Acute and sub-acute toxicity profiles of methanol leaf extract of *Psydrax subcordata* on swiss albino mice

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ABSTRACT

Background: *Psydrax subcordata* (DC) Bridson (Rubiaceae) shows a vast range of pharmacological activities as evident in its folkloric use with little information on its toxicity. The toxic effect of methanol extract of *P. subcordata* on the kidney biomarkers, structure and haematological parameters were determined using male Swiss albino mice.

Methods: Two groups of mice consisting of three mice each were administered with a single oral dose of 2000mg/kg and 5000mg/kg to determine acute toxicity. Forty mice (n = 10/group) were treated orally with distilled water, 250mg/kg b.w of extract, 500mg/kg b.w of extract and 1000mg/kg b.w of extract for 28 days. Lethality/mortality, haematological, biochemical and histological parameters were determined.

Results: The result of the acute study reveals that the LD50 is more than 5000mg/kg as no death was recorded in the animals. The biochemical parameters reduce with increase in dose same as the haematological parameters, except for the WBC which increases as the dose increases. The monocyte and eosinophils levels remain unchanged. The histopathological analysis of the kidney reveals vascular congestion with increase in dose.

Conclusion: *P. subcordata* extract causes increasing renal and liver histological deterioration as the doses increases, though no mortality was recorded. Thus, higher doses of the extract are not safe especially when administered for prolonged duration orally.

Keywords: Acute toxicity, Lethality, Methanol extract, *Psydrax subcordata*, Swiss albino mice

1. INTRODUCTION

Natural products have been in use for their health benefits as long as human civilization is concerned. Products from animals, plants and mineral were the main source of drugs and they still retain their historical importance [1]. All over the world, especially in developing countries herbal drugs are playing an important role in health care programs because they are cheap and locally available. There is a general belief amongst the consumers globally that herbal drugs are always safe because they are "natural". However, evidences suggest otherwise. The mere fact that a product is "natural" may not signify that the product is safe. Although limited evidence suggests that adverse effects associated with the use of herbal drugs are less likely to occur than with conventional drugs, they do occur though usually mild and only affecting a small number of people [2]. Recent evidence suggests that some of the herbs considered to be safe over the last many decades have proven to be associated with health hazards. Herbal remedies can act either as agonists or antagonists that potentiate some drug therapies. Therefore, an understanding of conventional drugs is an essential prerequisite for effective herbal therapeutics. The advancement of technology has enabled the scientists to detect minute amounts of carcinogenic and toxic chemicals in these herbs and recognize or evaluate potentially hazardous effects of some of the herbs which had been used in traditional medicine since centuries [2]. Toxicity testing is the determination of potential hazards a test substance may likely produce and the characterization of its action. Toxicity testing employed wide range of test in different species of animals with long term administration of drug, regular monitoring of physiological, biochemical abnormalities and detailed post mortem examination at

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the end of the trial to detect gross or histological abnormalities [3]. *Psydrax subcordata* (DC.) Bridson is an herbaceous plant belonging to the family of Rubiaceae (also known as *Canthium subcordatum*). It is a fruit bearing plant, commonly housing colonies of ants [4]. *Psydrax subcordata* is a plant widely distributed in the tropical region of West Africa. It has been used for the management of haemorrhoids, stomach ulcer, piles, abdominal pains, dyspepsia, enteritis, stomach aches, gastritis, heartburns and intestinal complaints [5]. *Psydrax subcordata* has been claimed to be effective based on is folkloric use for the treatment and management of several diseases of man, however, folks use this plant without any knowledge of the toxic effect of the plant if any or at which dose is safe for the effectiveness. It is therefore necessary to carry out this research to determine the acute and sub- acute toxicity of the plant *P. subcordata* and also evaluates toxic effect on the renal biomarkers, hematological parameters and as well the renal and liver histopathology of Swiss male albino mice.

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 *Plant material and preparation of plant extract*

Psydrax subcordata plant was collected from University of Lagos, Akoka Campus, Nigeria. The Plant was identified at herbarium of the Department of Botany, Faculty of Science, University of Lagos where a voucher specimen was deposited and assigned the voucher number LUH 7561. The leaves of the plants were washed well with clean water, dried at room temperature (25^{o}C ± 2^{o}C) for 14days and powdered using electric blender. A batch of 1500g of *P. subcordata* powdered leaves was macerated with absolute methanol with intermittent shaking at room temperature and changing of solvent every day (72 hours) for a week. The extracts were then filtered and concentrated using rotary evaporator, further drying was done at 40^{o}C and dried extract was stored in airtight bottle in a refrigerator a 4^{o}C. The extract of *P. subcordata* was re–dissolved in distilled water when ready for administration.

2.1.2 Experimental Animals

Male Swiss albino mice (inbreed stock), averagely weighing 20g were purchased from Komad Farm Limited, km. 27 Old Abeokuta-Lagos Expressway, Dalemo, Ogun State, Nigeria. All animal experiments were carried out as approved by the Health Research Ethics Committees of the College of Medicine University of Lagos, Idi-Araba (CMUL/ACUREC/02/20/726) and were acclimatized and fed with standard rodent diet from Livestock Feeds PLC, Ibadan, Oyo state, Nigeria, and water ad libitum for 14days at the Animal House of the College of Medicine, University of Lagos. The animals were housed in bottomed wire cages arranged in rows in standard environmental conditions of temperature (25 ± 2°C), humidity (55 ± 10%) and with 12-h cycle of day and night conditions. The cage beddings and water bottles were cleaned daily. The mice were carefully observed for any behavioural abnormalities such as changes in breathing, locomotors activity, convulsion, tremor, excessive salivation, diarrhoea, sedation and oedema.

2.2 Methods

2.2.1 Acute Toxicity Test

Acute toxicity test was performed using six (6) mice. Extract doses 2000 mg/kg body weights were administered to each of the three animals in the first group and subsequent dose of 5000 mg/kilogram body weight were administered to another group of three animals [6, 7, 8]. The animals were kept under the same natural condition and observed for toxicity signs and mortality for 72 hours and for the next fourteen days for any death or changes in general behaviour and other physiological activities [9]. The volume to be administered to each animal was calculated using the formula below:

Volume (ml) = (weight of animal (kg) x Dose (mg/kg))/(concentration(mg/ml))

2.2.2 Sub-Acute Toxicity Test

Sub-acute toxicity study was conducted with forty-eight (48) male Swiss albino mice divided into four groups of 6 mice each with doses of 250, 500 and 1000 mg/kg body weight administered per group representing low, intermediate and high dose groups respectively. These doses were chosen because they were higher than effective doses [10] and therapeutic dose [11] in other studies. The doses were administered orally once daily at 24 hour intervals for 28 days and the vehicle (distilled water) was administered to the control groups in the experiments. Biochemical analyses were carried out on the plasma while liver and kidney were examined histologically.

2.2.3 Animal Sacrifice and Sample Collection

At the end of the 28 days' treatment period, the animals were fasted of feed but left with drinking water ad libitum for 24 hours and blood samples were collected by retro-orbital plexus using capillary tubes into ethylenediamine

tetra acetic acid (EDTA) and non-heparinised tubes for the haematological and biochemical studies respectively. The kidney and liver of each animal was also harvested by longitudinal incision in the ventral surface of the abdomen after cervical dislocation into a formaldehyde solution and prepared for histological analysis.

2.2.4 Haematological analysis

The blood pooled from the animals in each groups were collected into bottles containing ethylenediamine tetra acetic acid (EDTA) as anticoagulant. The haematological composition of the blood was measured using 3 mice per group (n = 12). The haematological parameters viz., Red Blood Cells (RBCs) count, White Blood Cells (WBCs) count, Platelets cell count (PLT), Mean corpuscular volume (MCV), Mean platelet volume (MPV), Haemoglobin (Hb), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC) and Packed cell volume(PCV) were estimated by using the improved Swelab auto counter by Boule diagnostics. RBC, PLT, MCV, WBC, MPV was detected using the volumetric impendence method. Haemoglobin was detected using the photometric haemoglobin concentration method [12].

2.2.5 Biochemical analysis

Blood serum for the biochemical analysis was obtained by coagulation and centrifugation of the blood sample in the non-heparinized centrifuge tubes. Serum samples were analysed for alkaline phosphatase (ALP) (phenolphthalein method described by Babson et al., 1957[13]), aspartate transaminase (AST), alanine transaminase (ALT), albumin (ALB), total bilirubin (TBIL) and direct bilirubin (using Jandrassik and Grof technique as described by Tolman et al., 1999 [14]), using Mindray BS-120 automated analyzer.

2.2.6 Histopathological assessment

The harvested vital organs were fixed in 10% buffered formalin solution in labeled bottles to observe for possible histopathology changes and prevent autolysis and putrefaction. After fixation, the tissues were exposed to routine processing (re-sectioning, dehydration, infiltration), embedded in paraffin and section at 3–5 mm. Tissue sections were stained with hematoxyllin and eosin stain using Leica DM 500 microscope attached with camera Leica ICC50 HD (Leica Microsystems Ltd., Switzerland).

2.3 Statistical analysis

The data were analysed by using Graph Pad Prism version 7.0 (Graph Pad Software, Inc. San Diego, CA, USA) and the results expressed as Mean + SEM. The significant differences between and within the groups were analysed statistically by One-way ANOVA followed by multiple comparisons of their means using Turkey's post hoc test. Level of significance was considered at values of $p<0.05$.

3.0 RESULTS

3.1 Acute toxicity testing

After administration of the extract of *P. subcordata* at a dose of 2000 and 5000mg/kg according to OECD guideline 423 and observed for 28 days, mortality was not observed in the tested mice and no physically observable sign of toxicity was detected during the experimental period.

3.2 Effect of plant extract on biochemical parameters

The result presented in Table 1 show that only one parameter was statistically significant during the 28 days treatment period. A significant ($p<0.05$, 0.0456) reduction of albumin at the dose of 1000mg/kg was observed in the treated mice. Other biochemical parameters (AST, ALT, ALP, T.BIL, and D.BIL) of the treated mice were not significantly different compared to the control mice.

Table 1: Biochemical Parameters in Mice after Oral Administration of *Psydrax subcordata*

Biochemical Parameters	Control	250mg/kg	500mg/kg	1000mg/kg
AST (µ/L)	53.50 ± 24.04	46.00 ± 12.06	33.33 ± 3.33	50.00 ± 4.58
ALT(µ/L)	27.75 ± 6.95	29.67 ± 13.68	28.00 ± 6.03	18.00 ± 3.00
ALP (µ/L)	26.25 ± 2.96	29.67 ± 2.19	30.00 ± 0.58	26.00 ± 3.61
T.BIL umol/L	6.100 ± 0.40	6.867 ± 0.19	7.133 ± 0.09	6.133 ± 0.58
D.BIL umol/L	2.650 ± 0.13	2.367 ± 0.45	2.600 ± 0.84	2.633 ± 0.23
Creatinine (umol/L)	25.00 ±1.23	24.00 ±2.00	24.33±2.33	20.33 ±2.33
Urea (mmol/L)	9.50 ±1.24	8.43 ±0.43	7.97 ±0.03	7.63 ±0.59
Protein (g/L)	81.75 ±2.4	77.67 ±0.88	77.67 ±1.86	77 .33±1.33
Albumin (g/L)	43.75 ±2.10	39.33 ±0.33	39.00 ±1.00	37.00 ±1.00*

*Data expressed as Mean ± SEM (Standard Error of Mean), * p< 0.05.*

3.3 Effect of plant extract on hematological analysis

Table 2 shows the values of hematological parameters after the 28 days treatment period. Compared with the control mice, all measured serum hematological parameters were not significantly ($p < 0.05$) different.

Table 2: Hematological Parameters in Mice after Oral Administration of *Psydrax subcordata*

Hematological parameters	Unit	Control	250mg/kg	500mg/kg	1000mg/kg
WBC	103/µL	14650 ± 2661	13867 ± 2367	15600 ± 1447	18267 ± 1091
PCV	%	38.50 ± 1.190	42.33 ± 1.764	39.33 ± 1.333	35.67 ± 0.667
HGB	g/dL	12.60 ± 0.394	13.83 ± 0.524	12.97 ± 0.570	11.80 ± 0.153
RBC	106/µL	4.200 ± 0.091	4.467 ± 0.203	4.300 ± 0.322	4.167 ± 0.273
Neutrophil	%	0.750 ± 0.479	1.333 ± 0.882	0.667 ± 0.667	1.333 ± 0.667
Lymphocyte	%	99.25 ± 0.479	98.67 ± 0.882	99.00 ± 0.577	98.67 ± 0.667
Monocyte	%	0.00	0.00	0.00	0.00
Eosinophils	%	0.00	0.00	0.00	0.00
PLT	µL	36250 ± 2136	27667 ±666.7	43667 ± 6766	36667 ± 4327
MCHC	g/dL	32.75 ± 0.479	32.67 ± 0.333	32.67 ± 0.333	33.00 ± 0.577
MCH	Pg	91.75 ± 3.326	95.00 ± 0.577	92.33 ± 4.096	86.67 ± 5.840
MCV	fL	29.50 ± 1.190	31.00 ± 0.577	30.33 ± 0.882	28.67 ± 1.856

Data expressed as mean ± SEM.

PCV= Packed Cell Volume, HGB= Hemoglobin, RBC= Red Blood Cell, PLT= Platelet, WBC= White Blood Cell, N= Neutrophils, L= Lymphocyte, M=Monocyte, E=Eosinophils, MCHC=Mean Corpuscular Hemoglobin Concentration, MCH= Mean Corpuscular Hemoglobin, MCV= Mean Cell Volume.

3.4 Effect of plant extract on creatinine and urea

The extract of the plant did not produce any significant ($p < 0.05$) effect on creatinine and urea after 28 days' daily administration (Table 1).

3.5 Effect of plant extract on histopathological assessment in the sub-acute

The microscopic examination of the tissues of the kidney and liver of the mice showed some remarkable cellular appearance with alterations in the treatment groups as against the control group. Histopathological examinations of tissues from all the harvested organs indicated treatment-related changes as shown in figure 1.

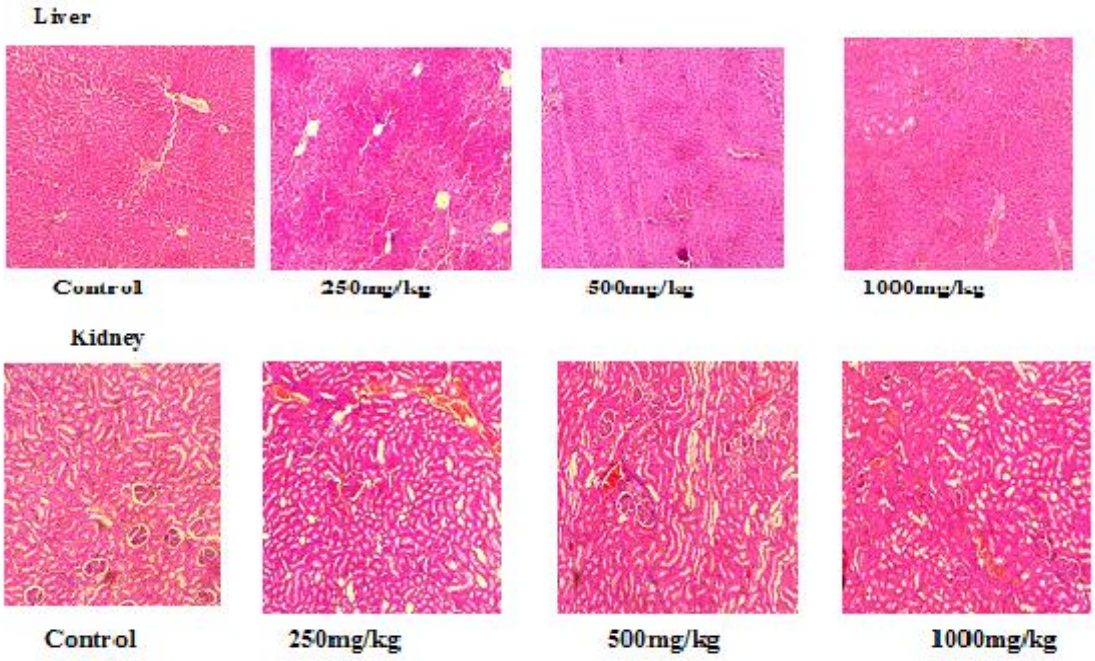

Figure 1: Histopathological examinations of tissues from the liver and kidney.

4.0 DISCUSSION

Natural products have been and are still a major plan in supporting the primary health systems. Their bio-activity is mainly associated with secondary metabolites, often elaborated for the plant defense. Some of these phytochemicals accidentally protect humans against pathogens and that is why they are a main target for drug

prospecting programs. These phytochemicals are known to have several properties important to cells including; prophylactic, therapeutic, nutritive and immune-modulative properties [15]. According to Teschke et al., 2014 [16], it is important to note that in a situation of limited evidence of therapeutic efficacy established toxicity concern in respect to herbal remedy, stringent evaluation of risk and benefit ratio is essential as well as experimental screening method in other to evaluate the claimed activity and to ascertain the safety and efficacy of these herbal products as well as to establish their active constituent [17]. Previously, five iridoid dimers, canthiumosides 1–5, together with nine known compounds, shanzhigenin methyl ester, 1-epishanzhigenin methyl ester, linearin, 1-epilinearin, mussaenoside, shanzhiside methyl ester, 3′,4′,7-trihydroxyflavone, betulinic acid, and oleanolic acid were isolated from the fruits of *Canthium subcordatum* DC (Syn. *Psydrax subcordata* (DC) Bridson) [18]. The phytochemical constituents of the methanol extract of *Psydrax subcordata* had been investigated. The findings of the phytochemical investigations revealed the presence of flavonoids, saponins and cardiac glycoside [19]. Phytochemical investigation of the leaves and bark of *Psydrax subcordata* has led to the isolation of six new iridoids, subcordatanols I–V and 1-O-methylcrescentin I, along with two known analogues [20]. In this present study, acute and sub-acute toxicity as well as the biochemical parameters, heamatological parameters and histopathological examination of kidney and liver of experimental mice were determined. In acute toxicity test, mortality was not observed in mice with doses of 2000 and 5000mg/kg throughout 28days of study. In sub-acute toxicity test, no mortality was observed in the animal with the maximum daily dose of 1000 mg/kg throughout 28 days of study. Significant changes were not found in breathing, sense of touch/sound, behavior pattern and locomotors activity. Convulsion, tremor, excessive salivation, diarrhea, sedation and edema were not observed. Evaluation of haematological abuses or its implications in animal models and humans are often carried out by evaluating the complete blood count or full counts in order to ascertain changes in erythrocytes, leucocytes and platelets which guide in the diagnosis of anemia, leucocytosis and thrombocytopenia often implicated in malaria infections. It is normally evaluated by testing for Red Blood Cell, Packed Cell Volume or Hematocrit, Hemoglobin, Mean Cell Volume of red blood cell, Mean Cell Hemoglobin, Mean Cell Hemoglobin Concentration, White Blood Cell levels and comparing the results with standard values. In mice, the normal range of HGB is 13.6 – 13.9 g/dL, MCV is 45 to 55 fL, MCHC is 30 to 38 g/dL, PCV is 35% to 52%, WBC is 2000 to 10,000 per microliter, PLT is 1,000–1,500 × 103 cells/µl, RBC is 7 x 106 to 13 x 106/mm3 [21]. The results and the test statistics done showed that there was no significance difference observed in WBC, HGB, RBC, PLT, MCHC, MCH, MCV, Neutrophil and lymphocytes when compared with the control, which suggests that the extract did not have any effect on these hematological parameters profile of the mice but there was a statistically significance difference for PCV which could suggest that the extract had an effect on the packed cell volume of the mice. Also multiple comparisons among the individual group showed a significant difference in PCV and HGB parameters between the doses of 250 and 1000mg/kg administered to the mice. The low dose group has the highest mean PCV when compared to the control group and other groups. It was observed that *P. subcordata* increased the PCV at a low dose (250mg/kg) beyond which the PCV begins to reduce gradually. The lowest PCV was observed in the high dose group. It is therefore suspected that low PCV observed could have been caused by one or a combination of these factors; Internal or external haemorrhage – bleeding, Complication of chronic renal failure – kidney disease, Pernicious anemia – vitamin-B12 deficiency [22] which is as a result of increase in dose of *P. subcordata* extract. Although there was no significant difference in PCV level when each group was compared with control group, but there was significant reduction in PCV volume between the low dose group and the high dose group. This signifies that beyond a certain dose (which is yet to be determine in human) *P. subcordata* would result in decrease in PCV. The values of MCHC, MCV, and MCH were consistent with the values observed in the PCV. Hematocrit can be estimated from measurements of the mean cell volume (MCV) or the mean corpuscular hemoglobin concentration (MCHC) [23]. The low dose group also has the highest mean hemoglobin when compared to the control group and other groups. It was observed that *P. subcordata* increased the hemoglobin at a low dose (250mg/kg) beyond which the hemoglobin begins to reduce gradually. The lowest PCV was observed in the high dose group. If a hemoglobin test reveals that the hemoglobin level is lower than normal, it means a low red blood cell count (anemia). This means that an increased dose of the plant extract could result in anemia. There was no significant difference in the hemoglobin level when each group was compared with the control group, but there was a significant different between the low dose group and high dose group. In the histopathological study of the liver, the histological sections of the liver tissue showed parallel radial arranged plates of hepatocytes with Central vein (CV), portal vein (PV) and the basophilic portion with nucleus and the acidophilic cytoplasm of the acinar cells. Mice treated with the dose 250mg/kg of the plant extract (*P. subcordata*) showed no difference when compared with the control, no abnormalities were seen. In the highest dose (1000 mg/kg) there was congested blood vessel seen, a mild vascular congestion as well as in the medium dose (500 mg/kg). This result shows that the highest dose had a toxic effect on the liver. In the control group, histological sections of kidney tissue show normocellular glomerular tufts disposed on a background containing renal tubules. No abnormalities are seen. In the Low dose group, histological sections of kidney tissue show normocellular glomerular tufts disposed on a background containing renal tubules. The interstitium is infiltrated by dense aggregates of inflammatory cells mild renal interstitial inflammation was observed. In the Medium dose, histological sections of kidney tissue show normocellular glomerular tufts disposed

on a background containing viable tubules. Aggregates of infiltrating red cells and congested blood vessels are seen. There is severe vascular congestion in the kidney. In the High dose group, histological sections of kidney tissue show normocellular glomerular tufts disposed on a background containing renal tubules. The interstitium is infiltrated by dense aggregates if inflammatory cells renal vascular congestion with interstitial inflammation. The enzymatic profile is one of the blood parameters with the greatest variability; in that case it is important to perform a more accurate evaluation and an appropriate interpretation of the biochemical profile [24]. The liver is known to play a major role in the metabolic processes of most substances. Thus any disturbance in the liver can affect the level of the biochemical parameters present in the liver. The activities of these enzymes are usually elevated during damage to liver cells [25]. Blood biochemical profiles reflect the physiological conditions of the animal and constitute a diagnostic tool of great interest [26]. Thus in order to analyze whether the extract influenced the function of the liver, the renal biochemical profile was evaluated with basis on the values of liver enzymes such as Albumin, Alanine transaminase Aspartate transaminase, Alanine Phosphatase, Total bilirubin and direct bilirubin. For the serum Albumin level, the comparison of the control and the dose of 1000 mg/kg were significantly elevated. It had a higher mean differential value than the other groups when compared with the control. This means that the plant extract had an effect on the albumin level at the highest dose. For AST, ALP, ALT, T.BIL and D.BIL, there was no statistically significant difference went compared with the control. The serum protein level decreases in the order of the control group, low dose, medium dose and high dose group with the mean values of 9.50 mmol/L, 8.43mmol/L, 7.97mmol/L, 7.63mmol/L respectively. This is consistent with what was observed in the urea and creatinine mean level observed in the mice. The highest level of protein was seen in the control group and the lowest level was observed in the high dose group. This means that *P. subcordata* helps to reduce serum protein level as Serum β -Trace Protein has been found to be elevated in patients with renal diseases [27]. The control group has the highest mean value of 25µmol/L and the high dose group has the lowest mean value of creatinine (20.33µmol/L). The low dose and medium dose groups have the mean creatinine values of 24µmol/L and 24.33umol/L respectively which are also lower than the control group. Although the high dose group has the lowest creatinine level compared to the control group and the other groups, there is no statistical significant different in the level of creatinine between the groups. This suffices to say that there is improved creatinine clearance by the kidney as the dose of *P. subcordata* increases, but there is no statistically significant clearance. The urea level decreases from the control group, to the low dose, medium dose and the high dose group in that order. This shows that the extract of *P. subcordata* causes a reduction in serum urea level as the doses increase from the low dose group to the high dose group. This means that *P. subcordata* helps in urea clearance by the kidney. Increased blood urea nitrogen (BUN) is seen associated with kidney disease or failure, blockage of the urinary tract by a kidney stone, congestive heart failure, dehydration, fever, shock and bleeding in the digestive tract [28]. Although there was a reduction in the low dose group down to the high dose group there was no significant reduction between the groups and when each group is compared to the control group.

5.0 CONCLUSION

The present study has demonstrated that the Methanol extract of *Psydrax subcordata* is safe when administered orally to mice. It did not cause mortality, abnormal behavior, in the animal at a dose of 2000 and 5000mg/kg which indicates a high safety profile of the plant extract. After prolonged administration, however the histological sections of the liver tissue showed mild vascular congestion at a dose of 500 and1000 mg/kg while dose 250 mg/kg maintained normal liver with no abnormalities. The histopathological analysis shows that there was increase in the kidney's vascular congestion as the dose of *P. subcordata* increases across the groups.

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Conflict of Interest

There is no conflict of interest

Contributions of the authors

Bamisaye O. Oyawaluja: Research concept and design, writing the article and critical revision of the article
Aminat A. Oyawaluja: Research concept and design; critical revision of the article, data analysis and interpretation; Gladys N. Ogbogu: Collection and/or assembly of data; Emmanuel O. Olayinka: Collection and/or assembly of data; Olanrewaju Salako: Data analysis and interpretation; Olukemi A. Odukoya: Research concept and design; final approval of article.; Herbert A.B. Coker: Research concept and design; final approval of article.

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