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Toxicity assessment of methanol extract of *Parinari curatellifolia* Planch ex. Benth

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Abstract: *Parinari curatellifolia* is mostly employed in the treatments of leukemia, anemia and malaria. The study was to determine the hematological, biochemical and histopathological effects of methanol stem bark extract of *Parinari curatellifolia* (PCME) on liver and kidney of adult female Wistar rats. The oral acute (Lorke's method) and sub-chronic toxicity of PCME were evaluate. Adult female Wistar rats were grouped into group I (n=6), normal control (5mL/kg of distilled water) and groups II-IV (100, 200 and 400mg/kg/day of PCME, n=6 each) for 30 days. On 31st day, biochemical, hematological and histopathological parameters were assessed. The LD_{50} was found greater than 5000mg/kg. In hematological parameters, RBC showed an increase in the treatment groups, however, the increment was not significant. HCT, PLT, MCH and MCHC levels were significantly increased (p<0.05) while WBC levels in all PCME groups were reduced (p<0.05). Amongst the liver biochemical parameters, only the ALP activity was significantly (p<0.05) raised. In kidney biochemical parameters, serum potassium and chloride were significantly (p<0.05) reduced. Histopathological findings on the liver showed mild infiltrating leukocytes, vascular congestion and piece meal necrosis compared to the normal anatomic features while that of the kidney appeared normal. In conclusion, PCME may be slightly toxic to the liver on repeated administration.

Keywords: *Parinari curatellifolia*, liver, kidney, histopathology, biochemical, hematology, toxicity.

INTRODUCTION

Medicinal plants (MP) are major sources of treatments for emerging diseases. Owing to people's quests for healing and other health-related issues, a high percentage of global population particularly in the rural communities patronise herbal medicine for healthcare needs without being aware of their possible adverse effects (Dougnon *et al*., 2021). The common perception that MP being natural in origin are without adverse effects is untrue and misleading (Gamde *et al*., 2019). Safety of MP is a major issue in traditional medicine. Ascertaining their toxicity profile is of paramount importance (Ugwah-Oguejiofor *et al*., 2019).

Parinari curatellifolia Planch. ex Benth, also known as Mobola plum, Fever tree or hissing tree belongs to the family Chrysobalanaceae. *P. Curatellifolia* is an evergreen medium to large tropical tree found in many deciduous woodlands of Africa including Nigeria where the indigenous names in Hausa, Yoruba, Fulani and Tiv languages are Rura, Idofun, Nawarre-badi and Ibua respectively (Halilu *et al*., 2018). Ethnobotanical uses of *P. curatellifolia* include anemia, leukemia, diarrhea and malaria (Kundishora *et al*., 2020). Previous scientific researches have shown that *P. curatellifolia* possesses cytotoxic activities (Anesu *et al*., 2020), anticonvulsant and sedative activities (Mshelia *et al.,* 2019), antimicrobial (Halilu *et al*., 2018), anti-snake venom and analgesic activities (Halilu *et al*., 2020). Some phytochemicals identified in *P. curatellifolia* which accounted for its medicinal properties were cardiac glycosides, tannins, anthraquinones, saponins, terpenoids and flavonoids (Halilu *et al*., 2018; Mshelia *et al*., 2019).

Despite the enormous nutritional and therapeutic properties of *P. curatellifolia*, there is no study on the risk awareness which guaranteed the safety of its use. Although reports of serious health injuries or mortality due to adverse effects of traditional medicines are scanty, several researches have revealed that they could be injurious to the body (Osagie-Eweka *et al*., 2021). The study was aimed to ascertain safety of methanol stem bark extract of *Parinari curatellifolia* (PCME) on liver and kidney of female Wistar rats (FWR).

MATERIALS AND METHODS

Plant preparation

Fresh stem bark of *P. curatellifolia* (FSB) was obtained from Zaria town of Kaduna State, Northwest Nigeria. It was identified by Mallam Musa of Department of

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Biological Sciences, Faculty of Science, ABU Zaria where voucher sample (903) was kept for reference. FSB was dried under shade and powdered with wooden pestle and mortar. The powdered plant material (3kg) was macerated in 6L of methanol for 24 hours and filtered using Buckner filter. The filtrate was evaporated using a rotary evaporator. The dried extract was then weighed and the percentage yield calculated.

Experimental animals

FWR were purchased from animal centre in UDUS, Nigeria. FWR were feed with rat feed and tap water *ad libitum*. Housing conditions were maintained at 25±2°C at 12h day/night cycles. Ethical Committee of Department of Pharmacology and Toxicology, UDUS approved the study (PTAC/PC(OA)/OT/007-18). Protocol for care and animal handling were as established in public health guidelines.

Acute toxicity

Oral acute toxicity evaluation of PCME was conducted in two phases to determine the LD_{50} using Lorke's method (Lorke, 1983). First phase of the study involved nine (9) FWR (n=3) which were divided into three different doses (10, 100 and 1000mg/kg). The FWR were monitored for 24h for behavioural signs of harmfulness and/or death. Next, the 2nd phase of the study involved three (3) FWR divided into three (3) doses (n=1) (1600, 2900 and 5000mg/kg). Observations were made as before.

Sub-chronic toxicity studies

The animals were allotted into 4 groups (n=6) as follows; group I, which is the normal control (5mL/kg of distilled water) and PCME groups II-IV (100, 200 and 400mg/kg/day of PCME) for 30 days. All FWR were treated orally. Twenty-four hours after last dose was given, FWR were anesthetized and blood samples drawn via cardiac puncture using a 10ml syringe.

About 2.5ml were poured into the EDTA bottles for hematological analysis while the rest of the blood was poured into the plain bottles for biochemical analysis (Yannick *et al*., 2022). The abdomens of animals were dissected and kidneys and livers excised and fixed in buffered 10% formal saline solution for histopathological assessment.

Hematological analysis

White blood cells (WBC), neutrophils, red blood cell (RBC), hemoglobin concentration (HBG), granulocytes (GRA), lymphocytes (LYM), MID cells (MID), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet distribution width (PDW), platelet (PLT) count, plateletcrit (PCT) and mean platelet volume (MPV) were measured using an auto-analyzer machine (Genesis™ HA6000) following protocols from the manufacturer.

Biochemical analysis

The blood collected from FWR were placed in plain bottles and centrifuged at room temperature at 3000 rpm for 5 min for serum assay. Serum albumin, total protein, total and direct bilirubin and the liver enzymes (Aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) were assessed using a chemistry analyzer machine (Raytochemray 120, Germany) following protocols from the manufacturer as described in the respective assay kits. Serum sodium, potassium, calcium, bicarbonate, urea and creatinine (kidney function test) were determined following standard procedures as described in the respective assay kit inserts.

Histological analysis

The fixed livers and kidneys tissues were processed in 70%, 80%, 90% and absolute alcohol following standard procedures. They were cut at 5µm (Surgcare microtome, model 335A, USA) and dewaxed for staining with hematoxylin and eosin (H&E). Olympus microscope equipped with a digital camera for photomicrographs was used to examine the tissues at x10 and x40 objectives (Diallo *et al*., 2020).

Qualitative and quantitative phytochemical screenings

Qualitative phytochemical screening of PCME for the presence of saponins, alkaloids, phenolics, flavonoids, tannins, steroids/triterpenoids, cardiac glycosides, cyanogenetic glycosides and anthraquinones was carried out using the methods described by Roghini *et al.*, (2018). The quantitative determination of phytochemicals was conducted according to the methods described by Neşe *et al*. (2022).

STATISTICAL ANALYSIS

Data were analyzed by SPSS 23.0 version software and presented as mean ± SEM. The differences amongst means were analysed using two way ANOVA and Dunnett's posthoc test for comparison between treated and control groups at $p \leq 0.05$ level of significance.

RESULTS

Percentage yield of PCME

The percentage yield of PCME was 8.2 % (w/w).

LD_{50} of PCME in FWR

Treatment of FWR with PCME at doses of 10 to 5000mg/kg using Lorke's method showed no obvious, adverse effect on the rats (table 1). PCME produced no visible toxicity/mortality signs in FWR. The LD_{50} was thus above 5000mg/kg.

Effect of PCME on hematological parameters after 30 days treatment

While RBC, PLT and MCHC levels that were increased, HBG, LYM, MID, GRA, MCV, PCT, MPV and PDW levels were not affected (table 2). WBC levels were significantly (p<0.05) reduced compared to normal control group.

Effect of PCME on biochemical parameters

The effect of PCME on serum liver function parameters is presented in figs. 1, 2 and 3. The mean activities of serum AST and ALT were not statistically different from normal control group (fig. 1). Again, serum levels of TP, ALB, ALP, TBIL and DBIL were not statistically different from control group (figs. 2 and 3).

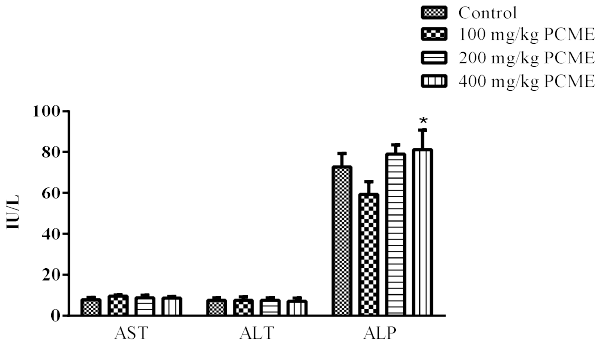


Fig. 1: Effect of PCME on serum AST, ALT and ALP levels in female Wistar rats. Values were expressed as mean ± SD *p<0.05

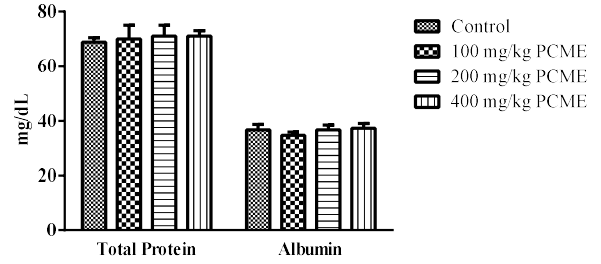


Fig. 2: Effect of PCME on serum Total protein and Albumin levels in female Wistar rats. Values were expressed as mean ± SD

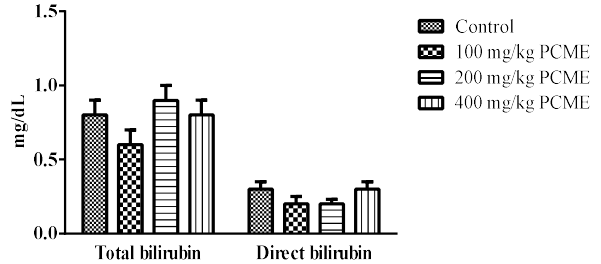


Fig. 3: Effect of PCME on serum Total and Direct bilirubin levels in female Wistar rats. Values presented as mean ± SD

In the kidney function test, serum potassium and chloride levels in all test groups were reduced in a dose-dependent manner (table 3) and significantly (p<0.05) at 400mg/kg with respect to normal control.

Histopathological effects of PCME on the liver and kidney

Histopathological findings from the liver treated with PCME showed normal hepatocytes in all groups (fig. 4A-D) but with mild infiltrating leukocytes and piecemeal necrosis in all the rats treated with PCME (fig. 4B-D) while that of the kidney showed normal regular glomeruli tuft, urinary space, renal tubules and interstitium in all the groups (fig. 5A-D).

Qualitative phytochemical screening

The various tests conducted revealed the presence of some classes of secondary plant metabolites with others not identified (table 4).

Quantitative phytochemical analysis

The quantity of the flavonoid and saponins were expressed in grams and as percentages in the whole plant extract (table 5).

DISCUSSION

Toxicity profile of any MP is evaluated to find and establish adverse effect, its significance and exposure level at which this effect is observed. LD_{50} is used to determine the safety or toxicity of a substance (Renata and Patrick, 2022). Our results from the acute toxicity study revealed that PCME administered via oral route to FWR at 5000mg/kg following Lorke's method of testing acute toxicity, failed to produce any sign of toxicity and/or mortality. Therefore, the LD_{50} was greater than 5000mg/kg and thus classified as relatively safe (Renata and Patrick, 2022). Previous investigation by Halilu *et al*., (2020) on the LD_{50} of the methanol and ethyl acetate extracts following i.p. route were 113mg/kg and 471.17mg/kg respectively revealing toxicity. The deviation from the result of the current finding may be attributed to the differences in the route of administration. However, information from acute toxicity is usually of limited clinical application hence the necessity of sub-chronic toxicity study.

Substances that are dosed frequently in the course of disease treatment may require repeated dosing toxicological evaluation (sub-chronic study). This is because daily use may amount to drug accumulation in the body leading to impairment of tissues and organs (Bariweni *et al*., 2018). Therefore, sub-chronic toxicity testing is essential in assessing the impact of the substance on the target organ, haematological or biochemical parameters which are not assessed in acute toxicity study (Ugwah-Oguejiofor *et al*., 2019). Hence,

Table 1: LD_{50} of PCME

Phase	Extract	Dose (mg/kg)	Mortality	Toxicity observed
Phase I	PCME	10	0/3	None
		100	0/3	None
		1000	0/3	None
Phase II		1600	0/1	None
		2900	0/1	Reduced activity
		5000	0/1	sedation

Table 2: Effect of PCME on hematological parameters

Parameter	Control	100 mg/kg	200 mg/kg	400 mg/kg
RBC ($\times 10^{12}$/L)	1.57±0.91	0.59±0.32	1.75±1.18	3.33±3.22
HGB (g/dL)	0.68±0.10	0.50±0.04	0.35±0.02	0.45±0.02
HCT (%)	6.25±0.83	9.00±5.50	27.40±12.34*	1.45±0.86
MCHC (g/L)	4.90±0.73	4.58±1.29	9.25±2.80*	6.00±2.12
MCH	0.87±0.16	4.52±1.46	16.48±3.37*	0.95±0.23
MCV	159.05±7.45	154.85±5.65	135.25±6.81	99.50±8.90*
RDW (%)	15.95±3.12	17.53±2.34	7.58±0.71*	13.73±3.71
WBC$\times 10^9$/L)	17.73±4.91	10.70±1.58*	4.84±1.31*	3.19±0.28*
LYM (%)	52.08±1.91	50.38±0.47	48.73±1.57	50.25±1.18
GRA (%)	26.88±1.13	25.73±1.17	28.35±0.67	28.65±0.58
MID (%)	20.90±1.03	22.00±0.18	22.93±1.44	21.10±0.63
PLT (10^9L)	268.78±94.63	2039.80±70.68*	2229.50±43.91*	1548.80±24.85*
PCT (%)	0.20±0.03	0.71±0.42	1.00±0.32	0.49±0.25
MPV (fL)	7.10±0.06	6.75±0.99	6.73±1.03	6.63±0.73
PDW (fL)	11.35±0.09	13.45±1.94	13.58±1.08	14.83±2.09

Values are mean ± SEM, n = 6. *p<0.05, red blood cell (RBC), hemoglobin concentration (HBG), hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), red cell distribution width (RDW), white blood cells (WBC), lymphocytes (LYM), granulocytes (GRA), MID cells (MID), platelet (PLT) count, plateletcrit (PCT), mean platelet volume (MPV) and platelet distribution width (PDW).

Table 3: Effect of PCME on serum renal function parameters

Treatment	Dose (mg/kg)	Na (mmol/L)	K (mmol/L)	Cl (Mmol/L)	HCO_3 (Mmol/L)	Urea (Mmol/L)	Creatinine (mg/dL)
Distilled water	5 ml/kg	139.25±1.38	4.15±0.26	100.2±2.14	26.50±1.71	4.30±0.35	0.70±0.09
PCME	100	135.50±2.60	3.50±0.25	93.7±2.78	27.50±1.32	4.55±0.55	0.63±0.09
	200	139.75±3.33	3.43±0.25	92.0±2.35	25.25±2.87	4.30±0.38	0.60±0.13
	400	133.75±4.71	2.75±0.12*	89.2±0.63*	25.75±2.95	5.28±0.33	0.70±0.15

Values are mean ± SEM, n = 6. *p<0.05

the sub-chronic toxicity profile of PCME was evaluated in rats using gross assessment, haematological, biochemical and histological parameters.

Sub-chronic administration of PCME caused no observable marks of toxicity or mortality in the rats. Haematological parameters are frequently used to evaluate functional characteristics of blood and blood forming organs such as the liver and kidney (Olayode *et al*., 2019). The extent of toxicity of an agent/drug are often determined through assessment of these parameters. In our study, PCME caused an increase in RBC and platelet counts. This suggests that PCME may possess constituents capable of causing stimulatory activities in the hematopoietic system which could lead to the rise in the production of RBC and Platelets. The reduction in white blood cell count observed suggests that PCME may possess some bioactive agents that could cause destruction or impaired production of white blood cells. Thus, administration of PCME may predispose to infection consequent of the reduction of WBC (Ononamadu *et al*., 2020).

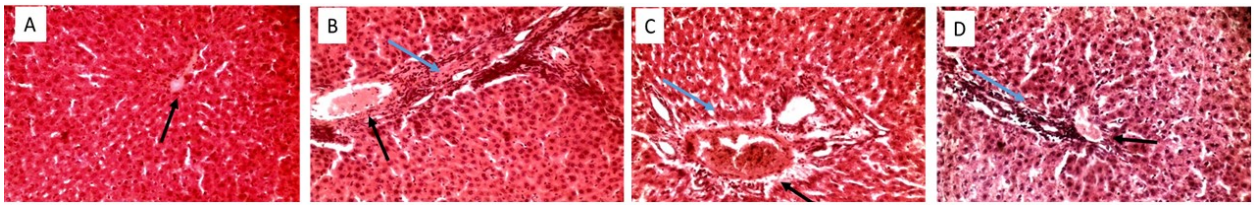
Table 4: Secondary plant metabolites

Metabolite/Test	Result
1. Saponins Frothing test & Hemolysis	+
2. Alkaloids Mayer's, Dragendorff's & Hager's	-
3. Phenolic hydroxyl group Ferric chloride	+
4. Flavonoids Alkali test	+
5. Tannins Lead aceate	+
6. Phlobatannins Hydrochloric acid	-
7. Steroids/triterpenoids Salkowski's	+
8. Cardiac glycosides Keller-Kiliani's	-
9. Quinones Concentrated H_2SO_4	+
10. Anthraquinones Borntrager's	+
11. Coumarins Ammonia and Sodium Hydroxide solution	+
12. Cyanogenetic glycosides Guignard's	+

In table 4, '+ ' means identified while '–' means not identified

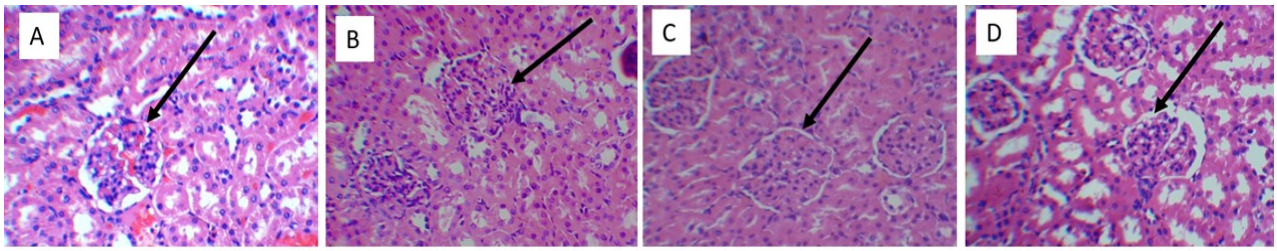
Table 5: Quantitative phytochemical analysis

Metabolite	Weight (g)	Quantity (%)
Saponins	0.46	2.30
Flavonoids	1.86	18.6



A (control): liver revealing healthy hepatocytes (black arrow). B (100mg/kg PCME): liver revealing hepatocytes with mild infiltrating leukocytes and piecemeal necrosis (blue arrow). C (200mg/kg PCME): liver revealing mild infiltrating leukocytes and piecemeal necrosis (blue arrow). D (400 mg/kg PCME): liver revealing mild infiltrating leukocytes and piecemeal necrosis (blue arrow).

Fig. 4: Photomicrograph of the livers of Wistar rats treated with PCME



A= (Control), B= (100 mg/kg), C= (200 mg/kg) and D= (400 mg/kg). All Kidney sections showed normal regular glomeruli tuft (black arrow) (H&E. X 400).

Fig. 5: Effect of PCME on the kidney histology of the treated Wistar rats

AST, ALP and ALT are classical biomarkers for evaluating liver injury (Kaid *et al*., 2019). The serum concentrations of these enzymes are index of damage or changes in the permeability of hepatocytes membrane (Mega *et al*., 2021). In our study, the levels of AST and ALT were similar to the control. However, ALP activity was significantly increased. Iso-enzymes of ALP abound in the various organs of the body including the bones, placenta, kidneys, intestines and liver. There are many clinical states (including bone and liver diseases) in which the activity of the ALP is elevated (Dana *et al*., 2020). With significant elevation in serum ALP activity and normal AST and ALT activities, ALP upsurge may likely emanate from other ALP iso-enzyme synthesising tissues apart from liver (Osagie-Eweka *et al*., 2021). Again, biliary duct obstruction may be implied (Alkali *et al*., 2018). It is also imperative to note that the mild infiltrating leukocytes and piecemeal necrosis observed in

the liver of rats treated with PCME might have caused hepatobiliary obstruction, resulting in increased ALP activity. Normal levels of serum total proteins and albumin imply that PCME failed to compromise the liver's synthesizing function. Therefore, toxicity caused by the extract to the liver may be mild. These findings on liver enzymes are in accordance with previous reports on the seeds and polyherbs of *Parinari curatellifolia* (Dana *et al*., 2020). There is often preservation of the metabolic and secretory functions of the liver amidst hepatic cells damaged. Our extract induced mild histopathological effect in the liver even though the liver enzymes AST and ALT were apparently similar to the normal control. This result was similar to the necro-inflammatory and edematous changes caused polyherbal mixture of *Parinari curatellifola* (Maha and Eman, 2022). These histopathological changes give credence to the deleterious effects of the stem bark extract of *Parinari curatellofolia* on prolonged use.

The kidneys play critical role in maintaining overall health. Estimation of urea and creatinine levels are often used to evaluate kidney function (Friedrich, 2020). In our study, urea and creatinine levels were similar to the control. The implication of the data obtained is that the ability of the kidney to carry out its functions were not compromised by PCME (Friedrich, 2020). The extract however caused a decrease in potassium and chloride levels in the animals. This is indicative of adequate renal (electrolyte regulatory) function. Increased serum potassium is indicative of renal failure (Friedrich, 2020). Histopathological evaluation also supported these findings.

CONCLUSION

LD_{50} of PCME per oral was calculated to be high (>5000mg/kg) in FWR. Sub-chronic administration of PCME in Wistar rats decreased WBC and increased platelet count but did not affect serum biomarkers for liver and kidney functions except potassium and chloride ions. Histopathological effects in the liver were mild infiltrating leukocytes, vascular congestion and piece meal necrosis while the kidneys appeared normal. Thus, PCME may cause liver injury on prolonged use and therefore caution should be exercised.

REFERENCES

Alkali YI, Jimoh AO and Muhammad U (2018). Acute and sub-chronic toxicity studies of methanol leaf extract of *Cassia singueana* F. (Frensen) in wistar rats. *J. Herb. Med.,* **4**(2): 1-6.

Anesu K, Simbarashe S and Stanley M (2020). Determination of the cytotoxic effect of different leaf extracts from *Parinari curatellifolia* (Chrysobalanaceae), *Hindawi J. Toxicol.* **2020**: Article ID 8831545, 11 pages.

Bariweni MW, Yibala OI and Ozolua RI (2018). Toxicological studies on the aqueous leaf extract of *Pavetta crassipes* (K. Schum) in rodents. *J. Pharm. Pharmacog. Res*., **6**(1): 1-16.

Dana M Zaher, Mohammed I El-Gamal, Hany A Omar, Sarah N Aljareh, Salma A Al-Shamma, Aya J Ali, Sumera Zaib and Jamshed Iqbal (2020). Recent advances with alkaline phosphatase isoenzymes and their inhibitors, *Arch. Pharm. Chem. Life Sci.*, **353**(5): e2000011.

Diallo MS, Dial CM, Poaty H and Faye O (2020). Histopathologic profile of miscarriages during first trimester of pregnancy in teaching hospital of grand yoff in dakar (Senegal). *Open J. Pathol.,* **10**(1): 56-65.

Dougnon TV, Hounsa E, Agbodjento E, Koudokpon H, Legba B, Fabiyi K, Afaton A, Sintondji K, Akpode B, Kloto´e, JR, Tchobo F, Bankole H and Dougnon TJ (2021). Toxicological characterization of ten medicinal plants of the beninese flora used in the traditional treatment of diarrheal diseases. *Hindawi-Evid.-Based Complement. Altern. Med.* 2021: 1-13.

Egbung GE, Odey OD and Atangwho IJ (2017). Effect of *Vernonia calvoana* extract on selected serum kidney function biomarkers of acetaminophen treated wistar rats. *Asian J. Biochem.* **12**(3): 99-104.

Firas RA and Wathiq AHA (2017). Effect of some environmental factors on hematological parameters in apparently healthy Iraqi Awassi sheep. *J. Entomol. Zool. Stud.* **5**(3): 1668-1671.

Friedrich CL (2020). Biomarkers and predicting acute kidney injury. *Acta Physiologica*, **231**(1): e13479

Gamde SM, Kabiru H, Abdulazziz A, Abubakar KA, Musa AA and Perede A (2019). Histopathological and biochemical effects of aqueous leaf extract of *Cadaba farinosa* on the liver of adult wistar rats. *Int. J. Res. Med. Sci*., **7**(10): 3716-3721.

Halilu ME, Ifeanyi GI, Salvia TM, Ungokore HY, Dibal MY and Mathias SN (2018). Antimicrobial activity of saponins from *Parinari curatellifolia* (Planch. ex benth) root bark extract, *Nig. Res. J. Chem. Sci.* **4**(1): 28-51.

Halilu ME, October N, Balogun M, Namrita L and Abubakar MS (2013a). Studies of *in vitro* antioxidant and cytotoxic activities of extracts and isolated compounds from *Parinari curatellifolia* (Chrysobalanaceae). *J. Nat. Sci. Res.* **3**(13): 149-154.

Halilu ME, October N, Ugwah-Oguejiofor CJ, Jega AY and Nefai MS (2020). Anti-snake venom and analgesic activities of extracts and betulinic and oleanolic acids isolated from *Parinari curatellifolia*. *J. Med. Plants Econ. Dev.* **4**(1): 1-8.

Kaid F, Alabsi AM, Alafifi N, Ali-Saeed R, Al-koshab MA, Ramanathan A and Ali AM (2019). Histological, biochemical and hematological effects of goniothalamin on selective internal organs of male

sprague-dawley rats. *Hindawi J. Toxicol.* **2019**: Article ID 6493286, 13 pages. https://doi.org/10.1155/2019/6493286

Kundishora A, Sithole S and Mukanganyama S (2020). Determination of the cytotoxic effect of different leaf extracts from *Parinari curatellifolia* (Chrysobalanaceae). *J. Tox.***2020**: Article ID 8831545.

Lorke D (1983). A new approach to practical acute toxicity testing. *Arch. Toxicol.* **54**(4): 275-287.

Maha MG and Eman S (2022). Efficacy of *Cuminum cyminum* (L.) seed oil on acute toxoplasmosis: An experimental study on albino mice, *Parasitol. Unit. J.*, **15**(1): 98-109.

Mega A, Marzi L, Kob M, Piccin A and Floreani A (2021). Food and nutrition in the pathogenesis of liver damage. *Nutrients,* **13**(4): 1326

Mshelia HE, Ugwah-Oguejiofor CJ, October N and Abubakar K (2019). Isolation of oleanolic acid from *Parinari curatellifolia* (Planch ex. benth) stem bark and evaluation of its anticonvulsant and sedative activities in rodents. *Trop. J. Nat. Prod. Res.*, **3**(1): 17-21.

Neşe BC, EmineY, Kultigin C and Selin SK (2022). Qualitative and quantitative phytochemical screening of Nerium oleander L. extracts associated with toxicity profile. *Sci. Rep.*, **12**(2022): 21421

Olayode OA, Daniyan MO and Olayiwola G (2019). Biochemical, hematological and histopathological evaluation of the toxicity potential of the leaf extract of *Stachytarpheta cayennensis* in rats. *J. Tradit. Complement. Med*., **10**(6): 544-554.

Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, Lilly P, Sanders J, Ononamadu CJ, Alhassan AJ, Ibrahim A, Imam AA, Ihegboro GO, Owolarafe AT, Ezeigwe OC, Atiku MK and Sule, MS (2020). Toxicological study of aqueous-methanol solvent fraction of methanol extract of *Dacryodes edulis* leaves. *Toxicol. Rep.,* **7**: 909-918.

Osagie-Eweka SE, Orhue NEJ, Omogbai EKI and Amaechina FC (2021). Oral acute and sub-chronic toxicity assessment of aqueous leaf extract of *Simarouba glauca* DC (Paradise tree). *Toxicol. Rep*., **8**: 239-247.

Renata RK and Patrick M (2022). Quantitative measurements of pharmacological and toxicological activity of molecules, *Chemistry*, **4**(4): 1466-1474.

Roghini R and Vijayalakshmi K (2018). Phytochemical screening, quantitative analysis of flavonoids and minerals in ethanolic extract of *Citrus paradisi, Int. J. Pharm.l Sc. & Res.* **9**(11): 4859-4864.

Sipes, G, Bracken W and Dorato M (2000). Concordance of the toxicity of pharmaceuticals in humans and in animals. *Regulatory Toxicol. Pharmacol*, **32**(1): 56-67.

Ugwah-Oguejiofor CJ, Okoli, CO, Ugwah MO, Umaru ML, Ogbulie CS, Mshelia HE, Umar M and Njan AA (2019). Acute and sub-acute toxicity of aqueous extract of aerial parts of *Caralluma dalzielii* N. E. Brown in mice and rats. *Heliyon.*, **5**(1): e01179.

Yannick CT, Danielle CB,Yolande SMN, Paul DDD, Pascal EO, Ronald B, Rodrigue N F, Lohik MN, Michel N, Théophile D and Pierre K (2022). Acute and subchronic toxicity studies on the aqueous extract of the plant mixture (*Bidens pilosa* and *Cymbopogon citratus* aerial parts) in rat model. *J. Toxicol.*, **20**(5): 1-13.