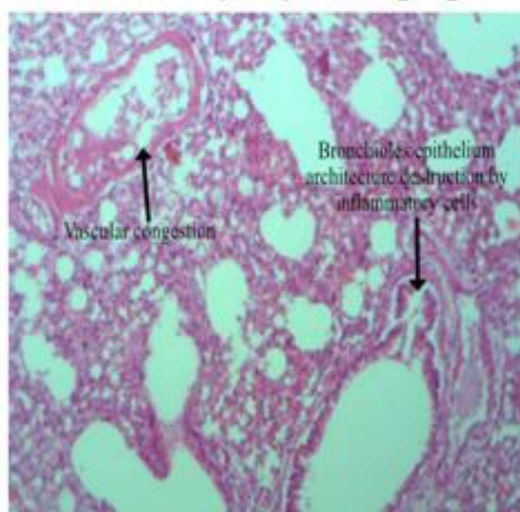


Plate 5C: (x40) Cisplatin

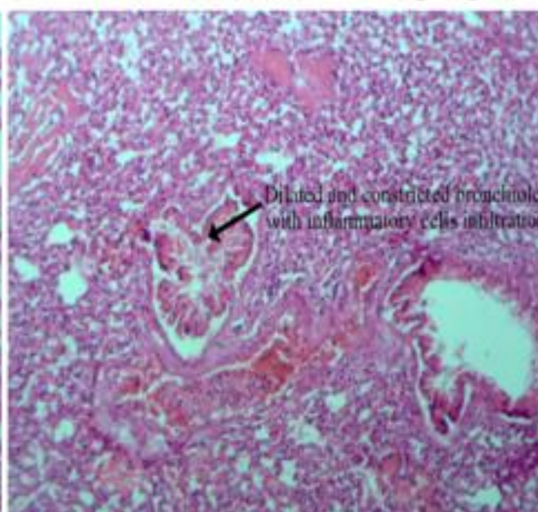


Plate 5D: (x40) NMU Only

Effect of *Ficus platyphylla* Ethanolic Extract on the Bone Marrow of *Mus musculus* induced with NMU

Table 9, below shows the findings observed on Polychromatic Erythrocytes of mice treated with *F. platyphylla* ethanol extract. Although there was no statistical significance, The 200 mg kg^{-1} and 100 mg kg^{-1} treatment demonstrated a reduction in most types of abnormal nuclei when compared to Group administered NMU only.

Between the 200 mg kg^{-1} and 100 mg kg^{-1} treatment, there was a potential dose-dependent increase in the normal (NM) counts with the higher dosage of the extract.

Table 9: Polychromatic Erythrocytes (PCE) observed in the bone marrow of mice treated with *F. platyphylla* Ethanol Extract for 12 weeks

Treatment	NM	PM	SM	KN	BLN	BN	MN	VC
200 mg kg^{-1}	202.00 ± 76.33	27.25 ± 3.32	5.00 ± 3.69	5.50 ± 2.50	4.50 ± 2.59	2.50 ± 1.44	7.25 ± 3.40	5.25 ± 4.02
100 mg kg^{-1}	199.75 ± 31.23	37.75 ± 8.37	7.75 ± 5.17	2.25 ± 1.31	5.00 ± 3.39	5.25 ± 2.28	9.00 ± 3.31	10.00 ± 5.90
Cisplatin	197.00 ± 62.53	84.5 ± 66.66	28.75 ± 23.27	9.25 ± 4.87	1.75 ± 1.18	4.00 ± 1.87	7.00 ± 2.97	17.75 ± 14.17
NMU only	126.25 ± 44.27	96.50 ± 9.04	9.00 ± 4.04	73.75 ± 41.39	109.5 ± 70.38	32.00 ± 29.07	16.00 ± 9.27	40.25 ± 34.21
Control	213.00 ± 65.09	17.50 ± 2.33	5.00 ± 4.06	5.25 ± 4.02	2.00 ± 2.00	2.25 ± 1.43	4.00 ± 4.00	0.00 ± 0.00

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

Table 10, presented the average counts of various types of normochromatic erythrocytes (NCEs) observed in the bone marrow of mice subjected to different treatment regimens. (Plate 6 a, b,c &d). The 200 mg kg^{-1}, Cisplatin, and NMU treatments all showed a significant increase in the count of polymorphic nuclei (PM). However, the PM levels in the 200 mg kg^{-1} and cisplatin treatments were lower compared to those treated with NMU only. 100 mg kg^{-1} had a significant increase in the counts of segmented nuclei (SM), kidney-shaped nuclei (KN), and blebbed nuclei (BLN) relative to the control. However, these counts were lower compared to mice treated with NMU only. Additionally, the Cisplatin treatment experienced a significant increase in SM counts. NMU treatment had a significant increase in binucleated (BN) counts. The 200 mg kg^{-1} treatment had a higher count of normal cells (NM) when compared with the 100 mg kg^{-1} treatment demonstrating a dose-dependent relationship.

Table 10: Normochromatic Erythrocytes (NCE) observed in the bone marrow of mice treated with *F. platyphylla* Ethanol Extract for 12 weeks

Treatment	NM	PM	SM	KN	BLN	BN	MN	VC
200 mg kg^{-1}	214.75 ± 27.94	14.75 ± 5.45[a]	2.50 ± 1.50	0.00 ± 0.00	0.00 ± 0.00	8.50 ± 6.13	0.00 ± 0.00	0.00 ± 0.00
100 mg kg^{-1}	158.00 ± 26.49	5.00 ± 2.54	5.75 ± 2.28[a]	3.50 ± 1.44[a]	4.75 ± 1.60[a]	0.00 ± 0.00	5.75 ± 3.61	3.00 ± 2.04
Cisplatin	155.00 ± 13.22	16.75 ± 4.92[a]	26.00 ± 5.83[a]	5.25 ± 1.18[a]	2.00 ± 1.41	15.0 ± 12.39	7.00 ± 2.86	1.75 ± 1.75
NMU only	149.00 ± 20.77	34.75 ± 11.26[a]	12.50 ± 7.53	12.75 ± 7.11	7.00 ± 4.06	11.00 ± 2.94[a]	19.25 ± 12.58	14.25 ± 6.61
Control	241.25 ± 76.19	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

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

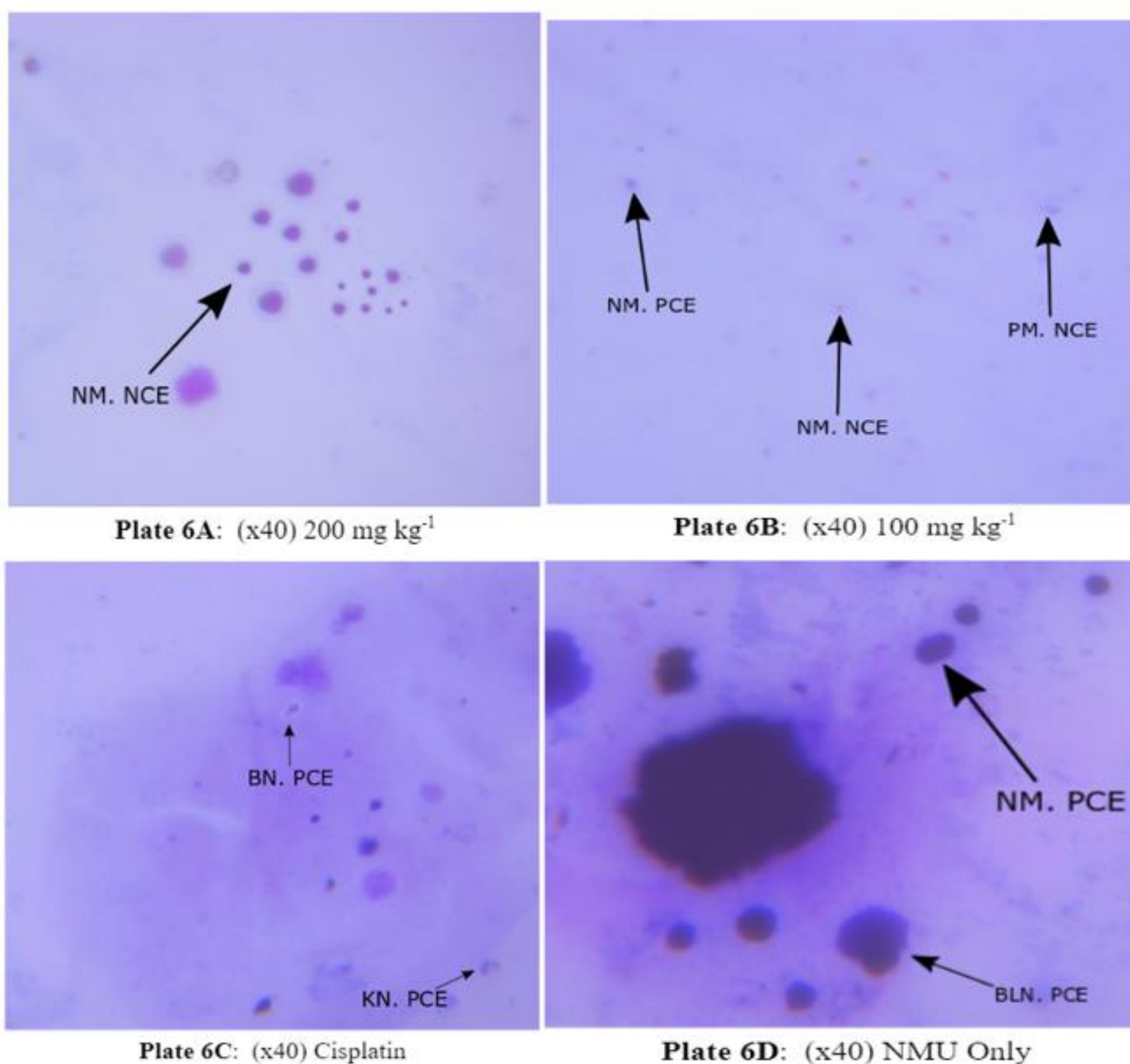


Plate 6A: (x40) 200 mg kg^{-1} **Plate 6B**: (x40) 100 mg kg^{-1}

Plate 6C: (x40) Cisplatin **Plate 6D**: (x40) NMU Only

A; NM.NCE- Normal Normochromatic Erythrocytes (mature red blood cells without RNA). B; PM.NCE- Polymorphic Normochromatic Erythrocytes (mature red blood cells exhibiting varied shapes). C; BN.PCE- Binucleated Polychromatic Erythrocytes (immature red blood cells characterized by having two nuclei), KN.PCE- Kidney Shaped Polychromatic Erythrocytes (immature red blood cells with a distinctive kidney-like shape). D; BLN.PCE- Blebbed, Lobed or Notched Polychromatic Erythrocytes (immature red blood cells displaying irregular membrane protrusions). E; NM.PCE- Normal Polychromatic Erythrocytes (immature red blood cells possessing RNA).

DISCUSSION

In assessing the ameliorative effects of a medicinal herb on the toxicity induced by a specific cytotoxic substance, body weight serves as a critical measure (Ferreira *et al.,* 2014). Variations in body weight can provide tangible evidence of the extract's effectiveness in counteracting the toxic impacts (Wanjiru *et al.,* 2022). The significant increased weight in 200 mg kg^{-1} suggest that the extract counteracts the toxicity of NMU, supporting overall better health and thus contributing to weight gain (Wanjiru *et al.,* 2022). Conversely, in 100 mg kg^{-1} treatment, the initial significant increase in weight followed by a plateau and subsequent decline suggest that the lower dose may only be partially protective against the effects of NMU or that its protective effects wear off over time (Wanjiru *et al.,* 2022).

Additionally, the study data suggested that the protective effects of *F. platyphylla* extract may be dose-dependent as the higher dose provided a sustained protective effect throughout the study period, while the lower dose was only effective up to a certain point (Ndatsu *et al.,* 2020).

Assessing the safety and toxicity of plant extracts requires evaluating the activities of marker enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) (Oloyede *et al.,* 2020a). These were assessed in this study and are vital markers for identifying and evaluating cellular damage, particularly in the liver (Leser *et al.,* 2023). Although they can also be found in muscle tissue, red blood cells, and heart cells, they are mainly concentrated in the liver (Jalili *et al.*, 2022). Elevated levels of these enzymes typically indicate cellular damage or stress (Zhang *et al.,* 2021). The decreased level of AST in the breast tissue for the 200 mg kg^{-1} and 100 mg kg^{-1} treatments suggest that the extract potentially offer protective effects against NMU-induced cytotoxicity (Zhang *et al.,* 2021).

Furthermore, ALT is a key marker of liver inflammation, hence, the lower ALT levels for mice treated with 100mg kg^{-1} may imply that the treatment at that dosage improves liver health (Oloyede *et al.,* 2020a). However, the higher levels in the 200 mg kg^{-1} treatment could indicate a dosage-dependent response where higher concentrations of the extract might induce stress or toxicity, counteracting its beneficial effects at lower doses (Ishaq *et al.,* 2021). ALP is widely utilized in evaluating the integrity of the plasma membrane in liver cells (Oloyede *et al.,* 2020a). Lower ALP levels in the breast tissue of mice treated with 200mg kg^{-1} and 100mg kg^{-1} might reflect a protective stabilization effect of the extract on cellular membranes (Oloyede *et al.,* 2020a). However, increased liver ALP could suggest hepatic stress or increased bile flow, potentially as a response to the treatments (Gholamhosseini *et al.,* 2021).

Serum creatinine, an endogenous product of creatine phosphate metabolism in skeletal muscles, is one of the most widely used biomarkers for assessing kidney function primarily because it is filtered by the glomeruli (Mach *et al.,* 2022). The lack of significant difference in serum creatinine levels in the 200 mg kg^{-1} and 100 mg kg^{-1} treatments may indicate that the treatment had minimal adverse effects on the kidney (Oloyede *et al.,* 2020a). However, elevated creatinine levels in breast tissue across the 200mg kg^{-1} and 100mg kg^{-1} treatments could indicate increased metabolic activity or cellular turnover that might reflect adaptive or stress responses at the cellular level possibly due to the treatment (Kazak and Cohen, 2020).

Moreover, the decreased albumin levels in the kidneys for the 200 mg kg^{-1} and 100 mg kg^{-1} treatments may suggest improved kidney function indicating that the treatment enhances the kidneys' ability to retain albumin, thereby improving their filtration efficiency (Schmidt *et al.,* 2023).

Analyzing haematologic and biochemical parameters is essential for diagnosing the root causes of diseases and evaluating the impact of toxic substances on biological systems (Oloyede *et al.,* 2020b). Changes in these parameters can indicate alterations in cellular integrity, membrane permeability, and metabolic activity (Oloyede *et al.,* 2020b). Additionally, these analyses are crucial for assessing the efficacy of substances with potential remediating effects on toxicity (Kumar and Banerjee, 2016). The decreased monocytes in the 100mg kg^{-1} treatment suggest a modulation of the immune response, which could be indicative of the extract's potential to manage inflammation and other immune-related aspects of NMU toxicity (Kwiecień *et al.,* 2021). *Ficus platyphylla* contains saponins, which may provide a defense against a wide range of inflammatory diseases (Mujeeb *et al.,* 2014). Thus, its anti-inflammatory effects could be attributed to these compounds (Mujeeb *et al.,* 2014).

Antioxidants are compounds that protect cells by neutralizing free radicals and

reactive oxygen species (ROS), functioning as hydrogen donors, radical scavengers, and enzyme inhibitors (Zehiroglu and Ozturk, 2019). The increased GSH levels in the breast tissue of the 100mg kg^{-1}, and increased GPx levels for the breast tissue of 200 mg kg^{-1} treatments suggest a potential protective role of the extract due to its antioxidant properties, which might enhance the endogenous antioxidant defense system (Ighodaro and Akinloye, 2018). Insignificant increase in GSH in the kidney further supports this theory (Ighodaro and Akinloye, 2018). GPx is important in inhibiting the lipid peroxidation process, thereby protecting cells from oxidative stress (Ighodaro and Akinloye, 2018). Moreover, GSH, a crucial intracellular antioxidant made up of thiol groups, effectively reduces H_2O_2, hydroperoxides (ROOH), and oxidative stress overall (Elmaidomy *et al.,* 2022). Furthermore, increased SOD levels in the liver tissue for 100 mg kg^{-1} suggests an active antioxidant mechanism and cellular protection in the liver (Younus, 2018). This could be attributed to the antioxidative phytochemicals in the extract, such as flavonoids and tannins, which are known to mitigate oxidative stress and enhance cellular resilience. Flavonoids are widely known for their antioxidant properties, directly scavenging free radicals, neutralizin highly reactive particles before they can damage cells (Mujeeb *et al.,* 2014).

Conversely, the decreased CAT levels in the kidney, breast, and liver of the 100 mg kg^{-1} treatment, alongside decreased GSH levels for the kidney and liver of the same treatment, and decreased SOD levels in the kidney, suggest a rise in the deleterious effects of free radicals (Sureshbabu *et al.,* 2015). These findings might indicate a selective enhancement of certain antioxidant pathways in preference to others (Sies *et al.,* 2022).

The investigation of histological status is crucial for diagnosing the underlying causes of diseases (Sabino and Wiederhold, 2022). Histological changes can arise from a variety of factors including infections, inflammation, exposure to chemicals or toxins, autoimmune responses, radiation, and genetic or congenital conditions (Miller *et al.,* 2017). Histologic parameters offer vital insights into the structural integrity and cellular morphology of tissues, aiding in the assessment of both health and pathological conditions of the body (Sabino and Wiederhold, 2022; Miller *et al.,* 2017). Thus, when evaluating the remedial effects of plants through histological studies, it is essential to analyze the roles and changes of the cellular components, such as tissue structure, cell type, and the presence of pathologic features (Kumar *et al.,* 2023).

The preservation of ductal architecture despite the presence of mild inflammatory infiltration for mice in 200 mg kg^{-1} suggest that at this dosage, the extract might possess properties that help maintain structural integrity in the breast tissue under toxic conditions induced by NMU (Konaré *et al.,* 2020). The presence of atrophic keratinized stratified squamous epithelium and loose fibrous collagenous stroma indicates some degree of tissue response to the NMU challenge, yet the overall preservation of key structures like breast ducts and skin adnexal features points towards a protective effect of the higher extract dosage (Giroux and Rustgi, 2017). Contrastingly, the marked inflammatory infiltration leading to the destruction of the ductal lining epithelium in 100mg kg^{-1} may imply that at lower concentrations, the protective effect of the extract might be less pronounced, potentially allowing NMU-induced damage to prevail over any protective effects offered by the extract (Zhao *et al.,* 2021).

Despite some sinusoidal and vascular congestion and inflammatory infiltration in the 200 mg kg^{-1} treatment, the partial preservation of hepatic structure suggests a potential hepatoprotective effect at higher doses (Yadav *et al.,* 2023). However, some degree of bile duct destruction and lymphocytic infiltration observed in the 200 and 100 mg kg^{-1} treatments might indicate an incomplete protection against NMU-induced hepatic damage (Yadav *et al.,* 2023). In the 200and 100 mg kg^{-1} treatments, the reduced nuclei to cytoplasmic ratio and milder inflammatory responses observed in cardiac tissues, alongside glomerular congestion, tubular destruction in renal tissues, and congested alveoli with reactive bronchioles in

pulmonary tissues can largely be attributed to the carcinogenic effects of NMU pretreatment (Ojedapo *et al.,* 2022). However, the less severe pathological changes noted specifically with the 200 mg kg^{-1} treatment suggest a dose-dependent protective effect of *Ficus platyphylla* (Ndatsu *et al.,* 2020). This indicates that higher doses of the extract may provide more effective mitigation against NMU-induced organ damage across these systems (Ndatsu *et al.,* 2020).

Bone marrow micronucleus (MN) assay is a cytogenetic technique used to evaluate the genotoxic potential of various substances by detecting the formation of micronuclei in bone marrow cells, particularly erythrocyte precursor cells (Sommer *et al.,* 2020). MN, which form from chromosomal fragments not included in the daughter nuclei during cell division, are recognized as biomarkers for chromosomal damage in both young and new erythrocytes (Oloyede *et al.,* 2020a). The observed decrease in micronucleated polychromatic and normochromatic erythrocytes in groups treated with 200 and 100 mg kg^{-1} of the extract compared to those treated only with NMU (N-nitroso-N-methylurea) suggests that the extract may contain phytochemicals that mitigate NMU-induced genotoxicity by preventing or repairing chromosomal damage (Hayashi, 2016). These beneficial components, particularly flavonoids, are known for their protective effects against DNA damage (Alcaraz *et al.,* 2021). Flavonoids can enhance the ability to repair damaged DNA and counteract the harmful effects of oxidative stress, thereby reducing the incidence of mutations that lead to micronuclei formation (Alcaraz *et al.,* 2021). However, this trend, while noticeable, was not statistically significant.

The concurrent development of MN, and multiple nuclear abnormalities such as polymorphic nuclei (PM), segmented nuclei (SM), kidney-shaped nuclei (KN), and blebbed nuclei (BLN) in erythrocytes exposed to a test substance is regarded as a more effective indicator for assessing both cytotoxicity and genotoxicity as this approach provides a comprehensive monitoring method for the effects of various treatments (Oloyede *et al.,* 2020a). The significant increase in the frequency of binucleated erythrocytes observed in mice treated with the NMU group may suggest that the toxin constituents of NMU block cytokinesis in normal dividing cells during the M phase of the cell cycle, leading to cytotoxicity (Bakare *et al.,* 2013). Additionally, the significant increase in the frequency of polymorphic nuclei observed in mice treated with 200 mg kg^{-1} in contrast to the control may suggest a genetic imbalance that has the potential to contribute to genotoxicity (Bakare *et al.,* 2013). However, the decreased levels of polymorphic nuclei in the 200 mg kg^{-1} treatment compared to the NMU group strongly imply that the extract possess the potential for mitigating genotoxic effects, effectively reducing genetic imbalance, and ameliorating cytotoxicity induced by NMU (Bakare *et al.,* 2013). Moreover, the significant increase in blebbed nuclei observed in 100 mg kg^{-1} compared to control is likely linked to aneuploidy, which may have its origins in tubulin dysfunction, leading to protrusions from the nucleus as a result of damage (Bakare *et al.,* 2013). However, its decreased frequency when compared to the NMU treatment suggest an improvement in tubulin function, counteracting the cytotoxic and genotoxic effects of NMU (Bakare *et al.,* 2013). Similarly, observed increased kidney-shaped nuclei in 100 mg kg^{-1} compared to control is highly indicative of mitotic errors, or structural abnormalities in chromosomes, such as translocations or deletions, resulting in irregular nuclear shapes (Canedo *et al.,* 2021). The decrease frequency of counts compared to NMU group suggest that the extract improved these effects, resulting in relatively lesser structural abnormalities (Canedo *et al.,* 2021).

CONCLUSION

The findings from the study suggest that *Ficus platyphylla* extract demonstrates potential protective effects against NMU-induced toxicity, evident in its modulation of body weight, enzyme markers, and histological changes across various organ systems. Notably, higher doses of the extract seem to offer more consistent protective benefits, mitigating both

cytotoxic and genotoxic effects. The study highlights the promise and efficacy of *Ficus platyphylla* as a therapeutic agent.

Declarations:

Ethical Approval: The ethical approval was issued by Mr Adebiyi of College of Medicine University of Lagos with reference no CMUL/HREC/0887/19.

Competing interests: The authors declare there are no conflicts of interest regarding the publication of this article.

Author's Contributions: The authors have equal distributions.

Funding: This research did not receive specific grants from funding agencies in the public, commercial, private, or not-for-profit sectors.

Acknowledgments: We want to express our gratitude to Mr. Adesola in Biological Garden for routine care provided to the animals.

References

Abubakar, A. and Haque, M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. *Journal of Pharmacy and Bioallied Sciences*, 12(1), 1.

Adamu, F. U., Abubakar, B. Y. and Musa, A. O. (2022). Morphological Characteristics of Different Ficus Species Found in Samaru-Zaria, Nigeria. *Dutse Journal of Pure and Applied Sciences*, 7(3a), 176–183.

Adisa, J., Olatunji, G., Akanbi, H., Fabiyi, O. and Zakariyah, R. (2022). Determination of pathophysiological anti-diarrhoeal activity of the aqueous stem bark extract of *Ficus platyphylla*. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 70(1): 51-58.

Alcaraz, M., Olivares, A., Achel, D. G., García-Gamuz, J. A., Castillo, J. and Alcaraz-Saura, M. (2021). Genoprotective effect of some flavonoids against genotoxic damage induced by X-rays in vivo: Relationship between structure and activity. *Antioxidants*, 11(1):94. https://doi.org/10.3390/antiox11010094

Bakare, A., Alabi, O., Gbadebo, A., Ogunsuyi, O. and Alimba, C. (2013). In vivo cytogenotoxicity and oxidative stress induced by electronic waste leachate and contaminated well water. *Challenges*, 4(2):169–187. https://doi.org/10.3390/ challe4020169

Canedo, A., de Jesus, L. W. O., Bailão, E. F. L. C. and Rocha, T. L. (2021). Micronucleus test and nuclear abnormality assay in zebrafish (*Danio rerio*): Past, present, and future trends. *Environmental Pollution*, 290:(118019).https://doi.org/10. 1016/ j.envpol.2021.118019

Chindo, B., Schroder, H. and Becker, A. (2015). Methanol extract of *Ficus platyphylla* ameliorates seizure severity, cognitive deficit, and neuronal cell loss in Pentylenetetrazole-kindled mice. *Phytomedicine*, 22(1): 86-93.

Chindo, B. A., Ya'U, J., Danjuma, N. M., Okhale, S. E., Gamaniel, K. S. and Becker, A. (2014). Behavioral and anticonvulsant effects of the standardized extract of *Ficus platyphylla* stem bark. *Journal of Ethnopharmacology*, 154(2): 351–360.

Elmaidomy, A. H., Mohamed, E. M., Aly, H. F., Younis, E. A., Shams, S. G. E., Altemani, F. H., Alzubaidi, M. A., Almaghrabi, M., Harbi, A. A., Alsenani, F., Sayed, A. M. and Abdelmohsen, U. R. (2022). Anti-inflammatory and antioxidant properties of *Malapterurus electricus* skin fish methanolic extract in arthritic rats: Therapeutic and protective effects. *Marine Drugs*, 20(10):639. https://doi.org/10. 3390/md 20100639

Ferreira, S. A., Guimarães, A. G., Ferrari, F. C., Carneiro, C. M., Paiva, N. C. and Guimarães, D. A. (2014). Assessment of acute toxicity of the ethanolic extract of *Lychnophora*

pinaster (Brazilian arnica). *Revista Brasileira de Farmacognosia: Orgao Oficial Da Sociedade Brasileira de Farmacognosia*, 24(5):553–560. https://doi.org/ 10. 1016/j.bjp.2014.09.005

Gholamhosseini, A., Hosseinzadeh, S., Soltanian, S., Banaee, M., Sureda, A., Rakhshaninejad, M. and Anbazpour, H. (2021). Effect of dietary supplements of *Artemisia dracunculus*extract on the haemato-immunological and biochemical response, and growth performance of the rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Research*, 52(5):2097-2109.

Giroux, V. and Rustgi, A. K. (2017). Metaplasia: tissue injury adaptation and a precursor to the dysplasia–cancer sequence. *Nature Reviews Cancer*,17(10): 594–604.

Hassan, M., Bala, S. Z., Bashir, M., Waziri, P. M., Musa Adam, R., Umar, M. A. and Kini, P. (2022). LC-MS and GC-MS Profiling of Different Fractions of *Ficus platyphylla* Stem Bark Ethanolic Extract. *Journal of Analytical Methods in Chemistry*, 22: 6342.

Hayashi, M. (2016). The micronucleus test—most widely used in vivo genotoxicity test—. Genes and Environment: *The Official Journal of the Japanese Environmental Mutagen Society*, 38(1). https://doi.org/10.1186/s41021-016-0044-x

Ighodaro, O. and Akinloye, O. (2018). First line defence antioxidants- superoxide dismutase (SOD), catalase, (CAT), and glutathione peroxidase (GPx): their fundamental role in the entire antioxidant defence grid. *Alexandria Journal of Medicine*, 54(4): 287-293.

Ishaq, A., Gulzar, H., Hassan, A., Kamran, M., Riaz, M., Parveen, A., Chattha, M. S., Walayat, N., Fatima, S., Afzal, S. and Fahad, S. (2021). Ameliorative mechanisms of turmeric-extracted curcumin on arsenic (As)-induced biochemical alterations, oxidative damage, and impaired organ functions in rats. *Environmental Science and Pollution Research International*, 28(46):66313–66326. https://doi.org/10. 1007/ s11356-021-15695-4

Jalili, V., Poorahmadi, Z., Hasanpour Ardekanizadeh, N., Gholamalizadeh, M., Ajami, M., Houshiarrad, A., Hajipour, A., Shafie, F., Alizadeh, A., Mokhtari, Z., Shafaei, H., Esmaeili, M. and Doaei, S. (2022). The association between obesity with serum levels of liver enzymes, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and gamma-glutamyl transferase in adult women. *Endocrinology, Diabetes and Metabolism*, 5(6). https://doi.org/10.1002/edm2.367

Kazak, L. and Cohen, P. (2020). Creatine metabolism: energy homeostasis, immunity and cancer biology. *Nature Reviews, Endocrinology*, 16(8):421–436. https://doi.org/ 10.1038/s41574-020-0365-5

Konaré, M.A., Diarra, N., Cisse C., Karim, A., Togola, Adounigna KassoguÃ, Rokia Sanogo, and Aboubacar Sidiki Ouattara. (2020). Evaluation of the biological activities of leaf and bark extracts of Ficus platiphylla Delile, a medicinal plant used in Mali. *Journal of Medicinal Plants Research*, 14(3): 118–128.

Kumar, R. and Banerjee, T. K. (2016). Arsenic induced hematological and biochemical responses in nutritionally important catfish *Clarias batrachus* (L.). *Toxicology Reports*, 3:148–152. https://doi.org/10.1016/j.toxrep.2016.01.001

Kumar, G., Virmani, T., Sharma, A. and Pathak, K. (2023). Codelivery of Phytochemicals with Conventional Anticancer Drugs in Form of Nanocarriers. *Pharmaceutics*, 15(3): 889. https://doi.org/10.3390/pharmaceutics15030889

Kwiecień, I., Rutkowska, E., Raniszewska, A., Rzepecki, P. and Domagała-Kulawik, J. (2021). Modulation of the immune response by heterogeneous monocytes and dendritic cells in lung cancer. *World Journal of Clinical Oncology*, 12(11):966–982. https://doi.org/10.5306/wjco.v12.i11.966

Leser, C., Dorffner, G., Marhold, M., Rutter, A., Döger, M., Singer, C., König-Castillo, D. M., Deutschmann, C., Holzer, I., König-Castillo, D. and Gschwantler-Kaulich, D.

(2023). Liver function indicators in patients with breast cancer before and after detection of hepatic metastases-a retrospective study. *PloS one*, 18(3): e0278454. https://doi.org/10.1371/journal.pone.0278454.

Mach, T., Qi, A., Bouganim, N., and Trinh, E. (2022). Targeted cancer therapies causing elevations in serum creatinine through tubular secretion inhibition: A case report and review of the literature. *Canadian Journal of Kidney Health and Disease*, 9:205435812211062. https://doi.org/10.1177/20543581221106246

Miller, M. A. and Zachary, J. F. (2017). Mechanisms and Morphology of Cellular Injury, Adaptation, and Death. *Pathologic Basis of Veterinary Disease*, 9(1): 2–43

Mujeeb, F., Bajpai, P. and Pathak, N. (2014). Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of Aegle marmelos. *BioMed Research International*, 2014:1–11. https://doi.org/10.1155/2014/497606

Ndatsu, S. and Abdullahi, H., (2019). Phytochemical screening and Biochemical evaluation of *Ficus platyphylla* stem bark extract of Nupe land origin in Albino rats. *Lapai Journal of Science and Technology*, 5(1): 177-189.

Ndatsu, Y., Usman, M., Abubakar, H., Mohammed, A.O. and Usman, H.B. (2020). Protective properties of *Ficus platyphylla* extract of Nupe land origin on oxidative stress in acetaminophen-induced albino rats. *Lapai Jornal of Applied and Natural Sciences*, 5(1): 103-113

Ojedapo, G. C., Minari, J. B. and Oloyede, A. M. (2022). Chemo-protective effects of dl-limonene unaided and in-blend with 6-Methyleneandrosta-1, 4-Diene-3, 17-Dione on N-nitroso-N-methylurea and estrogen sulfotransferase breast cancer-induced albino female rat. *Journal of Applied Sciences and Environmental Management*, 26(4):759–768. https://doi.org/10.4314/jasem.v26i4.28

Oloyede, A.M., Ottu, B., Ogunsanwo, K., Bolarinwa, K. and Makinde, K. (2020a). Evaluating the genotoxic and proximate analysis of ethanolic extract of *Lecaniodiscus cupanioides*. *Plant Biotechnology Persa*, 2(2):14–20. https://doi.org/10. 52547/ pbp.2.2.14

Oloyede, A., Ottu, B., Ogunsanwo, A., Sobiye, S., Bolarinwa, K., Aromolaran, C., Ogidi, E., Okafor, E. (2020b). Subchronic Toxicity of the Ethanolic Extract of Lecaniodiscus cupanioides on Albino Wister Mice (*Mus musculus*). *Herbal Medicinal Journal,* 5(4):145-52

Onuh, K., Onuh, K., Ozojiofor, U., Kereakede, E., Hassan, A., Yaro, F. and Ori, O. (2022). analgesic and anti-Inflammatory effect of the ethanolic Extract of the Leaf Of *Ficus platyphylla* on male albino mice. *FUW Trends in Science and Technology Journal*, 7(1): 728-735.

Sabino, R., and Wiederhold, N. (2022). Diagnosis from Tissue: Histology and Identification. *Journal of Fungi*, 8(5): 505

Schmidt, S., Barnas, U., Aigner, C., Wolf, P., Kozakowski, N., Kain, R., Scherer, T., Schmidt, A. and Sunder-Plassmann, G., (2023). Severe nephrotic syndrome and early end-stage diabetic kidney disease in ABCC8-MODY12: A case report. *Frontiers in Genetics,*14:1132772.

Sheidu, A., Zezi, A., Ahmed, A, Chindo, B. and Magaji, G. (2020). Antimicrobial and wound healing properties of the methanol extract of F*icus platyphylla* Del. (Moraceae) stem bark. *Journal of Pharmacy and Bioresources,*17(2): 121-130.

Sies, H., Belousov, V. V., Chandel, N. S., Davies, M. J., Jones, D. P., Mann, G. E., Murphy, M. P., Yamamoto, M. and Winterbourn, C. (2022). Defining roles of specific reactive oxygen species (ROS) in cell biology and physiology. *Nature Reviews. Molecular Cell Biology*, 23(7): 499–515. https://doi.org/10.1038/s41580-022-00456-z

Sommer, S., Buraczewska, I. and Kruszewski, M. (2020). Micronucleus assay: The state of

art, and future directions. *International Journal of Molecular Sciences*, 21(4):1534. https://doi.org/10.3390/ijms21041534

Sutter, J., Becker, A. and Chindo, B. (2019). Effects of a methanol extract of *Ficus platyphylla* stem bark on a two-way active avoidance task and body core temperature. *Behavioural Brain Research*, 367: 215-220.

Wanjiru, J. N., Maitho, T. E., Mbaria, J. M. and Moriasi, G. A. (2022). Subacute toxicity effects of the aqueous shoot extract of *Yushania alpina* (K. schum.) W.C.L in in Sprague Dawley rats: An appraisal of its safety in ethnomedicinal usage. *Journal of Toxicology*, 2022:6283066. https://doi.org/10.1155/2022/6283066

Yadav, R., Dubey, P. K. and Patel, A. K. (2023). Hepatoprotective effect of methanolic leaf extract of *Prosopis Cineraria* on paracetamol induced hepatotoxicity in Swiss albino mice. *International Journal of Pharmaceutical Sciences and Medicine*, 8(3):123–131. https://doi.org/10.47760/ijpsm.2023.v08i03.009

Younus, H. (2018). Therapeutic potentials of superoxide dismutase. *International Journal of Health Sciences*, 12(3): 88-93.

Zehiroglu, C. and Ozturk Sarikaya, S. B. (2019). The importance of antioxidants and place in today's scientific and technological studies. *Journal of Food Science and Technology*, 56(11):4757–4774. https://doi.org/10.1007/s13197-019-03952-x

Zhang, Z., Hu, S., Fan, P., Li, L., Feng, S., Xiao, H. and Zhu, L. (2021). The roles of liver inflammation and the insulin signaling pathway in PM2.5 instillation-induced Insulin Resistance in Wistar rats. *Disease Markers*, 2021:1–11. https://doi.org/10.1155/2021/2821673

Zhao, H., Wu, L., Yan, G., Chen, Y., Zhou, M., Wu, Y. and Li, Y. (2021). Inflammation and tumor progression: signaling pathways and targeted intervention. *Signal Transduction and Targeted Therapy*, 6(1).