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Haematotoxicity and Histotoxicity Studies of Ethanolic Leaves Extract of *Piliostigma thonningii* and *Lophira lanceolata* in Wistar Albino Rats

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Abstract:- The use of medicinal plants as source of health remedies in malaria endemic regions has significantly increased as a result of increase malaria parasite resistant to some synthetic drugs currently in used. Extracts prepared from *Piliostigma thonningii* and *Lophira lanceolata* leaves are used for curing malaria and other related human diseases in Nigeria. The aim of this study was to investigate the possible haematotoxicity and histotoxicity profile of *P. thonnigii* and *L. lanceolata* in Wistar albino rats. Graded doses of Ethanolic extracts prepared from *P. thonningii* and *L. lanceolata* leaves were administered day-to-day (1000, 1500 and 2000 mg/kg) orally to rats for 14 days respectively and the effects on body weight, relative organ weight, haematology, serum biochemical and histopathology parameters were evaluated. The relative weights of the sampled organs of treated rats were unaffected. No significant deviations in haematological profile of treated animals. Both extracts elicited significant decline in BUN levels at 1000 mg/kg dose. Histopathological findings reveal evidence of some mild and focal lesions. This study indicates that *P. thonnigii* and *L. lanceolata* leaves ethanolic extracts have no obvious negative effects when applied orally under a controlled dosage of 2000mg/ kg body weight. However, the histopathological studies therefore calls for watchfulness or caution to constant/repeated exposure to the extracts.

Keywords:- *Piliostigma thonningii, Lophira lanceolata, haematotoxicity, histotoxicity, Wistar albino rats.*

I. INTRODUCTION

Malaria is an endemic parasitic infectious disease spread through the bites of Anopheles mosquitoes infected with Plasmodium species (WHO, 1998; 2008). It is one of major public health challenge in Africa, affecting more than 40% of the populace (Snow et al., 2005). Nigeria accounts for one-quarter of all malaria cases in Africa (WHO, 2008a; Federal Ministry of Health, 2004).The global strategy for reducing the morbidity and mortality of malaria mainly focuses on case management by providing drugs capable of reducing or eradicating the parasites (WHO, 1993; Schapira, 1993).

The emergence of Plasmodium species resistant to anti-malarial drug poses a serious threat to the parasite control scheme thereby causing not only the spread of malaria to new areas but also its recurrence in regions where it had earlier been eliminated (Collins and Jeffery, 1996). This encouraged many communities in endemic regions to begin search for malaria medications free of adverse side effects in plants within their local settings (Gbadamosi *et al*., 2011) as a result of increase malaria resistant to a number of synthetic drugs currently in used (Falade *et al*., 1997).

Seeking remedies for humans health from medicinal plants within the environment as an alternative therapy has moved from fringe to mainstream (Maikai *et al*., 2008;Olowokudejo *et al*., 2008;Amadi *et al*., 2011), due to the erroneous belief that these medicinal product from plants are safer, free from adverse effects and causes less injury to the human body compared to synthetic drugs in the treatment of diseases (Ernst, 2005; Atere and Ajao, 2009; Alam *et al*., 2011).

Chronic use of herbal products may result in high chances of adverse effects and/or organ toxicities wrongly attributed to certain diseases due to dearth scientific confirmation about the safety and efficacy profiles to support the acclaimed therapeutics of such plants. Considering the immense significance and unparallel impact of plant medicines on human life in Nigeria, the toxicological screenings of widely used plant preparations are highly pertinent.

In this research we prepared extracts from the leaves of *Piliostigma thonningii* and *Lophira lanceolata* used in malaria cure and other related human diseases in Nigeria (Tor-Anyiin *et al*., 2003; Igoli *et al*., 2005; Ajaiyeoba *et al*., 2006; kayode, 2006; Olowokudejo *et al*., 2008; Adebayo and Krettli, 2011) to investigate and evaluate organ and heamatology toxicity of ethanolic extracts of the leaves of these plants using experimental animals (rats).

P. thonningii usually recognized as Monkey bread or Camel's foot in English; Abefe in Yoruba, Kargo in Hausa, Nyiral in Fulani, Okpoatu in Igbo, Atakpa-efa in Igala, Omepa in Igede and Nyihar in Tiv (Madara *et al*, 2010) While *L. lanceolata* is generally recognized as red ironwood in English, Namijin kande in Hausa, Eki in Yoruba, Ada or Okopia in Igbo, Maganchi in Nupe, Okopi in Igala (Etuk and Muhammad, 2009).

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II. MATERIALS AND METHODS

A. The Plant Material Collection and identification

Fresh matured leaves of *P. thonningii* and *L. laceolata* were harvested as one batch from Ibadan Nigeria. Identification and authenticated were carried out at the Forestry Research Institute of Nigeria (FRIN), Jericho, Ibadan, Nigeria, the plant voucher samples with number FHI: 109666 and FHI: 101377 were placed at the Institute Herbarium for reference. The leaves were freed of extraneous materials, air dried for three weeks, then pulverized into a uniform powdery.

B. Preparation of Alcoholic (Ethanol) Plant Extract

300g each of the milled leaves were soaked in 2000ml of ethanol for 72 hours with constant shaking at intervals. The extracts were sieved using Whatmann No. 1 (Whatmann International Ltd, Maidstone, UK) paper. The extracts so obtained were made ethanol free under vaccorotatory evaporator and lyophilized to obtain a brownish crude extract and the dried extracts residue was stored in a refrigerator separately in airtight bottles at 4 ◦C as crude extracts until the time of use, each crude extract was carefully weighed and suspended in 5% and 10% dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, USA) respectively and made up to required quantity with distilled water in a sterile container of 50 ml capacity. The solution was vigorously stirred using Fisher Vortex Machine (Cat. No. 12-812) to aid completes dissolution and these now constitute the stock solutions used for administration at the required dose.

C. Animal stocks selection, maintenance and care

Pathogen free Wistar albino rats aged 6–8 weeks old were purchased from the Central Animal Breeding House, Department of Veterinary Medicine and brought to the Experimental Animal House, Zoology Department University of Ibadan. The animals were allowed to adapt for a period of seven (7) days. The animals were kept in group of five (5) in a suspended cage under suitable conditions and exposed to natural photoperiod of 12 hours light and 12 hours dark cycles. They stayed fed *ad libitum* with typical commercial feed (Ladokun Feeds, Ibadan, Nigeria) and had access tap water in bottles with stainless steel sipper tubes. The animals were handled humanely in agreement with international recognized ethics for laboratory animal use Protocol and practices (OECD, 2008; WHO, 1998; NIH, 1985).

D. Sub-Acute Toxicity Studies

The study was designed in accordance with the procedures provided by Aniagu *et al*., (2005), Builders *et al*., (2012) and Rajina and Shini(2013).Prior to dosing, animals were made to fast over night and after the administration, food was withheld for further 3-4 hours, to allow fast cellular absorption. The tested plant constituent was administered to animals in a sequential routine. The prepared stock leaves extracts were administered using syringe and canula via oral route. The animals were blindly allotted into four (4) groups for each extracts with each group having five animals (n =5). The first three groups received homogeneous suspensions of the extract once daily through oral gavaging for fourteen (14) days at the dosage rate of 1000, 1500, and 2000mg/kg body weight/day respectively while the last group served as control. Weights of the animals were verified on day 0, 7, and 14.

E. Sera samples Preparation

On Day 15 after the dosing period, samples of blood were collected from the rats through retro-orbital sinus puncture into two (2) different bottles for biochemical and hematological analysis. The sampled blood for haematology were kept in a labelled bottles containing ethylene diamine tetraacetic acid (EDTA), each sample bottle was shaken gently to mix the sampled blood with EDTA to avoid clotting. The blood samples for serum biochemistry were placed in a well labelled plain serum clinical sample bottles, then were centrifuge at 4000 rpm for 10 minutes to obtain sera. The sera were collected and transferred into another tubes, then kept refrigerated within 24-48 hours at 4°C before used for analysis (Buetow *et al*., 1999).

F. Haematology and biochemical parameter

The haematology parameters were analyzed using SYSMEX automated haematological analyzer. The parameters measured were Haemoglobin Concentration (HGB), Leucocyte/White Blood Corpuscles (WBC), Erythrocyte/Red Blood Corpuscles (RBC), Hematocrit (HCT), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Red Cell Distribution Width (RDW%), Lymphocyte % (LYM), Granulocyte % (GRAN) and Platelets (PLT) (Weingand *et al*., 1996).

The biochemical parameters determined includes Total Protein, Aspartate Aminotransferase (AST), Bilirubin, Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), Blood Urea Nitrogen (BUN), Creatinine (Creat) and Cholesterol(Weingand *et al*., 1996) using the standard commercial available kits (Randox Laboratory Ltd., County Atrim, U.K.) by abiding strictly to the manufacturer's instructions for each biochemical parameter during the investigations.

G. Relative organ weight (ROW)

All rats (control and treated) were sacrificed by cervical dislocation immediately after blood sampling at the end of experiment on Day 15. The organs (kidneys, liver, heart, and brain) were carefully resectioned, cleaned and weighed in grams (absolute organ weight). Each organ was then fixed in 10% buffered formalin fixative for subsequent histopathological investigation or analysis. The relative organ weight (ROW) of each organ was then calculated according to the following equation:

$$\text{ROW} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100$$

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H. Histopathology analysis

Selected organs (kidneys, liver, heart and brain) were resection from sacrificed experimental animals for macroscopic analysis and stored in 10% formalin. The tissues specimens from the organs were processed with an automated tissue processor. Subsequent dehydration and embedding, serial sections of 4µm thickness were obtained on glass slides using rotatory microtome, deparaffinised and stained using haematoxylin and eosin (H & E) (Lison, 1960; Drury *et al*., 1976; Galighor and Koziff, 1976; Kiernan, 1981; Halim *et al.*, 2011) and examined microscopically.

I. Statistical analysis

Data were tabulated and analyzed statistically by one way analysis of variance (ANOVA) and subjected to Dunnett's post hoc Test, using the Statistical Package Sciences (SPSS version 17). Data are expressed as the mean ± standard error of mean. The calculated variables were compared with the control to determine significant differences at $p<0.05$.

III. RESULTS

❖ *Sub-Acute Toxicological Assessment*

In sub acute toxicity study the following parameters were evaluated in details.

A. Clinical signs and mortality:

Experimental rats used for this research study appeared normal before and throughout the 14 consecutive days of the experiment. No behavioural deviations or toxicity signs and as well as death recorded at all dosage used during the 14 consecutive days except for group exposed to 2000 mg extract /kg *L. lanceolata* that recorded deaths of a single rat after day eleven (11)of oral extracts administration.

B. Effect of extracts Body Weight

During the 14 consecutive days of exposure, the body weight of all the rats improved significantly with respect to their initial weight. At 2000 mg/kg dose the extract presented significant ($P<0.05$) increase in body weight, while the 1500 mg/kg dose presented significant ($P<0.05$) decrease in body weight at the last day. The results gives an expression that the *P. thonningii* leaves extract elicited a dose-dependent significant increase in body weight compared to the control. On the contrary, *L. lanceolata* leaves extract treated rats showed no significant changes in the body weights when compared to the control rats. The results show that the *L. lanceolata* leaves extract elicited a constant increase in body weight (Table not shown).

C. Effect of extract on Relative organs weight

There was no significant ($P<0.05$) changes in the relative weight of the organs of the rats treated with ethanol extract of *P. thonningii* and *L. lanceolata* leaves when compared with the control groups (Table 1).

D. Effect of extract on hematological parameter

The result of oral administration of the extracts on the hematological parameters is summarized in table 2. There was no significant ($P<0.05$) change in the hematological profile of the treated rats when compared with the control. Although, the WBC and LYMPH of the groups administered 2000mg/kg of *L. lanceolata* extract was slightly higher, at same time there is a slight constant reduction of GRAN in all groups when compared to the control group but not statistically significant. In the groups treated with *P. thonningii* leaves extract, only the WBC and GRAN were slightly but not significantly lower as compared to the controls.

E. Extract effect on serum biochemical parameter

The result of oral administration of *P. thonningii* and *L. lanceolata* ethanol leaves extracts on the serum biochemical parameters is summarized in table 3. There was no significant ($P<0.05$) difference as most of the serum biochemical parameters were not altered by the extracts. Both extracts only produced significant ($P<0.05$) decrease in BUN levels at 1000 mg/kg dose when compared to control groups.

F. Histopathology of selected vital organs

All rats in the control groups showed normal architectures and no structural changes were identified in all the selected organs. These suggest that all the animals were healthy and the experiment was conducted under conditions which were proper. However, some mild and focal lesions were observed in animals treated with *P. thonningii* and *L. lanceolata* at all dose levels used in the study (Figure 1-4).

Treatment (mg/kg/day)		Heart (g)	Liver (g)	Kidneys (g)	Brain (g)
Control	*P.t*	1.06±0.05	9.17±0.26	1.65±0.09	1.83±0.11
	L.l	0.54±0.13	4.57±0.30	0.95±0.12	1.48±0.39
1000	*P.t*	0.97±0.10	10.05±1.47	1.75±0.21	2.04±0.11
	L.l	0.39±0.03	4.99±0.16	0.89±0.04	1.29±0.08
1500	*P.t*	0.95±0.13	8.83±0.76	1.63±0.15	2.09±0.09
	L.l	0.43±0.02	4.67±0.08	0.88±0.23	1.24±0.07
2000	*P.t*	0.82±0.09	6.65±0.69	1.41±0.15	1.91±0.09
	L.l	0.48±0.04	5.22±0.40	0.89±0.06	1.29±0.09

Table 1:- Effect of extracts on relative organ weight (Mean±SEM, n=5)
KEY: *P.t- Piliostigma thonningii L.l- Lophira lanceolata*

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Parameter		Control	1000	1500	2000
HCT (%)	*P.t*	50.72±1.06	52.14±2.46	46.90±1.19	49.72±1.56
	L.l	43.90±2.74	47.60±2.38	45.37±1.87	46.00±0.40
WBC (× 10^3/µl)	*P.t*	14.38±1.40	12.02±1.81	11.30±0.78	12.48±1.68
	L.l	8.93±0.66	8.53±1.59	8.53±0.29	10.25±0.85
HGB (g/dl)	*P.t*	15.30±0.30	15.64±0.64	14.42±0.34	15.10±0.41
	L.l	12.57±1.07	13.77±0.46	13.07±0.66	13.65±0.15
MCV (fl)	*P.t*	61.10±2.11	61.02±0.89	59.54±1.39	59.32±1.18
	L.l	66.83±3.13	66.60±0.59	67.57±3.71	65.60±4.80
MCH (pg)	*P.t*	18.34±0.45	18.36±0.38	18.30±0.33	18.24±0.23
	L.l	19.07±0.44	19.30±0.32	19.50±0.95	19.40±1.00
MCHC (g/dl)	*P.t*	30.18±0.23	30.08±0.36	30.74±0.32	30.78±0.27
	L.l	28.57±0.74	29.00±0.50	28.87±0.29	29.60±0.60
RBC(×10^6/µl)	*P.t*	8.53±0.33	8.54±0.37	7.88±0.22	8.28±0.16
	L.l	6.63±0.71	7.14±0.32	6.78±0.61	7.04±0.46
PLT(× 10^3/µl)	*P.t*	685.00±7.39	629.20±33.58	596.00±43.60	670.80±49.72
	L.l	708.00±86.89	628.33±171.54	669.00±82.02	701.50±18.50
LYM(×10^3/µl)	*P.t*	10.48±1.54	9.90±1.14	8.74±0.85	10.04±1.39
	L.l	7.00±1.15	7.43±1.37	7.53±0.32	9.20±0.60
GRAN(×10^3/µl)	*P.t*	2.00±0.32	1.64±0.46	1.36±0.15	1.12±0.11
	L.l	0.67±0.22	0.40±0.12	0.30±0.06	0.30±0.00
RDW (%)	*P.t*	15.94±0.12	16.24±0.07	15.62±0.25	15.64±0.17
	L.l	17.90±1.70	16.17±0.87	16.40±0.40	16.85±0.75

Table 2:- Effect of extracts on Hematological Parameters (Mean±SEM, n=5)

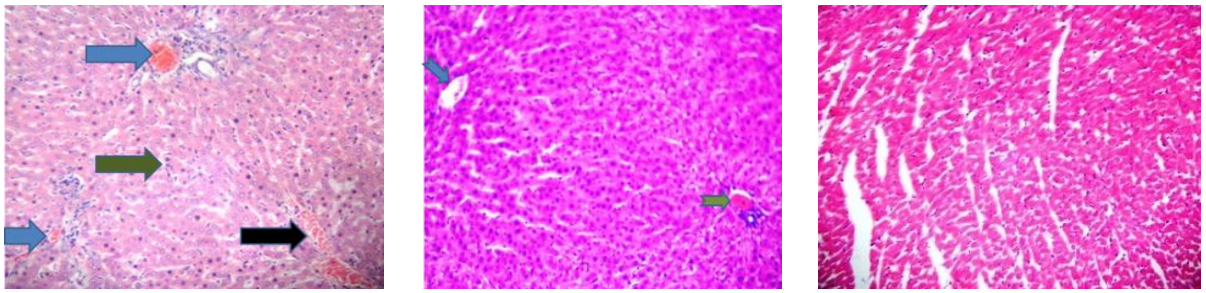
KEY: HCT- Haematocrit; WBC- White Blood Cell; HGB- Haemoglobin Concentration; MCV- Mean Corpuscular Volume; MCH- Mean Corpuscular Haemoglobin; MCHC- Mean Corpuscular Hemoglobin Concentration; RBC- Red Blood Cell; PLT- Platelets; LYM-Lymphocyte; GRAN- Granulocyte; RDW- Red Cell Distribution Width.
P.t- Piliostigma thonningii L.l- Lophira lanceolata

Parameter		Control	1000	1500	2000
Tot. Prot.(g/dl)	*P.t*	6.84±0.12	7.08±0.12	7.16±0.12	7.12±0.26
	L.l	6.33±0.55	6.10±0.25	6.23±0.15	6.45±0.25
AST (U/l)	*P.t*	41.20±0.97	39.40±1.03	39.00±1.14	41.00±1.64
	L.l	41.33±2.19	41.00±3.06	39.33±0.67	45.00±0.00
ALT (U/l)	*P.t*	28.80±0.66	28.80±0.74	27.80±0.92	30.00±0.89
	L.l	30.67±0.88	28.33±2.40	28.67±1.45	32.50±0.50
ALP (U/l)	*P.t*	116.80±2.96	113.20±3.25	112.00±6.25	110.20±3.01
	L.l	122.00±1.16	125.67±5.04	123.00±1.53	116.00±4.00
Tot. Bil(mg/dl)	*P.t*	6.00±0.32	2.90±1.70	5.82±1.48	5.64±1.53
	L.l	1.87±1.57	2.13±1.93	2.20±1.90	2.20±1.80
BUN (mg/dl)	*P.t*	15.40±0.25	14.40±0.25*	15.00±0.00	14.80±0.37
	L.l	16.33±0.33	14.33±0.33*	15.00±0.58	15.00±0.00
CREAT(mg/dl)	*P.t*	0.44±0.25	0.46±0.05	0.48±0.06	0.42±0.06
	L.l	0.63±0.19	0.50±0.10	0.57±0.03	0.55±0.05
CHOL (mg/dl)	*P.t*	45.80±1.56	45.20±3.97	51.80±2.80	45.20±4.83
	L.l	51.33±7.31	44.33±4.37	42.67±1.76	59.00±6.00

Table 3:- Effect of extracts on serum biochemical Parameters (Mean±SEM, n=5)

*: Values are statistically significant at $p<0.05$ when compared to control group.
KEY: Tot. Prot.-Total Protein; AST-Aspartate Aminotransferase; ALT-Alanine Aminotransferase; ALP-Alkaline Phosphatase; Tot. Bil.- Bilirubin; BUN-Blood Urea Nitrogen;Creat- Creatinine; Chol- Cholesterol.

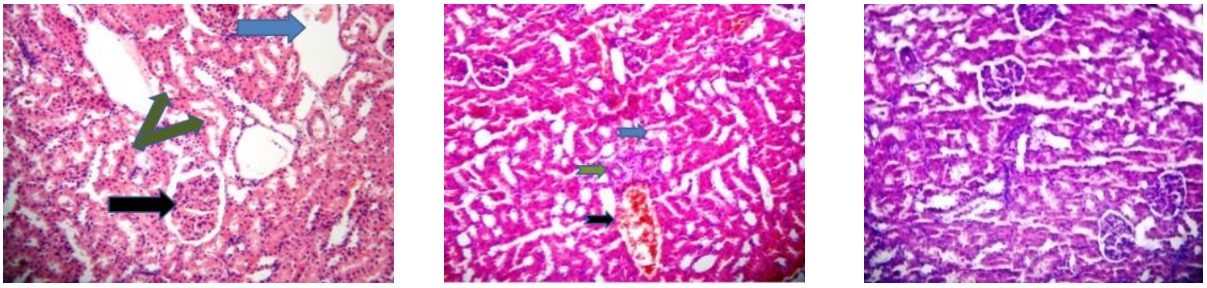
P.t- Piliostigma thonningii L.l- Lophira lanceolata



A B C

Figure 1: Photomicrographs of liver tissue of rats (X 40 magnifications)

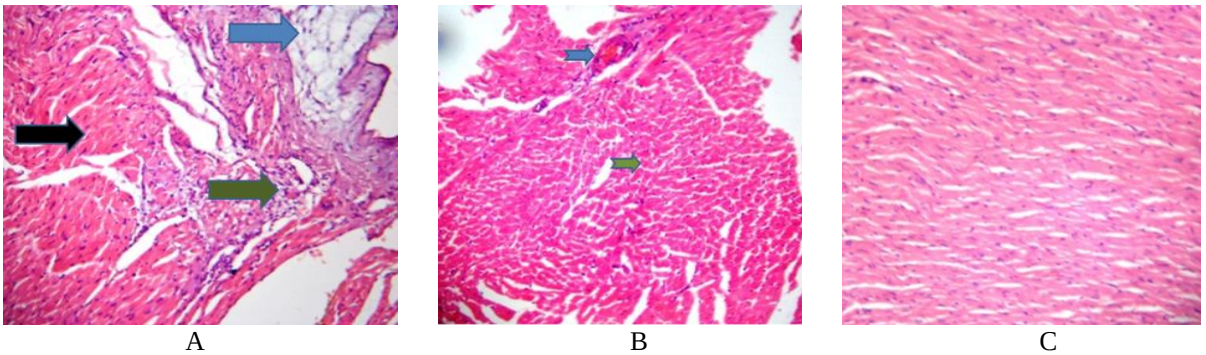
A. Section of rat treated with ethanolic extract of *P. thonningii* showing normal architecture with periportal inflammation (blue arrow); hepatocyte degeneration (green arrow); sinusoidal dilation with congestion (black arrow).
B. Section of rat treated with ethanolic extract of *L. lanceolata* showing normal architecture with normal central vein(blue arrow); focal periductal inflammation (green arrow).
C. Control



A B C

Figure 2: Photomicrographs of kidney tissue of rats (X 40 magnifications)

A. Section of rat treated with ethanolic extract of *P. thonningii* showing normal architecture with dilated vessel (blue arrow); tubular degeneration (green arrow); mesangial cell proliferation (black arrow).
B. Section of rat treated with ethanolic extract of *L. lanceolata* showing normal architecture with degenerate tubule (blue arrow); thickened vascular wall (green arrow); dilated and congested vessel (black arrow)
C. Control



A B C

Figure 3: Photomicrographs of heart tissue of rats (X 40 magnifications)

A. Section of rat treated with ethanolic extract of *P. thonningii* showing normal architecture with pericardial fat (blue arrow); pericardial inflammation (green arrow); normal myocardium (black arrow).
B. Section of rat treated with ethanolic extract of *L. lanceolata* showing normal architecture with congested vessel (blue arrow); normal cardiac myocyte (green arrow).
C. Control

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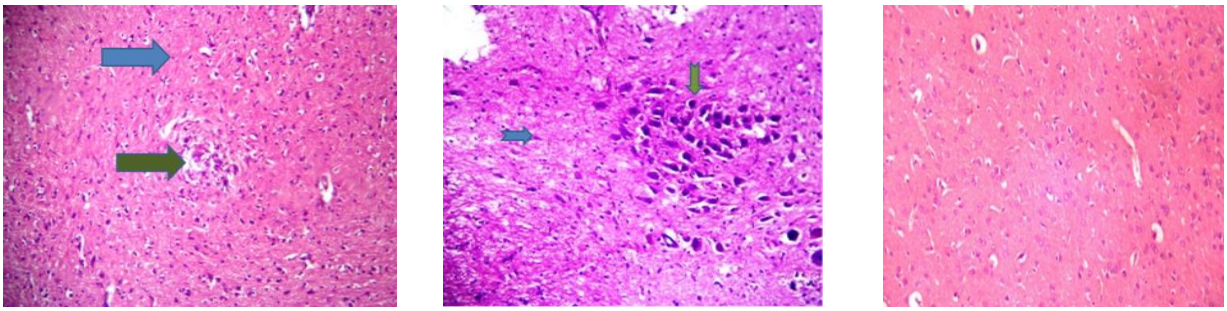

A B C

Figure 4: Photomicrographs of brain tissue of rats (X 40 magnifications)

A. Section of rat treated with ethanolic extract of *P. thonningii* showing normal architecture with cerebral oedema (blue arrow); focal gliosis (green arrow).
B. Section of rat treated with of ethanolic extract of *L. lanceolata* showing normal architecture with degenerating changes withcerebral oedema (blue arrow); focal gliosis (green arrow).
C. Control

IV. DISCUSSION

The reliance of humans on nature to manage ailments is a common practice in Nigeria as medicines from plants are even sold in pharmaceutical stores (Amadi *et al.*, 2011). In this present study to investigate the potential toxic effect of *P. thonningii* and *L. lanceolata* plants leaves extracts in human using animal model with repeated graded dosage administration the extracts failed to produce clinical signs of toxicity except for rats exposed to 2000mg/kg of *L. lanceolata* with recorded single death after day eleven (11) meaning that the crude extract might have metabolised to a more toxic end product that interferes with the cellular function and decrease cellular efficiency. However, the viscera of the dead rat show no macroscopic changes that could point to the cause of the death.

The administration of the plants leaves extracts were found to result in general body weights gain of all the rats meaning that the administrated extracts does not have any untoward actions that affect the growth of animals, as body weight change is used to assess the response to therapy of drugs and to indicate adverse effects of drugs; decrease in body weight is an index of detrimental toxic effect of drugs. In general toxic nature of the drug leads to abnormalities in body weight (Teo *et al*., 2002; Etuk and Muhammad, 2010).

In toxicology studies organ weight changes is an important endpoint for detecting harmful effects of chemicals (Rani, *et al*., 2007). In this study, the relative weights of the isolated organs indicated no significant change after treatment with the extracts, suggesting that the extracts did not induce any toxic effect on any of the organs. The macroscopic examination of these target organs showed no significant changes in colour, shape and texture in relation to that of the control groups, this probably may rule out organ toxicity at the doses tested. Furthermore, the histopathological results revealed no severe damage to the organs and this may indicate that both extracts was not toxic to the organs, since they all exhibited normal architecture.

In this study, the extracts generally disclosed no noticeable changes in the haematological target parameters at all tested doses, all the changes in the parameters remained within the normal limits of expected range for the rats used in this study when compared with control rats. However, the WBC count showed a slight increase in the *L. lanceolata* treated rats that received 2000mg/kg, but this was not statistically significant, such an increase in WBC level directly indicates the strengthening of the organism defences (Chang-Gue *et al*., 2003; Stanley *et al*., 2005). This elevation in WBC suggests that *L. lanceolata* contains an immune potentiating effect at 2000mg/kg. There is also a non-significant decrease in the level of GRAN for both plant extracts, which might be an indication of the nontoxic beneficial effect of the plants leaves extracts.

In the assessment of the biochemical parameters, the results in this study revealed that there were no significant changes/ alterations in the levels of most target parameters. The extracts only produced significant decrease in Blood Urea Nitrogen (BUN) levels at the lowest dose(1000 mg/kg) but the value was still within normal range (Semler *et al.*, 1992).Decrease in the levels of BUN indicates that both extracts at 1000mg/kg enhanced the clearance functioning of the kidney. The lack of significant alterations in the levels of ALT, AST, ALP, Cholesterol, Bilirubin and creatinine, are good indicators of liver and kidney functions and thus the results imply that the plants leaves extracts are non-hepatotoxic and non-nephrotoxic at all doses used. The normal levels of serum bilirubin concentrations at the graded doses are indication of non-adverse effects of both plant extracts on haemoglobin metabolism pathways.

V. CONCLUSION

The ethanolic extracts of *P. thonningii* and *L. lanceolata* leaves reveals no obvious worrisome significant toxic effects in the graded doses and have demonstrated that both plant leaves extracts are relatively safe when administered orally, since there is no alteration in the haematology, serum biochemical (except BUN at 1000mg/kg) body weight and relative organ weight. The

histopathological studies with normal architectures showed that no prominent damages/injuries in the target organs, however, the disclosure of some degree of lesions calls for watchfulness to constant exposure to these plants extract. The results in this study showed that these plants leaves extracts at the graded doses is not toxic in relation to the extraction solvent and route of exposure. However their toxicity profile cannot be concluded as this study provides the basis for further study on the detailed toxicity and pharmacological effects of the plants via other extraction method, exposure routes and more specific toxicity assays so as to further furnish information regarding the toxicity of these plants extract to ascertain their safety.

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