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Original Research Article

Comparative Acute Oral Toxicity of *Landolphia owariensis P. beauv*. Leaf Extracts in Wistar Rats

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ABSTRACT

Landolphia owariensis is a liana used traditionally to treat malaria and other conditions in parts of sub-Saharan Africa. This study was aimed at comparing the acute oral toxicity of various leaf extracts of the plant in Wistar rats. Crude extracts of *L. owariensis* leaves were prepared by extracting successively with n-hexane, dichloromethane, ethylacetate, methanol, and water. In the acute toxicity test, a single dose of 2000 mg/kg body weight of each extract was administrated orally to groups of female Wistar rats. On day 14, blood samples and the organs (heart, lungs, liver, kidney, and spleen) of each animal were collected after they had been sacrificed. The effects of the various extracts on haematological parameters and organ histology were then evaluated. Data were analyzed using one-way ANOVA followed by Tukey's test. The n-hexane extract significantly increased the leucocyte count ($P < 0.05$) compared with the control. It was further observed that treatment with the n-hexane, dichloromethane, and ethylacetate extracts of the plant produced significant changes ($P<0.05$) in platelet larger cell ratio (P-LCR), Haemoglobin (HGB) and haematocrit (HCT) respectively as compared with the control group. Histopathological examination of the organs revealed rhabdomyolysis in the heart and mild liver inflammation in the groups that received the n-hexane and dichloromethane extract of *L. owariensis*. The study revealed a median lethal dose (LD_{50}) of greater than 2000 mg/kg body weight for all the extracts of *L. owariensis* since no mortality was observed at 2000 mg/kg. The n-hexane and dichloromethane extracts of the plant were found to be the most toxic

Keywords: *Landolphia owariensis*, Acute toxicity, Haematology, Histopathology, and Rats

Introduction

Landolphia owariensis is a climbing herb belonging to the family Apocynaceae. It is well distributed from Guinea to West Cameroon and extends across Central Africa to Sudan, Uganda, and Southern Tanganyika.[1] Of the sixty (60) known species of the Landolphia genus, *Landolphia owariensis* is recognized as one of the most studied and useful species.[2] It is known as "panukuru" in the Yoruba Language.[3] Microscopic examination of the powdered leaves of *L. owariensis* showed stomatal cells, starch grains, and fibers are present.[4] Studies on its pharmacological properties have shown it to have a host of diverse activities including antioxidant,[5,6] antiulcer, and antisecretory effects with the leaves.[7] The aqueous, methanol, and chloroform leaf extracts have shown anti-inflammatory and analgesic properties[8] while the ethanol extract of the leaf and root showed antimicrobial activities.[9–11] Similar antimicrobial activity has also been established with the fruits.[12] The root is soaked in local gin and given for the treatment of gonorrhea.[13]

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The stem bark had been reported as a vermifuge[14] while the aqueous decoction of the dried liana bark was found to have anti-haemorrhoidal activity.[15] The plant has been used over the years in traditional medicine for the treatment of malaria.[16] The methanol extract of the plant displayed good activity against the plasmodium parasite in a murine model.[17]

There are several reports of serious toxicity and adverse effects resulting from the use of herbal medicines. This has been linked to their indiscriminate use on the presumption that since they are of natural origin, they will automatically be less toxic and safe. Sixty percent (60 %) of the respondents in a survey conducted in South-East Nigeria believed that herbal medicines are safe and effective.[18] This assumption is not always true. Some documented reports of adverse effects due to the use and consumption of herbal medicines include: hepatotoxicity due to Pyrrozolidine alkaloids found in some herbal medicines containing *Gynura segetum* and other plants.[19–21] Several phytochemicals such as Aristocholic acid derivatives found in *Aristolochia*, *Bragantia* and *Asarum* species,[22] the triterpene glycoside Glycrrrhizin found in *Glycyrrhiza glabra*[23,24] and anthraquinone derivatives found in plants such as *Rheum officinale*[25] have all been linked to kidney injury. There are other reports of death from Cardiac arrest caused by the alkaloid Aconitine found in various *Aconitum* species.[26,27] The toxic effects associated with herbal medicines have been linked to both their inherent toxicity and toxicities induced by the presence of contaminants.[28]

There are a few studies that have reported the toxicological properties of some extracts of *Landolphia owariensis*. For example, the acute toxicity of the dried liana bark of the plant was evaluated in Wistar rats and the LD_{50} was found to be above 5,000 mg/Kg.[29] The methanol leaf extract of the plant was similarly found to have an LD_{50} of greater than 5,000 mg/Kg[17] while the aqueous leaf extract of the plant was found to

have an LD_{50} of 3370 mg/Kg.[30] It has been established that the part of the plant, as well as the extraction solvent used, can influence the toxicity observed and therefore the value of LD_{50} obtained.[31] To the best of our knowledge, there is no study where the acute oral toxicity of the leaf extracts of *L. owariensis* obtained using various solvents was evaluated comparatively. If *L. owariensis* is to stand any chance of being developed further into a useful medicine for human use, it is important to fully establish its safety and toxicological profile. This study aimed at evaluating the acute oral toxicity of various extracts of the leaves of the plant obtained through extraction with solvents of varying polarity in rats. This information when combined with information on the biological activities of the plant leaf extracts will help guide the choice of the specific solvent extracts of the plant with a better safety margin.

Materials and Methods

Collection and identification of the plant
The leaves of *Landolphia owariensis* were collected from their natural habitat in Toro LGA in Bauchi State, Nigeria in August 2020. The plant was identified and authenticated by a taxonomist (Mr. J.J. Azila) at the Federal College of Forestry, Jos, Plateau State. A Voucher specimen (with voucher number FHJ 301) was also deposited at the Herbarium of the same institution.

Preparation of plant material
The leaves were washed with water and air dried under shade to constant weight at room temperature. Dried leaves were pulverized with the aid of an electric blender. The pulverized leaves (2 Kilograms) were extracted successively by maceration with 5 Liters each of n-Hexane, Dichloromethane, Ethylacetate, Methanol, and Water. The extracts were then concentrated using a rotary evaporator (40-50°C) while the aqueous extract was lyophilized and stored at 4°C before use.

Phytochemical screening
The major classes of phytochemical constituents present in the various leaf extracts were determined according to established methods.[32]

Preparation of animals
Female Wistar rats weighing between 20 -30 grams each were randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days before dosing to allow for acclimatization to the laboratory conditions. The temperature in the experimental animal room was 25ºC ± 3ºC and relative humidity ranged between 30% - 70%. Lighting was 12 hours light and 12 hours dark cycles. The animals were fed conventional laboratory diets with free access to drinking water.

Acute Oral Toxicity Studies of the Extracts
The experimental protocol was approved by the Ethical committee animal experimental unit of the Department of Pharmacology and Toxicology, University of Jos with reference number: UJ/FPS/F17-00379. The procedure described by the Organization of Economic Cooperation and Development (OECD) in the guideline for testing of chemicals; Acute Oral Toxicity – Acute Toxic Class Method[33] was used for the acute toxicity study. Seven groups of three (3) female Wistar rats each were administered orally a single dose of 2000 mg/kg of the various extracts of *Landolphia owariensis* leaves. The two (2) control groups received distilled water and diluted dimethylsufoxide (0.1 %) of the same volume respectively. After the treatment, animals were observed for 1, 2, and 4 hours, then over 24 hours, and subsequently daily for 14 days for signs of toxicity. No death was recorded in the first step and the procedure was repeated in the second step. The observations made included changes in the skin and fur, eyes and mucous membrane, respiration, urination, drowsiness, sedation, tremor, diarrhea, change in body weights, food intake, water intake, behavioural changes, and mortality for a period of 14 days post-treatment.[34, 35] The body weights of the rats in all the groups were determined on Day 1 of the study (before extract administration), Day 7, and Day 14 representing the end of the experiment. Thereafter, all surviving animals were euthanized with chloroform and sacrificed on day 14 for sample collection.

Sample collection
Blood samples were collected before sacrificing the animals in EDTA-treated sample bottles by retro-orbital puncture using capillary tubes for haematological studies. After the rats were sacrificed, the heart, lungs, liver, kidney, and spleen were excised for histopathological assessment.[36]

Histopathological examination
The heart, lungs, liver, kidney, and spleen were fixed in NaCl buffer containing 10% formalin. The processed tissues were embedded in paraffin wax and sections (5 µm thickness) were cut with a microtome, stained with haematoxylin and eosin dyes then assessed on a microscope at X100 magnification. Changes in tissue architecture as compared to the normal structure were recorded.

Haematological analyses
Haematological analyses of the blood were performed using an Automated haematology analyzer (SM9000, England) to measure the following haematological parameters: red blood cell (RBC) count, leukocyte (WBC) count, haemoglobin (Hb), haematocrit (HCt), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet and lymphocyte counts.

Statistical analysis
Statistical analysis was performed using GraphPad Prism software. Results were presented as mean ± SEM. The differences between samples were analyzed by one-way analysis of variance (ANOVA). Tukey's post-hoc test was performed to determine statistical significance at p-value of 0.05.

Results and Discussion

The use of medicinal plants to treat human diseases is almost as old as mankind. They are recognized to play an important role in meeting the healthcare needs not just in low- and middle-income countries where the majority of the populace depend on them but also increasingly in developed countries where their popularity is on the rise. Despite the foregoing, toxicity due to the use of medicinal plants remains a problem that is sometimes under-reported. It has been estimated that about 2 % of intoxications and 0.2 % of toxicity deaths are linked to plants.[37] Also, the use of herbal supplements and medicines has been implicated in up to 20 % of all drug-induced hepatotoxicity in developed countries.[38] It is, therefore, necessary to carry out an in-depth evaluation of the potential toxicological effects that may be caused by plants used in traditional medicine.
In this study, the n-hexane, and dichloromethane extracts of *L. owariensis* had percentage yields of 2.91 and 2.38 % respectively (Table 1) after extraction but linking this to the results of the phytochemical screening, it was observed that these extracts only contained a limited number of phytochemical classes as seen by the observation that the n-hexane extract contained mostly fats and oils in addition to steroids and cardiac glycosides while the dichloromethane extract contained only steroids and cardiac glycosides (Table 2).

Table 1: Yield of the various extracts of *Landolphia owariensis* leaves

Extract	% Yield
n-Hexane	2.38
Dichloromethane	2.91
Ethylacetate	0.75
Methanol	2.50
Water	1.36

Table 2: Phytochemical constituents of the leaves of *Landolphia owariensis*

Constituent	n-Hexane	Dichloromethane	Ethylacetate	Methanol	Water
Alkaloids	-	-	+	-	-
Saponins	-	-	-	+	+
Tannins	-	-	+	+	+
Anthraquinones	-	-	-	-	-
Cardiac glycosides	+	+	+	+	+
Steroids & Triterpenoids	+	+	+	+	+
Flavonoids	-	-	+	+	+
Carbohydrates	-	-	+	-	+
Fixed oil and Fats	+	-	-	-	-

Key: + = Present, - = Absent

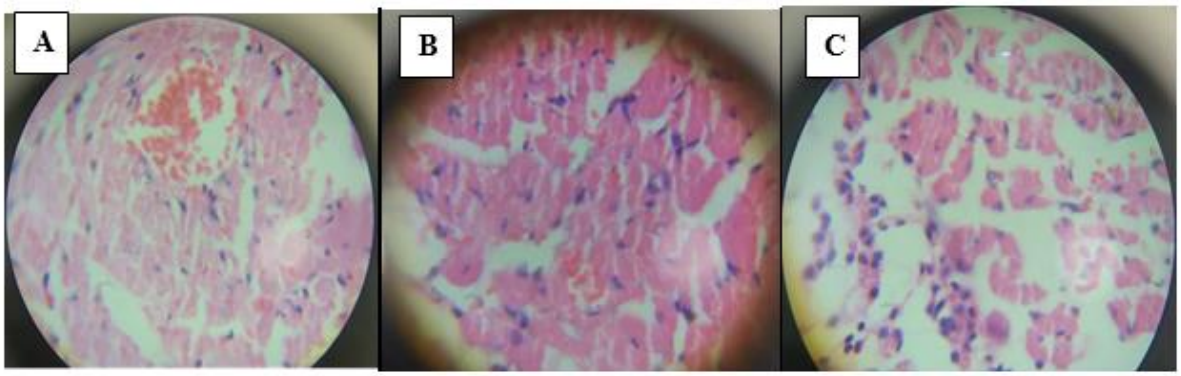

Figure. 1: Cross section of representative samples of heart tissue from experimental groups stained with hematoxylin and eosin stain 100 ×. (A) Control rat administered distilled water showing normal architecture, (B) Rat from the group that received n-Hexane extract of *L. owariensis* (LOHE) showing rhabdomyolysis (C) Rat from the group that received dichloromethane extract of *L. owariensis* (LODE) showing rhabdomyolysis.

On the other hand, the ethylacetate, methanol, and water extracts had lower extraction yields of 0.71, 2.50 and 1.36 % respectively though they contained a greater diversity of phytochemical classes as can be seen from the phytochemical screening results which showed that the ethylacetate extract contained alkaloids, tannins, flavonoids, carbohydrates and the methanol extract tested positive for saponins, tannins, cardiac glycosides, and flavonoids while the water extract contained saponins, tannins, cardiac glycosides, steroids, flavonoids, and carbohydrates. The diversity of phytochemicals obtained in these extracts may be linked to the fact that these solvents which range from intermediate polarity (ethylacetate) to strongly polar (water) have greater extractive power as compared to non-polar solvents. The result from this study agrees with a previous study on the methanol extract of the plant leaves which were found to contain alkaloids, flavonoids, saponins, tannins, and steroids.[17] Similar phytochemical constituents were also found in another study with a yield of 17.80 % however obtained from the methanol leaf extract.[10] The slight differences in the phytochemical constituents found in the present study as compared to previous studies may be due to differences in the soil quality and composition and also climatic conditions in the different geographical locations where the plant materials used were collected from.

In the acute toxicity studies, no mortality was observed in all the groups within 14 days after extract administration. The animals also did not display visible signs of acute toxicity such as salivation, aggression, rising furs, and writhing during this period. The median lethal dose (LD_{50}) of the extracts was estimated to be above 2000 mg/kg. This figure falls in category 5 of the Globally Harmonized Categorization System (GHS). The data obtained indicated no statistically significant differences between control and extract-treated rats in the amount of food and water consumed. There were also no statistically significant differences in their weights after 14-day treatment with the extracts as compared to their initial weights. The LD_{50} from this study agrees with a previous study on the alkaloid-rich fraction of the ethanol leaf extract of the plant where an LD_{50} of greater than 2,000 mg/Kg was similarly obtained.[39] In other studies, an LD_{50} of greater than 5,000 mg/kg was reported with the Ethanol, Methanol, Water, Hexane, and Acetone extracts of *L. owariensis* leaves[40] while an LD_{50} of greater than 3000 mg/Kg was obtained for the hydroethanolic bark extract of the plant when applied intraperitoneally.[41] It is pertinent to note that in all the previous reports in literature, there is no record of death of animals that received extracts of this plant. Another study on the aqueous leaf extract of the plant[30] indicates an LD_{50} of 3370 mg/Kg. This same study also found a slight increase in some liver enzymes (ALT, AST, ALP) in the serum but this increase was not significant when compared to the control.

Histopathological studies of the heart of the rats showed evidence of rhabdomyolysis in the groups administered the n-hexane and dichloromethane extract of *L. owariensis* while the other groups showed normal architecture (Figure 1). Similar results were obtained for the liver of the animals which demonstrated that the n-hexane and dichloromethane extracts were mildly toxic at the given dose revealing signs of inflammation (Figure 2), whereas the methanol extract produced hepatocyte necrosis at 2000 mg/kg in comparison with the control group. No histological abnormalities were observed in the kidneys of the animals in all the groups compared to the control. The lungs of the animals of the groups that received the n-hexane, methanol, and water extracts showed evidence of pulmonary congestion while the group that received the dichloromethane extract of the plant showed signs of inflammation (Figure 3). The spleen of the animals in the various groups was normal except for the group that received the n-hexane and methanol extract of the plant which showed signs of extramedullary haemopoeisis (Figure 4). Histological examination of tissues helps to reveal any damage to the normal architecture of the tissues. In this study, the heart of the rats showed evidence of

rhabdomyolysis in the groups that received the n-hexane and dichloromethane extract of *L. owariensis* while the other groups showed normal architecture. There are a few reports in literature on rhabdomyolysis due to herbal medicines or extracts.[42,43] Similar results were obtained in the liver of the animals which demonstrated that the n-hexane and dichloromethane extracts were slightly toxic at the given dose revealing signs of mild inflammation. Another important observation of the histopathological examination was that the methanol extract produced hepatocyte necrosis at 2000 mg/kg in comparison with the control group. This agrees with a previous study in which the activity of alkaline phosphatase in the liver of the rat that received *Landolphia owariensis* methanol leaf extract was found to decrease though this decrease was not statistically significant.[44] The kidneys of the animals in all the groups were normal as compared to the control. Upon examination of the lungs of the animals, it was found that the groups that received the n-hexane and methanol extracts showed evidence of pulmonary congestion while the group that received the dichloromethane extract of the plant showed signs of inflammation. Constituents of some medicinal plants such as *Avena sativa*, *Panax ginseng*, and *Sauropus androgynous* have previously been implicated in causing lung injury.[45] The spleen of the animals in the various groups were normal except for the group that received the n-hexane and methanol extract of the plant which showed signs of extramedullary haemopoeisis with giant cells. At this point, it may be difficult to conclude if these observed pathological changes in the various organs are transient in nature or if they are suggestive of long-term damage. This can only be ascertained after chronic toxicity studies hence there is need to exercise restraint in interpreting the results.

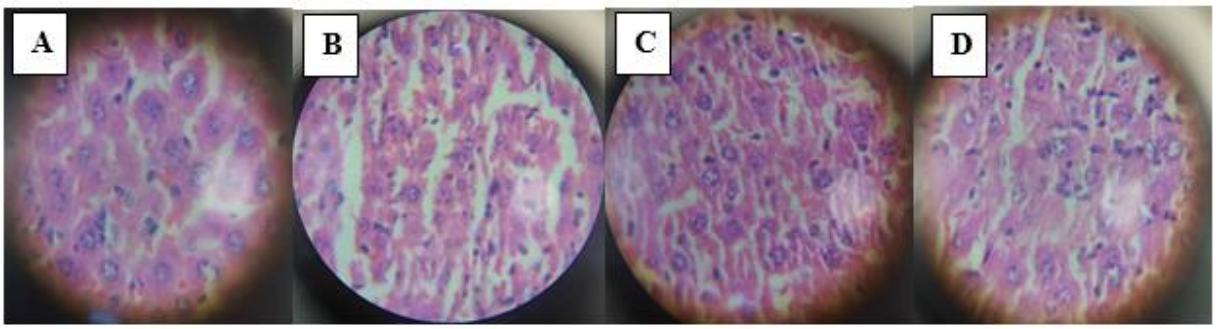

Figure. 2: Cross section of representative samples of liver tissue from experimental groups stained with hematoxylin and eosin stain 100 ×. (A) Control rat administered distilled water showing normal architecture, (B) Rat from the group that received n-Hexane extract of *L. owariensis* (LOHE) showing inflammation (C) Rat from the group that received dichloromethane extract of *L. owariensis* (LODE) showing inflammation. (D) Rat from the group that received methanol extract of *L. owariensis* (LOME) showing mild hepatocyte necrosis.

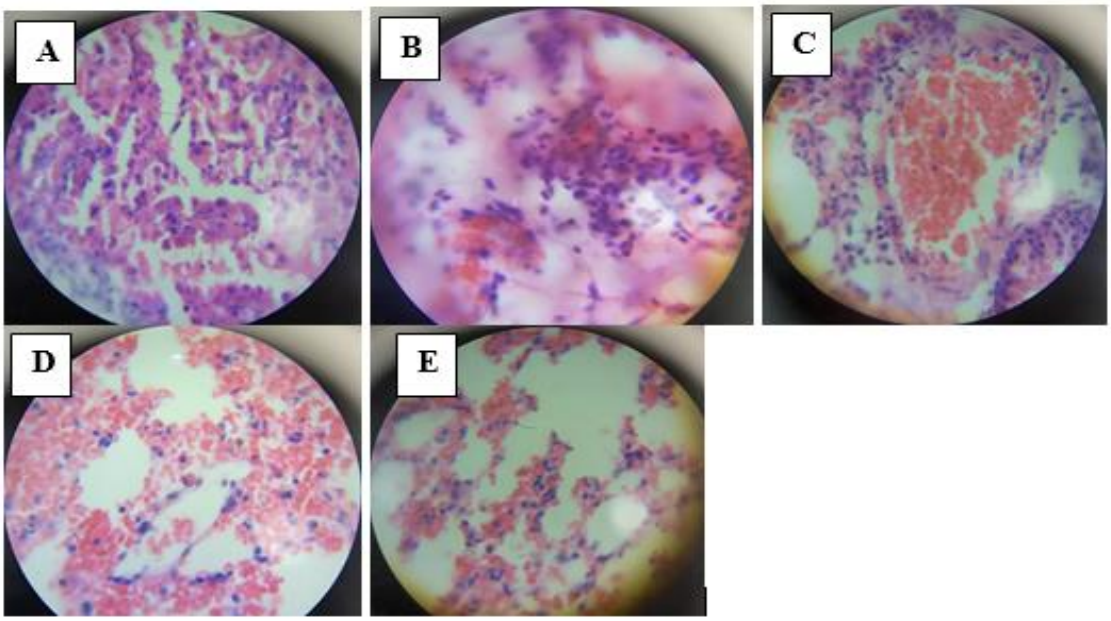

Figure. 3: Cross section of representative samples of lung tissue from experimental groups stained with hematoxylin and eosin stain 100 ×. (A) Control rat administered distilled water showing normal architecture, (B) Rat from the group that received n-Hexane extract of *L. owariensis* (LOHE) showing inflammation and pulmonary congestion (C) Rat from the group that received dichloromethane extract of *L. owariensis* (LODE) showing inflammation and lung abscess. (D) Rat from the group that received methanol extract of *L. owariensis* (LOME) pulmonary congestion. (E) Rat from group that received water extract of *L. owariensis* (LOWE) showing pulmonary congestion.

Hematological parameters obtained for the rats (Table 3) showed that there was a significant increase ($P<0.05$) in white blood cell (WBC) count for the group that received the n-hexane extract of the plant while the other groups did not experience any significant changes. In addition, it was observed that they were no significant changes in the platelet count for all the groups as compared to the control group. Also, there was no significant change in red blood cell (RBC), MCV, MCH, RDW-SD, RDW-CV, MPV, and PCT in the extract-treated groups of rats compared to the control. On the other hand, treatment with the n-hexane, dichloromethane, and ethylacetate extract of the plant produced

significant changes (P<0.05) in P-LCR, Haemoglobin (HGB), and HCT respectively as compared with the control group (Table 4). The n-hexane extract of the plant (LOHE) caused a statistically significant increase in WBC count. A condition known as leukocytosis which can occur in response to infection, injury, or acute stress.[46] It is likely that the n-hexane extract triggered an immune reaction which led to increased WBC production. It was also observed that all the extracts produced statistically significant changes in granulocyte count as compared to the control group. The lymphocyte count was similarly deranged for the groups that received LOHE, LODE, LOEE, and LOWE, though the derangements were to different extents. A review of existing literature on the plant shows that this study is the first report of extracts of *L. owariensis* producing such changes in these haematological indices and it is a cause for concern that may require further investigation. Furthermore, the n-hexane, dichloromethane, and ethylacetate extract of the plant produced significant changes (P<0.05) in P-LCR, Haemoglobin (HGB), and HCT respectively as compared with the control group while the water extract produced significant changes in MCHC and PDW. The changes in these parameters (P-LCR, HGB, HCT, MCHC, and PDW concentrations) may imply that the extracts interfered with the normal production of hemoglobin within RBCs which is an indication of toxicity.[47] The statistically significant decrease in Haemoglobin levels obtained for the LODE group is similar to the result obtained in a previous study[30] where rats that received aqueous extracts of *L. owariensis* leaves also showed decreased haemoglobin level and this was indicative of haemolysis of red blood cells.

Conclusion

The study indicates that the various extracts of the leaves of *Landolphia owariensis* did not produce severe toxicity when administered in a single dose following the OECD guidelines for acute oral toxicity. However, some changes in haematological parameters and histological presentation of some organs of animals that received the extracts were observed. The n-hexane and dichloromethane extracts of the plant consistently produced toxic effects and there may be need for caution and vigilance in the medicinal use of these particular extracts of the plant.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

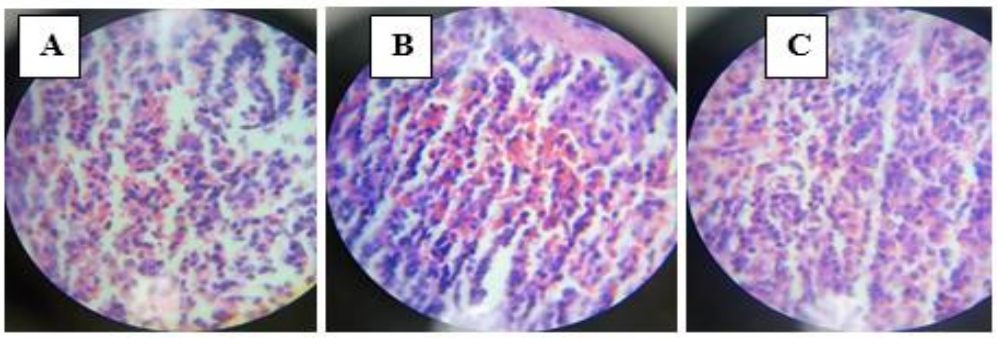

Figure. 4: Cross section of representative samples of spleen tissue from experimental groups stained with hematoxylin and eosin stain 100 ×. (A) Control rat administered distilled water showing normal architecture, (B) Rat from the group that received n-Hexane extract of *L. owariensis* (LOHE) showing extramedullary haemopoeisis (C) Rat from the group that received dichloromethane extract of *L. owariensis* (LODE) showing extramedullary haemopoeisis.

Table 3: Effect of various *Landolphia owariensis* extracts on white blood cells, platelets and differentials after acute toxicity studies

	LOHE	LODE	LOEE	LOME	LOWE	Control
WBC (10^9/L)	3.00 ± 0.28***	1.20 ± 0.10	1.60 ± 0.10	1.20 ± 0.10	1.60± 0.20	1.10 ± 0.10
LY (%)	76.35 ± 0.85*	79.35 ± 0.45**	80.95 ± 0.55**	70.50 ± 4.90	77.60 ± 4.70*	56.40 ± 0.70
MID (10^9/L)	7.35 ± 0.25**	6.50 ± 0.50**	7.30 ± 0.10**	9.70 ± 0.60#	9.75 ± 0.15#	11.95 ± 0.75
GR (%)	14.65 ± 0.55***	13.35 ± 0.15***	11.30 ± 0.20***	11.25 ± 0.45***	8.25 ± 0.45****	27.85 ± 2.35
Platelet (10^9/L)	67.50 ± 44.50#	414.50 ± 295.50#	157.50 ± 95.50#	155.50 ± 5.50#	144.00 ± 36.00#	438.00 ± 300.00

Note: p value (P < 0.05), Highly significant ***, Moderately Significant **, Significant *, Non-significant #, WBC = White Blood Cells, LY = Lymphocytes, GR = Granulocytes,

Table 4: Effect of various *Landolphia owariensis* extracts on red blood cells and other haematological indices after acute toxicity studies

	LOHE	LODE	LOEE	LOME	LOWE	Control
RBC (/L) X 10*12	0.38 ± 0.10	0.92 ± 0.67	0.15 ± 0.01	0.77 ± 0.00	0.37 ± 0.15	0.93 ± 0.16
HGB (g/dL)	12.80 ± 10.70	4.45 ± 0.15**	5.70 ± 1.80	9.30 ± 1.10	8.70 ± 0.40	12.05 ± 7.75
HCT (%)	2.00 ± 0.40	5.30 ± 3.60	0.95 ± 0.25***	2.15 ± 0.75	1.40 ± 0.30	5.30 ± 0.90
MCV (fL)	56.25 ± 3.95	64.00 ± 7.30	64.55 ± 12.85	60.15 ± 0.35	53.85 ± 2.45	57.85 ± 0.15
MCH (pg)	287.50 ± 212.50	100.55 ± 71.45	373.60 ± 95.10	322.85 ± 18.75	367.65 ± 9.55	148.25 ± 108.85

MCHC (g/dL)	555.15 ± 423.95	152.25 ± 100.65	591.05 ± 33.95	577.20 ± 8.50	729.95 ± 24.55***	259.65 ± 190.35
RDW-SD (fL)	39.55 ± 5.35	62.00 ± 17.10	45.95 ± 9.65	46.00 ± 1.00	39.85 ± 1.35	39.50 ± 3.20
RDW-CV (%)	15.30 ± 1.20	21.50 ± 4.00	17.00 ± 6.10	16.45 ± 0.95	15.85 ± 0.25	15.10 ± 1.20
MPV (fL)	7.30 ± 0.10	10.80 ± 0.40	10.65 ± 1.25	9.85 ± 0.35	7.50 ± 0.30	8.65 ± 0.45
PDW (%)	9.30 ± 0.40	14.60 ± 1.30	15.15 ± 3.85	12.90 ± 0.20	29.30 ± 0.50**	11.50 ± 0.00
PCT (%)	0.05 ± 0.04	0.43 ± 0.30	0.15 ± 0.08	0.15 ± 0.01	0.09 ± 0.02	0.39 ± 0.28
P-LCR (%)	1.80 ± 0.60***	28.80 ± 0.20	24.05 ± 8.65	17.70 ± 4.80	7.45 ± 1.15	14.10 ± 3.60

Note: RBC = Red Blood Cells; HGB =Hemoglobin; HCT = Hematocrit (or Packed Cell Volume, PCV); MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Hemoglobin; MCHC = Mean Corpuscular Hemoglobin Concentration; RDW-SD = Standard Deviation in Red Cell Distribution Width; RDW-CV = Coefficient of Variation in Red Cell Distribution Width; MPV = Mean Platelet Volume; PDW = Platelet Distribution Width; PCT = Procalcitonin; P-LCR = Platelet Larger Cell Ratio; LOHE = *L. owariensis* n-hexane extract; LODE = *L. owariensis* dichloromethane extract; LOEE = *L. owariensis* ethylacetate extract; LOME = *L. owariensis* methanol extract and LOWE = *L. owariensis* water extract;. Values are represented as mean + SD of triplicates (n = 3). *p < 0.05, **p < 0.01,***p < 0.001 indicate significant changes in comparison with the control

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