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Biosafety Profiling of "Makann", a Bi-herbal Formulation in Histopatology of the Brain and Uterus

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Abstract

The biosafety of *Garcinia kola* and *Carica papaya* aqueous root extract "makann," a bi-herbal formulation, on the brain and uterus of female mice was examined. Plant roots were harvested, washed, shade-dried, and processed into an aqueous formulation extract. The bi-herbal formulation extract was administered orally at a dose of 2 g/kg in the treatment groups, as a single dose for 24 hr and 14 days, with a daily dose of 2 g/kg for 14 days. The investigation measured body weight, temperature, hematological parameters, organ weights (brain and uterus), and histology of the organs using standard experimental techniques. The results revealed no significant changes in body weight and temperature in the treated animals compared to the control group in all treatment groups. Hematological parameters in the 24-hour treated group showed a significant increase in white blood cell count, lymphocytes, red blood cell count, hemoglobin levels, and mean corpuscular hemoglobin concentration compared to the control ($p<0.05$). However, in 24-hr single, 14-day single and 14-day daily doses, platelet count was significantly increased. The organ weights of the brain and utserus showed no significant difference in bi-herbal formulation extract-treated groups compared with the control group ($p<0.05$). Histopathological evaluations of the examined organs did not reveal

any lesions or signs of toxicity in the brain and uterus across all treatment groups compared to the control. In conclusion, the non-adverse changes in the parameters studied suggest that the bi-herbal formulation at 2 g/kg and for a period not more than 2 weeks may exhibit minimal or no toxicity, and its effect on long-term use should be investigated.

Keywords: biosafety, bi-herbal formulation, Garcinia kola, Carica papaya, hematological parameters

INTRODUCTION

Ensuring the safe and proper formulation of medicinal herbs is as important as proving their efficacy. According to the World Health Organization (WHO) 2019 Global Report on Traditional and Complementary Medicine, herbal products are often subjected to rigorous safety evaluations similar to those required for conventional medications, including post-marketing surveillance. Regulatory frameworks for traditional herbal medicines typically encompass contemporary scientific findings on similar market products (Lim *et al.*, 2021; The *et al*., 2022). In accordance with the Good Laboratory Practice (GLP) principles established by the Organization for Economic Co-operation and Development (OECD), regulatory bodies generally require various animal toxicity studies of differing durations before granting approval for an herbal medicine (Teng *et al.*, 2019).

The *Carica papaya* plant is from the Caricaseae family, a native to Central America and southern Mexico, yet it is extensively cultivated across various Asian nations, including the Philippines and Pakistan. This plant is recognized for its wide range of therapeutic uses, as documented in numerous scientific studies (Sobia *et al.*, 2016). Although research has primarily focused on the extract's effectiveness in treating dengue fever, studies also indicate its role as an anti-inflammatory agent, with a selective impact on cytokine production (Sheneni *et al.*, 2018). Furthermore, there is empirical evidence supporting its diverse therapeutic effects, including antibacterial, antimalarial, gastroprotective, hypoglycemic, hypolipidemic, antioxidant, hematological, anti-tumor properties, and wound-healing capabilities (Nariya and Jhala, 2017; Sathyapalan *et al.*, 2020). Various extraction techniques, from aqueous to ethanol-based methods, have been streamlined to enhance its medicinal application across multiple health conditions (Londet and Adoga , 2017).

The plant *Garcinia kola*, commonly referred to as bitter kola, male kola, and false kola, belongs to the Guttifera family. In Nigeria, it has various names among different tribes: Orogbo (Yoruba), Cida goro (Hausa), Aku ilu or Ugugolu (Igbo), Efiari (Efik), and Igoligo (Idoma). This evergreen, non-buttressed tree typically grows in moist forests and reaches a height of about 13-15 meters. Garcinia kola is recognized for its traditional medicinal applications, including the treatment of liver disorders, diarrhea (WHO, 2019; Phromnoi *et al*., 2022; Subramanian *et a*l., 2018), diabetes, bronchitis, and throat infections, as well as its use as an aphrodisiac (Jitareanu *et a*l., 2023; Rahmat and Damon, 2018; Gadhwal et al., 2016). The root is commonly used in traditional remedies for cough and appendicitis.

Toxicological assessments are fundamental to pharmacological research and the development of pharmaceuticals, aiding in the establishment of a safety profile for compounds prior to their clinical use in humans (Rahmat and Damon, 2018). Given this necessity, chronic toxicity studies complement acute evaluations, providing a thorough understanding of the long-term physiological, biochemical, hematological, and pathological effects of an herbal medicine.

MATERIALS AND METHODS

Plant Material and Authentication

Garcinia kola and *Carica papaya* roots were harvested from the Ovia North East Local Government Area of Edo State in November 2021. They were identified and authenticated at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State. They were given voucher numbers: *Garcinia kola* UBH-365 and *Carica papaya* UBH-C505.

Plant Extraction

The roots were washed, cut into small pieces and dried in the shade for two weeks. The dried roots were further dried in a hot air oven at 60 ^{o}C for 6 hr before pulverizing separately into powder using a laboratory milling machine. In the bi-herbal formulation, 50 g of *Garcinia kola* with 50 g of *Carica papaya* roots were combined (100 g) and was macerated in boiled water. They were left at room temperature (30 ^{o}C ± 2 ^{o}C) with frequent shaking for 72 hr. It was filtered using a glass funnel tightly plugged with cotton wool, and

the filtrate was concentrated in a laboratory oven at 60 °C. It was labeled and kept in the refrigerator at 4 °C until use.

Experimental Animals

Thirty albino adult female mice of weight 20 – 30 g were used for this experiment. The animals were maintained at the Animal Unit of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria. Ethical approval (EC/FP/021/20) was obtained from the Ethics Committee on the Use of Animals for Experimental Procedures, Faculty of Pharmacy, University of Benin. The animals were kept in well-ventilated cages and fed with commercial animal pelleted feed, with free access to clean water. All animals were handled carefully according to the Guide for the Care and Use of Laboratory Animals (2011). The animals were weighed on a S-Mettler electronic compact balance model K- 500BH (max=500, d=0.01g), weight was recorded in grams. Anal temperature was taken using C-Tone digital thermometer (Mode: GF502) in degree Celsius (°C).

Acute Toxicity

The modified method of OECD (2001); Bafor and Igbinuwen (2009) were used in a 24-hr single, 14-day single and 14-day daily doses toxicity study of the aqueous extract of the bi-herbal formulation of *Garcinia kola* and *Carica papaya* roots (BH).

Single Dose (24hr) Treatment

The extract group (n=5) were given 2 g/kg BH extract and the control group (DW) (n=5) received 10 ml/kg of distilled water orally using orogastric tube fitted on a 1ml syringe on the first day (D0). They were observed for any behavioral changes or death. After 24 hr (D1) the animals were anaesthetized by chloroform inhalation. The weight and temperature and were taken prior to administration and before anaesthetized.

Single Dose (14 Days) Treatment

The extract group (n-5) received 2 g/kg BH extract and control group (n=5) received 10 ml/kg of distilled water (DW) orally using orogastric and 1ml syringe on D0, they were observed for any abnormal behavior or death. The weight and temperature were taken D0, D7 and D13 before anaesthetized by chloroform inhalation on D14.

Daily Dose (14 Days) Treatment

A daily dose of 2 g/kg BH extract was administered orally to the extract group (n=5) and control (DW) group (n=5) received 10 ml/kg distilled water using an oral gastric tube every day for 14 days. The body weight and temperature were taken daily before administration and on the last day before being anaesthetized by chloroform inhalation. The animals were observed daily for signs of sickness or changes in behavior, or death.

Blood Sample and Vital Organs Collection

Blood (0.4-0.6 ml) was collected through cardiac puncture and from the abdominal aorta into EDTA anticoagulant blood sample bottle. The blood was properly mixed to avoid forming a blood clot. Mythic 18 auto-analyzer, 3 Parts differential (Germany) was used for blood cell count analysis (OECD, 2001).

The brain and uterus were harvested, weighed and immediately placed in Bouin's fluid fixative for histological investigations (Committee, 2011). Tissues were prepared in Leica TP1020 tissue processor and sectioned in Thermo Scientific Microm HM340E microtome, slides were stained with haematoxylin and eosin, mounted in DPX mountant and covered with cover slips. Prepared slides were viewed and photomicrograph taken with an S-Viewer digital camera (14M USB2.0, UHD-14-AGC) mounted on a LABO Microscope AXL (Germany) connected to an *hp* laptop with magnification x4000 (Bradbury, 1977).

Data Analyses

The results obtained were subjected to relevant statistical analyses. Data were presented as percentage of control in mean ± S.E.M (standard error of mean) of frequency (cycles/5 min) and amplitude (g) of uterine contractions. Comparisons were made using one-way repeated measures ANOVA with Dunnett's correction for multiple comparison or student's t-test where appropriate. $P \leq 0.05$ was used to indicate statistical significance. Graph pad prism 7.00 (California, USA) and Microsoft office excel 2013 were used.

RESULTS

Mouse Weight and Temperature

There was no significant increase in the body weight and temperature of 2 g/kg aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) and the control (DW) in each

of the treatment groups, 24 hr, 14 days single dose and 14 days daily doses as shown in Figure 1and Figure 2.

Haematology (24 hr) Treatment

The blood cell count analysis showed significant increase ($P < 0.05$) in white blood cells, lymphocytes, mean corpuscular haemoglobin concentration, red blood cells, haemoglobin and platelets count but significantly decreased in monocytes and granulocytes in 2 g/kg BH extract group compared to control group ($P < 0.05$), shown in Table 1.

Haematology Single Dose (14 Days) Treatment

There was a significant increase in platelet count and a significant decrease in mean corpuscular haemoglobin concentration (MCHC) in BH extract group compared to the control group. There was no significant change in all other blood cell parameters (Table 2).

Haematology Daily Dose (14 Days) Treatment

Granulocytes, mean corpuscular volume and platelet count were significantly increased in 2 g/kg BH extract group compared to the control group. All other parameters showed statistically insignificant changes when compared with control (Table 3).

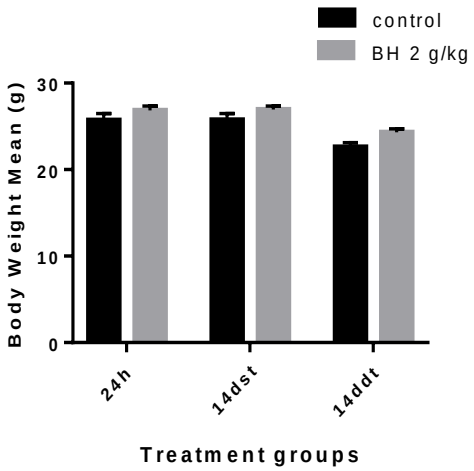

Figure 1: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya*

Key: 24h--- 24 hour group, 14dst--- 14 days single dose group, 14ddt--- 14 days daily dose group. (BH) on the body weight of female mice (n = 5).

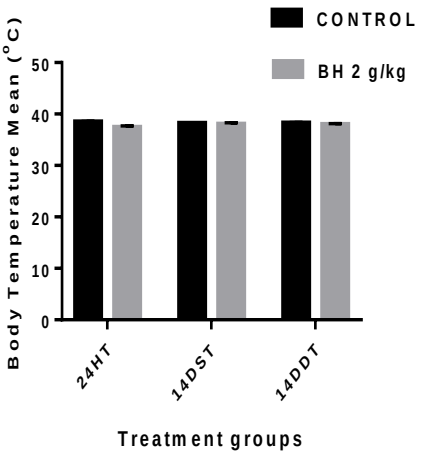

Figure 2: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya*

Key: 24HT--- 24 hour group, 14DST--- 14 days single dose group, 14DDT--- 14 days daily dose group. (BH) extract on the body temperature of female mice (n = 5).

Table 1: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) extract on haematological parameters in 24 hr treatment group

Haematological Parameters	Groups	
	Control	**BH (2 g/kg)**
WBC $x10^3mm^{-3}$	3.38 ± 0.53	5.18 ± 0.39*
LYM $x10^3mm^{-3}$	2.26 ± 0.23	4.62± 0.39*
MO $x\ 10^3mm^{-3}$	0.78 ± 0.06	0.22 ± 0.05#
GR $x\ 10^3mm^{-3}$	1.02 ± 0.24	0.34 ± 0.04#
RBC $x10^3mm^{-3}$	6.68±0.57	8.17±0.16*
HGB g/dl	10.64 ± 1.02	13.96 ± 0.26*
MCV fl	52.12 ± 0.63	52.34 ± 0.39
MCH pg	13.82 ± 1.73	17.02 ± 0.07
MCHC g/dl	30.14 ± 0.43	32.54 ± 0.19*
PLT $x\ 10^3mm^{-3}$	424.44 ± 40.19	747.41 ± 13.32*

WBC = white blood cell; **LYM** = lymphocytes; **MO** = monocytes; **GR** = granulocytes; **RBC** = red blood cell count; **HGB =** haemoglobin concentration; **MCV** = mean corpuscular volume; **MCH** = mean corpuscular haemoglobin; **MCHC** = mean corpuscular haemoglobin concentration; **PLT** = platelet count; * significant increase; # significant decrease relative to the control; $P < 0.05$; n = 5.

Table 2: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) on haematological parameters in 14 days single dose treatment group

Haematological Parameters	Groups	
	Control	**CA (2 g/kg)**
WBC $x10^3mm^{-3}$	3.88±0.53	5.38 ±0.85
LYM $x10^3mm^{-3}$	2.26±0.23	3.38±0.53
MO $x\ 10^3mm^{-3}$	0.78 ±0.06	0.92±0.07
R $x\ 10^3mm^{-3}$	1.02 ±0.24	1.24±0.32
RBC $x10^3mm^{-3}$	6.69±0.58	8.72±1.17
HGB g/dl	10.64±1.02	11.78±0.71
MCV fl	52.12±0.63	50.94±0.89
MCH pg	13.82±1.73	14.08±1.41
MCHC g/dl	30.14 ±0.43	27.82±3.03#
PLT $x\ 10^3mm^{-3}$	424.40±47.19	464.8±58.70*

WBC = white blood cell; **LYM** = lymphocytes; **MO** = monocytes; **GR** = granulocytes; **RBC** = red blood cell count; **HGB =** haemoglobin concentration; **MCV** = mean corpuscular volume; **MCH** = mean corpuscular haemoglobin; **MCHC** = mean corpuscular haemoglobin concentration; **PLT** = platelet count; * significant increase; # significant decrease relative to the control; $P < 0.05$; $n = 5$.

Table 3: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) on haematological parameters in 14 days daily dose treatment group

Haematological Parameters	**Groups**	
	Control	**BH (2 g/kg)**
WBC $x10^3mm^{-3}$	3.06 ± 0.70	4.64±0.28
LYM $x10^3mm^{-3}$	2.58 ±0.68	3.54±0.26
MO $x\ 10^3mm^{-3}$	0.16 ±0.05	0.34±0.10
GR $x\ 10^3mm^{-3}$	0.38 ±0.08	0.80±0.36*
RBC $x10^3mm^{-3}$	8.07±0.51	9.16±0.17
HGB g/dl	14.40±1.00	16.20±0.50
MCV fl	54.72±1.64	54.84±0.35*
MCH pg	17.80±0.16	17.62±0.30
MCHC g/dl	32.58±1.01	32.16±0.57
PLT $x\ 10^3mm^{-3}$	409.8±9.26	856.4±13.80*

WBC = white blood cell; **LYM** = lymphocytes); **MO** = monocytes; **GR** = granulocytes; **RBC** = red blood cell count; **HGB =** haemoglobin concentration; **MCV**

mean corpuscular volume; **MCH** = mean corpuscular haemoglobin; **MCHC** = mean corpuscular haemoglobin concentration; **PLT** = platelet count; * significant increase; # significant decrease relative to the control; $P < 0.05$; $n = 5$.

Relative Vital Organs Weight (24 hr)

There was no significant difference between the relative weight of brain and uterus in aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* **(**BH) and the control groups (Figures 3 and 4).

Relative Vital Organs Weight Single Dose (14 Days)

The relative weight of brain and uterus in the BH extract 2 g/kg and the control groups was not significantly different (Figures 5 and 6).

Relative Vital Organs Weight Daily Dose (14 Days)

The relative weights of brain and uterus in BH extract 2g/kg group were not significantly different from those in the control group (Figures 7 and 8)

Vital Organs histopathology (24 hr)

Photomicrographs revealed normal structural cell architecture. Brain revealed multiform layer and inner pyramidal layer and molecular layer (Plate 1G and 1H); and Uterus reveals normal endometrial glands, normal endometrial lining and normal endometrial stroma compared with the control (Plate 2K and 2L).

Vital Organs histopathology Single Dose (14 Days)

Photomacrograph of brain reveals normal multiform layer, normal granular layer and central sulcus (Plate 3H) compared with the control (Plate 3G) and uterus reveals normal endometrial glands and normal endometrial stroma with mild active vascular congestion (Plate 4L) compared with the control (Plate 4K).

Vital Organs histopathology Daily Dose (14 Days)

Photomicrographs brain revealed molecular layer, pyramidal layer and multiform layer compared to the control (Plates 5G and 5H) and uterus reveals normal endometrial glands, normal endometrial lining and normal endometrial stroma compared to control (Plates 6K and 6L)

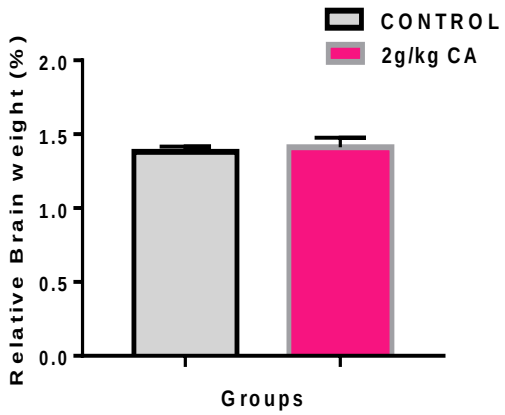

Figure 3: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) on relative brain weight in 24 hr single dose treatment group of female mice (n = 5).

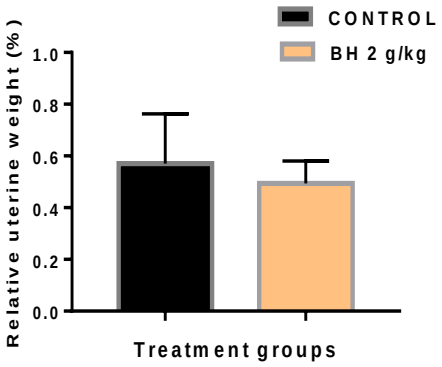

Figure 4: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) on relative uterus weight in 24 hr single dose treatment group of female mice (n = 5).

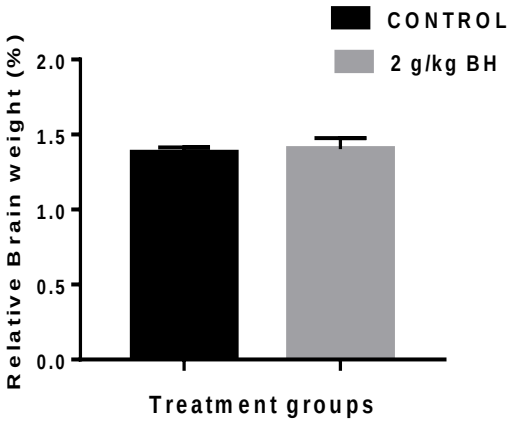

Figure 5: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) on relative brain weight in 14 days single dose treatment group of female mice (n = 5).

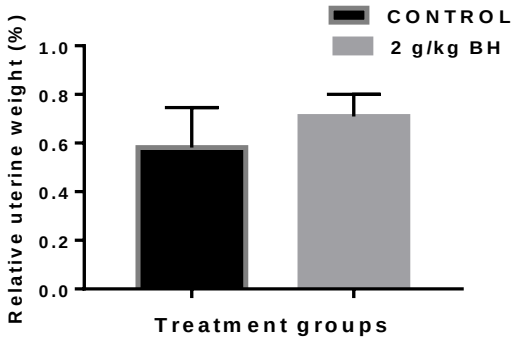

Figure 6: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) on relative uterus weight in 14 days single dose treatment group of female (n = 5).

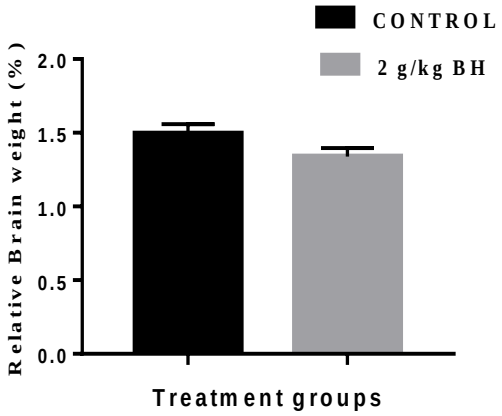

Figure 7: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) on relative brain weight of female mice in 14 days daily dose treatment group (n = 5).

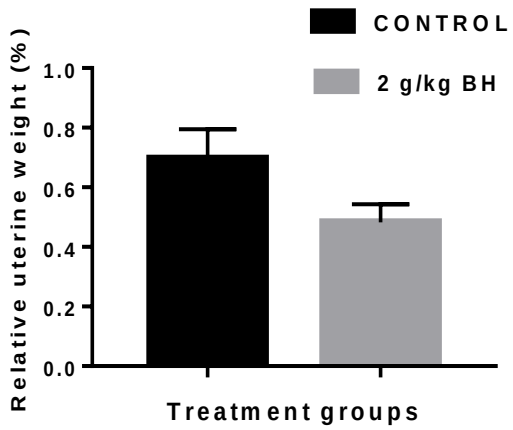

Figure 8: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) on relative uterus weight of female mice in 14 days daily dose treatment group (n = 5).

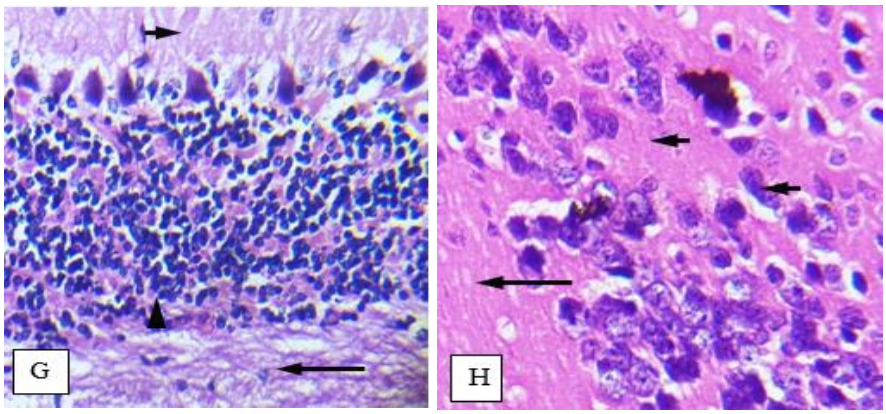

Plate 1: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH on the histopathological structure of the brain in 24 hr treatment group of female mice (H & E stain, X40 objective).

(G) Control Brain reveals multiform layer (long arrow), granular layer (arrowhead) and molecular layer (short arrow).

(H) BH Extract Brain reveals multiform layer (long arrow) and inner pyramidal layer, molecular layer (short arrow)

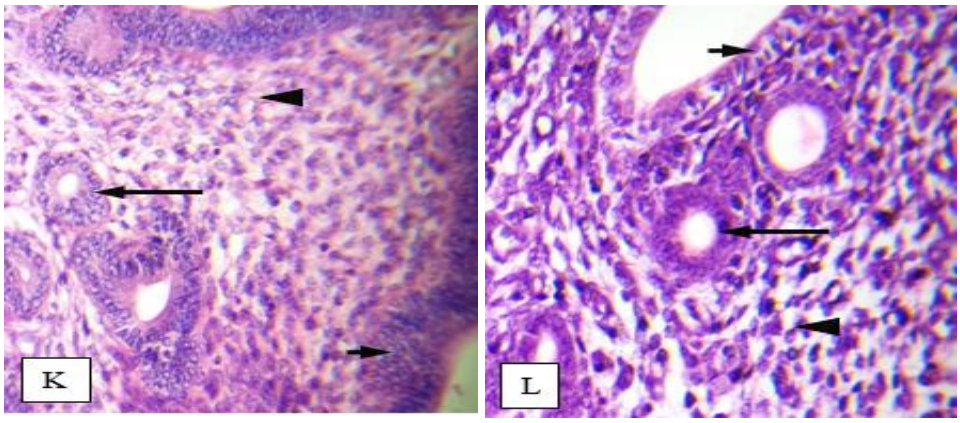

Plate 2: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH on the histopathological structure of the uterus in 24 hr treatment group of female mice (H & E stain, X 40 objective).

(K) Control Uterus revealed small endometrial glands in the proliferative phase of the oestrous cycle (long arrow), proliferating endometrial lining (short arrow) and plump endometrial stroma (arrowhead).

(L) BH Extract Uterus revealed normal endometrial glands (long arrow), normal endometrial lining (short arrow) and normal endometrial stroma (arrowhead).

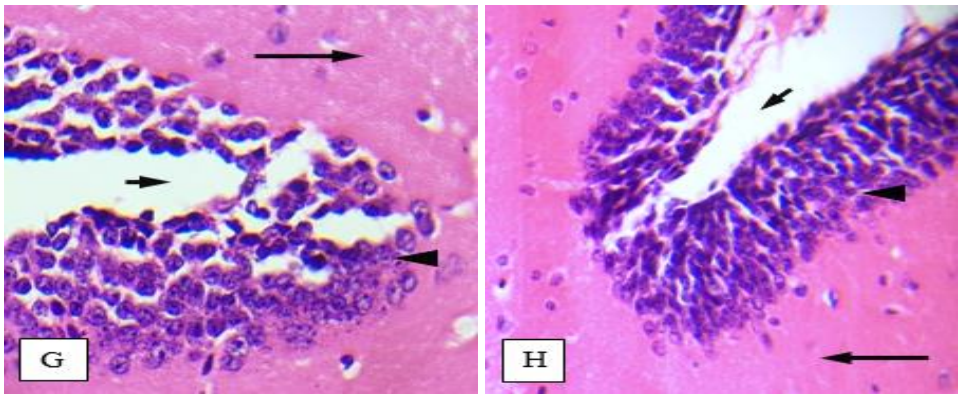

Plate 3: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH on the histopathological structure of the brain in 14 days single dose treatment group of female mice (H & E stain, X40 objective).

(G) Control Brain reveals normal multiform layer (long arrow), normal granular layer (arrow head) and central sulcus (short arrow).

(H) BH Extract Brain reveals normal multiform layer (long arrow), normal granular layer (arrow head) and central sulcus (short arrow).

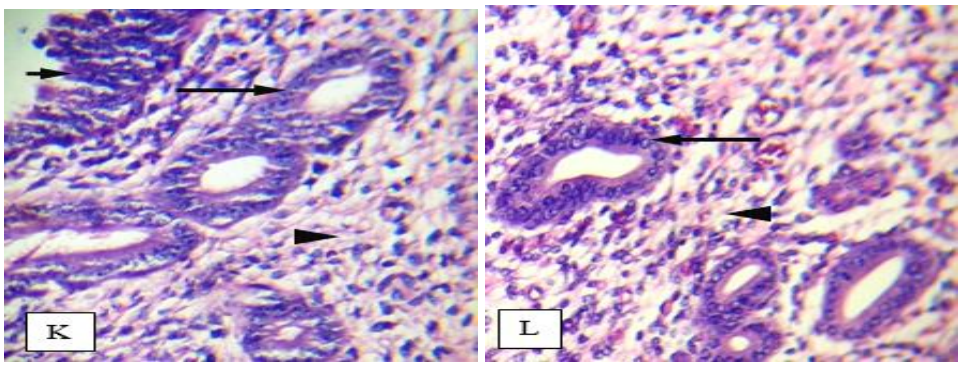

Plate 4: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH on the histopathological structure of the uterus in 14 days single doseTreatment group of female mice (H & E stain, X40 objective).

(K) Control Uterus reveals normal endometrial glands (long arrow), normal endometrial lining (short arrow) and normal endometrial stroma (arrowhead).

(L) BH extract Uterus normal endometrial glands (long arrow) and normal endometrial stroma with mild active vascular congestion (arrowhead).

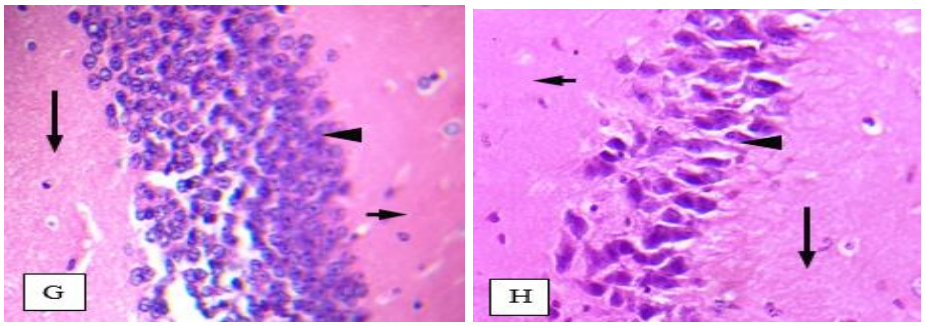

Plate 5: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) on the histopathological structure of the brain in 14 days daily dose treatment group of female mice (H & E stain, X 40 objective).

(G) Control Brain revealed multiform layer (long arrow), granular layer (arrow head) and molecular layer (short arrow).

(H) BH Extract Brain revealed molecular layer (short arrow), pyramidal layer (arrow head) and multiform layer (long arrow).

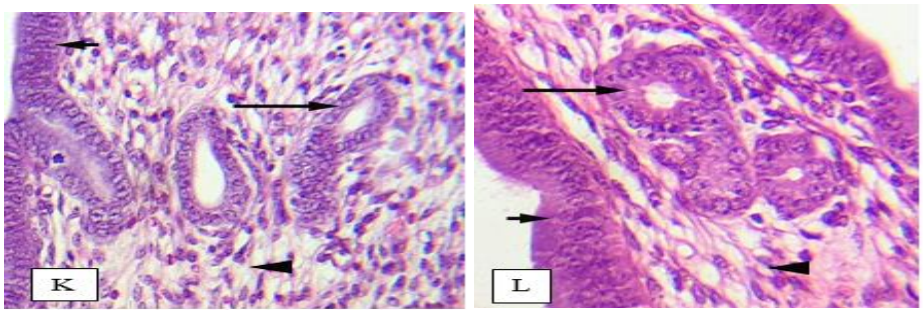

Plate 6: Effect of aqueous bi-herbal root extract of *Garcinia kola* and *Carica papaya* (BH) on the histopathological structure of the uterus in 14 days daily dose treatment group of female mice (H & E stain, X40 objective).

(K) Control Uterus reveals normal endometrial glands (long arrow), normal endometrial lining (short arrow) and normal endometrial stroma (arrowhead)

(L) BH Extract Uterus reveals normal endometrial glands (long arrow), normal endometrial lining (short arrow) and normal endometrial stroma (arrowhead)

DISCUSSION

The evaluation of herbal product toxicity is necessary for establishing safe consumption levels of herbal products in humans, ensuring safe application (Nair and Jacob, 2016). It's a standard procedure to perform preliminary acute and chronic toxicity assessments of herbal products using animal models before commencing clinical trials to evaluate the effectiveness of these products. This initial phase is crucial for determining optimal dosages and the appropriate duration of administration, providing a solid foundation for future human trials (Sireeratawong *et al.*, 2016; Balekundi and Mannur, 2020). The acute oral toxicity test is vital for immediate adverse effects following a single oral dose within 24 hours, as well as over 14 days and daily doses for 14 days. In our study, we administered a dose of 2 mg/kg of the biherbal formulation to evaluate its safety profile. This dosage was chosen based on previous research indicating that a 2 g/kg dose is suitable for screening, a method used to identify significant biological effects in medicinal plants, which had shown no toxic or lethal effects. This aligns with the results of Santana *et al.* (2019) and Hasan *et al.* (2018).

Monitoring changes in general behavior and body weight is crucial in identifying potential toxic products. A weight loss exceeding 10% of baseline in test animals could signal adverse effects and affect survival. In our study, results revealed no significant weight loss in the female mice receiving the biherbal extract compared to the control group, resulting in no significant changes in body weight and no mortality. Furthermore, the extract at 2 g/kg did not produce a difference in the body temperature of treated animals. This observation was supported by the findings of Norahmad *et al.* (2019).

Hematological analyses are vital for detecting potential tissue damage or physiological stress, providing insights into the cellular health of internal organs. Blood serves as a sensitive indicator of physiological and pathological conditions due to its role in distributing nutrients and foreign substances. Toxic agents can adversely affect essential blood components like red and white blood cells, platelets, and hemoglobin. The findings of Abdel-Halim *et al.* (2021); Ostermann and Joannidis (2016) supported these results. In this investigation, we noted a significant increase in the white blood cell count, lymphocyte, red blood cell count, hemoglobin level, platelet, and mean corpuscular hemoglobin concentration at the 24-hour single dose ($p<0.05$). Platelet count was significantly increased in all treatment groups. Variations in organ weight are an indicator for assessing the

impact of drug exposure on organ health. In toxicological studies, comparing organ weights of treated and untreated animals is essential. Our findings corroborate reports from Estella *et al.* (2020) and Diferelanko (2016). In our study, gross pathological examinations revealed no significant abnormalities in the internal organs of the biherbal extract-treated groups, consistent with the findings in the control groups. Following the OECD Test Guideline 420, the results from this study substantiate the non-toxic profile of the biherbal extract. Additionally, detailed histological examinations of the brain and uterus revealed no signs of tissue damage. Importantly, no fatalities were recorded in this study, consistent with the existing literature, which indicates an LD50 for the biherbal formulation extract well above 2 g/kg following a single oral dose over 24 hours, 14 days, and daily doses for 14 days. Our study showed no signs of toxicity or histopathological abnormalities in any treatment groups, particularly in the brain and uterus, corroborating the reports by Nghonguyi *et al.* (2016).

CONCLUSION

In conclusion, this study demonstrates significant evidence supporting the safety of the biherbal formulation. Acute toxicity tests conducted with a single oral dose of 2 g/kg over 24 hours and 14 days, and repeated daily doses for 14 days indicated no harmful effects on body weight, temperature, hematological parameters, organ weights (brain and uterus), or histopathological evaluations of the organs. Therefore, further research is necessary to explore chronic toxicity and therapeutic efficacy.

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