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Evaluation of sub-acute oral toxicity effects of *Phaulopsis falcisepala* C.B. Clarke (Acanthaceae) in rats

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Abstract

Phaulopsis falcisepala is a herb or undershrub found in forest zone of West Africa and used for a wide range of ethnomedicinal purposes. Despite the ethnomedicinal importance of *P. falcisepala*, detailed data about its safety and toxicity is lacking. This study was done to evaluate sub-acute toxicity of *P. falcisepala* in rats. Whole plant of *P. falcisepala* was extracted with methanol. Wistar rats (n = 6/group) were administered orally with *P. falcisepala* extract at doses of 250, 500 and 1000 mg/kg/day for 28 days. Control group received distilled water. Physical observations were recorded daily and weights of animals were recorded weekly. After 28 days, samples of blood, serum and vital organs were obtained for haematological, biochemical and histological evaluations. Results showed that daily oral doses of *P. falcisepala* extract up to 1000 mg/kg for 28-days did not cause any behavioral changes or mortality. The plant extract did not induce significant alterations in body-weights, haematological, hepatic and renal parameters and serum levels of sodium, potassium, and chloride ions. No significant differences ($p > 0.05$) were recorded in relative weights of ovaries, testes, heart and kidneys of experimental groups compared to control group. Changes were observed in lipid profile ($p < 0.05$) and histology of kidneys and liver of rats treated with plant extract, particularly at highest dose, 1000 mg/kg. These findings suggest that extract of *P. falcisepala* could be safely applied for its medicinal properties at low to medium doses. However, sub-acute administration of doses up to 1000 mg/kg/day could pose deleterious risk to liver and kidneys.

Keywords: *Phaulopsis falcisepala,* herbal medicine, sub-acute toxicity, hematology, biochemical parameters, histopathology

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INTRODUCTION

The use of herbs for the treatment of ailments and as remedies for health conditions has a long history and is indigenous to many cultures. Due to their cultural antecedents, herbal medicines are still largely the most preferred and available therapy in many societies, particularly in developing countries. In more developed societies, the use of herbal and other complementary alternative medicines is widely gaining acceptance. A study involving adults from an Australian state reported that nearly a quarter of the adult population used some form of herbal medicine (Zhang *et al*., 2008). In the United States, the use of complementary and alternative medicines had increased from 34% in 1990 to 42% in 1997 (Eisenberg *et al*., 1998). In 2014, the WHO's World Health Assembly noted "the heightened level of interest" and affirmed "the growing importance and value of traditional medicine in the provision of healthcare nationally and globally" (WHO, 2014). The Assembly further urged member States "to develop and implement, as appropriate, working plans to integrate traditional medicine into health services…" (WHO, 2014). There has been increase in research on therapeutic potential of plants and herbs, with large volumes of scientific reports on the considerable activities of many plants and herbs in the treatment of several conditions. However, while several studies have ascertained the medicinal properties of many plants, fewer plants have been thoroughly evaluated for their detrimental effects. Reports of efficacy are, by far, more numerous than those on toxicity (Chalut, 1999; Ekor, 2014). Thus, there is need for more studies on the short and long-term toxicities of plants with potential medicinal properties.

Phaulopsis falcisepala C.B. Clarke (Acanthaceae) is a herb or weak undershrub that is found in the forest zone of West Africa. The plant has been reported to have a wide range of ethnomedicinal purposes. In Southern Nigeria, it is used for the treatment of wound and as remedy for cancer (Oladipupo *et al*., 2021). *P. falcisepala* is also applied as laxative, anti-emetic, aphrodisiac and as remedies for rheumatic pain, fever, diabetes, parasitic and fungal infection (Burkill, 1985; Fongod *et al*., 2013). Previous studies have investigated the antioxidant, alpha-amylase and alpha-glucosidase inhibitory, anti-inflammatory, antimitotic and cytotoxic properties of *P. falcisepala* (Abiodun *et al*., 2018; Adesegun *et al*., 2009; Oladipupo *et al*., 2021; Usman *et al*., 2020). Despite these medicinal properties of *P. falcisepala*, detailed data about its safety and toxicity is lacking. Hence, this study was done to evaluate the sub-acute toxicity of *P. falcisepala* in rats.

MATERIALS AND METHODS

Plant material

Fresh samples of whole plant of *P. falcisepala* were collected from Forest Research Institute of Nigeria (FRIN), Ibadan. Botanical authentication was done by Mr Nodza G.I. at the Herbarium of the Department of Botany, University of Lagos, where a voucher specimen (LUH 7992) was deposited. The plant samples were air-dried at room temperature (24°C ± 2°C) and pulverized. About 500 g of the pulverized plant was extracted with methanol (3L) by maceration at room temperature for 72 h. The crude extract was concentrated under vacuum at 40°C to yield a mass of 30.4 g (6.08% w/w).

Phytochemical investigation

The extract was screened for the presence of phytochemical classes including alkaloids, flavonoids, terpenes, sterols, saponins, phenols, cardiac glycosides, and tannins as previously described (Evans, 2009; Farnsworth, 1996; Harborne, 1998).

Experimental animals

Wistar rats (weighing 100–120 g) of both sexes were obtained from Priceless Test Animal Venture, Badagry, Lagos and kept in the Animal House of the College of Medicine, University of Lagos, Nigeria. The animals were housed in a well-ventilated room at room temperature and 12 h light/dark cycle, fed with standard rodent pellets and water *ad libitum*. Animals were acclimatized to laboratory conditions for 7 days and fasted for 12 h before the start of experiment. Animals handling was in accordance with the National Research Council's 'Guide for the Care and Use of Laboratory Animals' (National Research Council, 1996). The study was conducted with approval of the Health Research Ethics Committee of College of Medicine, University of Lagos (CMUL/HREC/03/18/341).

Sub-acute toxicity study

Sub-acute toxicity study was carried out according to OECD guideline No. 407 (OECD, 2008). A total of twenty-four rats were randomly divided into 4 groups of 6 animals (3 males and 3 females) each. Animals of group 1 served as control and received 10 ml/kg body weight of distilled water while those of group 2, 3, and 4 were treated daily with methanol extract of *P. falcisepala* at 250, 500, and 1000 mg/kg body weight respectively, orally for 28 days. Animals were observed for mortality, changes in physical appearance, behaviour (sleepy, salivation, lethargy), and any injury or illness after dosing and up to 4 h after dosing throughout the 28 days. After 28 days, all surviving animals were fasted overnight and anesthetized with thiopental sodium (Alpa Laboratories Ltd., Indore, India). Blood samples were collected from the animals by cardiac puncture into EDTA and heparinised tubes for haematological and biochemical studies, respectively. Rats were euthanized after blood collection, and vital organs including liver, heart, kidneys, lungs, testes, and ovaries were harvested.

Measurement of body and organ weights

Body weights of the animals were measured before the start of the experiment and subsequently, weekly and on the day of sacrifice (OECD, 2008). The weight of each harvested organ was measured and the percentage relative organ weight was determined using the following expression.

$$\text{Relative organ weight (\%)} = \frac{Absolute\ Organ\ Weight\ (g)}{Body\ Weight\ of\ Rat\ on\ Sacrifice\ day\ (g)} \times 100$$

Haematological analysis

Blood samples were analyzed for parameters including haemoglobin concentration (HB), hematocrit (HCT), red blood cells (RBC) count, white blood cells (WBC), packed cell volume (PCV), platelets (PLT), neutrophils (NEU), monocytes (MID), lymphocytes (LYMP), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) using a complete blood count (CBC) Auto Hematology Analyzer (Mindray BC-3200).

Biochemical analysis

Serums were obtained by coagulation and centrifugation of blood samples collected into heparinised tubes and analysed for biochemical parameters using a Cobas C311 Automated Analyzer. Parameters investigated include aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), albumin (ALB), total bilirubin (TBIL), triglycerides (TG), total protein (TP) creatinine, blood urea, triglycerides (TG), total cholesterol (TC), high density lipoproteins (HDL), and very low density lipoproteins (VLDL). Serum electrolytes including sodium (Na^+), potassium (K^+), and chloride (Cl^-) ions were also determined.

Histopathological analysis

Histopathological examinations of the harvested organs were evaluated as previously described (Pieme *et al*., 2006). Offcuts of the organs were fixed in 10% formol-saline for 24 h, washed in running water, dehydrated in an Autotechnicon and then cleared in xylene. Cleared tissues were embedded in paraffin and cut into 3–5 µm thick sections. The slides were stained with hematoxylin-eosin for photomicroscopic assessment and examined by a histopathologist and a histologist.

Statistical analysis

The values were expressed as mean ± standard error of mean (SEM). For each parameter, the one-way analysis of variance (ANOVA) was used to detect significant differences ($p \leq 0.05$) between the groups. When significant differences existed, the post-hoc Tukey's test was used to compare the means.

RESULTS

Phytochemical constituents

The analysis revealed the presence of saponins, terpenes, phenols, tannins, and sterols in the plant extract. Flavonoids, alkaloids and cardiac glycosides were also found in the extract. However, resins, volatile oil and anthraquinones were absent.

Sub-acute toxicity study

Effect of extract of *P. falcisepala* on general behavior

There was no mortality in animals at all doses of the extract up to 1000 mg/kg. It was observed that both male and female rats that received 250 mg/kg body weight of *P. falcisepala* extract did not show symptoms of toxicity. However, rats that were dosed with 1000 mg/kg of the extract exhibited little signs of lethargy, weakness and slow reflex activities. The symptoms of lethargy started manifesting around days 19–21 and days 25–26 respectively in the groups treated with the 1000 and 500 mg/kg doses. At day 28, the symptoms were no longer evident. Mortality and changes in respiratory rhythm and fur patterns were not observed during the 28 days experimental period in the aforementioned groups. At day 21 of the study, one of the male rats in the group administered with 500 mg/kg had an inflammation on left side of the face. This was then noticed to be healed at day 25 of the study, indicating that *P. falcisepala* extract has exerted anti-inflammatory effect on the rat. This corroborates the findings of Usman *et al*. (2020), who reported the anti-inflammatory activities of *P. falcisepala* extract in mice and rats.

Effect of extract of *P. falcisepala* on body weight

Weights of all the animals in the different groups were compared with their relevant weights on day 1. The mean weight of the animals in the control and experimental groups experienced a change at each time interval (Figure 1). There was an increase in weight between day 1 and 7 for both the control and the experimental group; and then a steady increase was observed between day 14 and 21. Between day 7 and 14, there was a significant increase ($p < 0.05$) in weight in the group treated with 1000 mg/kg of the extract compared to the control group. Between day 14 and 28, decline in weights were recorded in the control and experimental groups. Overall, there was no significant changes in body weights of the animals treated with the plant extracts compared to the control group at the end of the study.

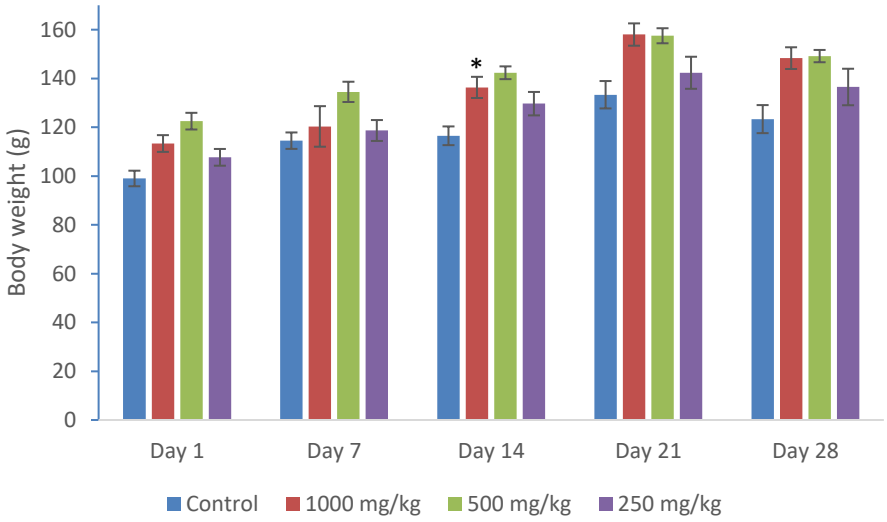

Figure 1. Body weight changes in rats in the sub-acute toxicity study. Values presented as mean ± SEM. * represents significant difference ($p < 0.05$) from control.

Effect of extract of *P. falcisepala* on relative organ weight

The female rats treated with the extract at the doses of 250, 500 and 1000 mg/kg body weight had lungs weights significantly ($p < 0.05$) lower than those of the control. Furthermore, significant difference ($p < 0.05$) in the weights of the liver was observed in the female rats treated with 500 and 1000 mg/kg of extract of *P. falcisepala* compared with the control group. The male rats treated with 500 and 1000 mg/kg body weight of the extract

showed significant decrease in the weights of the lungs ($p < 0.05$). No significant differences were recorded in the measurements of the weights of other organs compared to their respective controls. The results of relative organ weights of female and male rats are presented in Table 1.

Effect of extract of *P. falcisepala* on biochemical parameters

Liver and kidney parameters

The effects of extract of *P. falcisepala* on hepatic and renal indices following the 28 days daily oral administration are presented in Table 2. Bilirubin level was significantly lower ($p < 0.05$) in the group treated with 500 mg/kg of *P. falcisepala* extract compared to the control group. However, there were no significant differences ($p > 0.05$) in the serum levels of aspartate transaminase (AST), alkaline phosphatase (ALP), alanine transaminase (ALT), total protein (TP), albumin, creatinine and urea between all the experimental and control groups.

Electrolytes

The effects of *P. falcisepala* extract on selected serum electrolytes are presented in Table 3. *P. falcisepala* extract did not produce any significant effect on the levels of sodium, potassium, and chloride ions in the treated animals compared to the control group.

Lipid profile

As shown in Table 4, *P. falcisepala* extract produced significant changes in the lipid profiles of the treated rats at 250 and 500 mg/kg doses. Significant dose-dependent reductions in very low density lipoproteins (VLDL) and triglycerides (TG) were recorded in the groups dosed with 250 and 500 mg/kg of the plant extract. Conversely, significant increase in total cholesterol (TC) and low density lipoproteins (LDL) was observed in the group treated with 250 and 500 mg/kg of the plant extract, respectively. There were no significant changes in high density lipoproteins (HDL) in all the experimental and control groups. No significant alteration in lipid profile was also noted in the group that received 1000 mg/kg of the plant extract.

Effect of *P. falcisepala* extract on haematological parameters

The results of haematological parameters of rats treated with *P. falcisepala* extract and control rats are presented in Table 5. At the end of the 28 days treatment, *P. falcisepala* extract did not produce any significant effect on haematological indices at doses up to 1000 mg/kg compared to the control group.

Table 1. Effect of *P. falcisepala* on organ weights (per 100 g body weight) in the sub-acute study

Organ	Females				Males			
	Control	1000 mg/kg	500 mg/kg	250 mg/kg	Control	1000 mg/kg	500 mg/kg	250 mg/kg
Liver	3.05 ± 0.025	2.82 ± 0.241*	2.72 ± 0.197*	3.23 ± 0.403	2.79 ± 0.121	2.59 ± 0.092	2.53 ± 0.005	2.68 ± 0.215
Heart	0.37 ± 0.018	0.41 ± 0.115	0.35 ± 0.023	0.43 ± 0.128	0.37 ± 0.030	0.39 ± 0.073	0.32 ± 0.004	0.35 ± 0.043
Lungs	0.82 ± 0.141	0.62 ± 0.002*	0.57 ± 0.034*	0.72 ± 0.106*	0.71 ± 0.067	0.45 ± 0.126*	0.59 ± 0.051*	0.72 ± 0.047
Kidneys	0.31 ± 0.040	0.30 ± 0.045	0.29 ± 0.031	0.28 ± 0.013	0.35 ± 0.010	0.28 ± 0.030	0.26 ± 0.008	0.28 ± 0.018
Ovaries/ Testes	0.06 ± 0.004	0.06 ± 0.004	0.05 ± 0.009	0.05 ± 0.010	0.77 ± 0.012	0.71 ± 0.015	0.72 ± 0.006	0.84 ± 0.045

Values are presented as mean ± SEM. * represents significant difference ($p < 0.05$) from control.

Table 2. Effect of *P. falcisepala* on liver and kidney parameters

Parameters	Groups			
	Control	250 mg/kg	500 mg/kg	1000 mg/kg
Bilirubin (mg/dL)	2.68 ± 0.19	1.75 ± 0.39	1.23 ± 0.20*	2.80 ± 0.41
Albumin (mg/dL)	32.13 ± 9.24	42.7 ± 1.92	41.03 ± 1.88	40.18 ± 1.56
ALP (IU/L)	160.18 ± 27.53	179.83 ± 26.52	187.93 ± 34.67	151.45 ± 9.94
AST (IU/L)	161.45 ± 7.13	170.02 ± 5.66	272.8 ± 66.22	170.13 ± 17.33
ALT (IU/L)	62.63 ± 3.08	54.13 ± 7.72	109.13 ± 62.49	78.15 ± 2.90
TP (g/L)	83.73 ± 3.34	78.73 ± 1.36	76.8 ± 2.60	84.93 ± 1.49
Creatinine (mg/dL)	52.43 ± 5.01	49.55 ± 1.53	84.55 ± 24.32	55.85 ± 2.63
Urea (mg/dL)	7.30 ± 0.80	6.0 ± 0.55	10.2 ± 4.28	7.95 ± 0.55

Values are presented as mean ± SEM. * represents significant difference ($p < 0.05$) from control.

Table 3. Effect of *P. falcisepala* on serum electrolytes

Parameters	Groups			
	Control	250 mg/kg	500 mg/kg	1000 mg/kg
Sodium (meq/L)	138.25 ± 0.25	147 ± 4.71	138.25 ± 0.85	136 ± 1.23
Potassium (meq/L)	4.53 ± 0.38	4.58 ± 0.37	5.32 ± 0.48	5.55 ± 0.70
Chloride (meq/L)	98.88 ± 1.15	102.08 ± 2.46	99.05 ± 1.11	99.03 ± 0.87

Values are presented as mean ± SEM. No statistically significant difference ($p > 0.05$) between treated and control groups.

Table 4. Effect of *P. falcisepala* on lipid profile

Parameters	Groups			
	Control	250 mg/kg	500 mg/kg	1000 mg/kg
HDL (mmol/L)	0.76 ± 0.11	0.89 ± 0.18	0.44 ± 0.04	0.50 ± 0.03
LDL (mmol/L)	0.33 ± 0.14	0.94 ± 0.19	1.21 ± 0.14**	0.46 ± 0.10
VLDL (mmol/L)	0.41 ± 0.06	0.14 ± 0.05**	0.15 ± 0.03**	0.27 ± 0.04
TG (mmol/L)	0.91 ± 0.14	0.30 ± 0.10**	0.31 ± 0.05**	0.58 ± 0.08
TC (mmol/L)	1.5 ± 0.10	1.96 ± 0.09*	1.80 ± 0.11	1.22 ± 0.10

Values are presented as mean ± SEM. * represents significance ($p < 0.05$), ** ($p < 0.01$) from control.

Effect of *P. falcisepala* extract on histology of liver and kidneys

Liver

Microscopic examination of liver of both male and female rats in the control group showed normal hepatic features (Figure 2). The male rats administered 250 mg/kg of *P. falcisepala* extract showed comparable hepatic features to the control group. However, necrosis and slight dilation of lobules were observed in the female rats with the same dose (Figure 2). The livers of male rats administered 500 mg/kg of extract showed focal necrosis while those of female rats with same treatment did not show any treatment-related changes (Figure 3). The photomicrographs of livers of both male and female rats administered 1000 mg/kg of *P. falcisepala* extract showed zonal and periportal necrosis (Figure 3).

Kidneys

Sections from kidney of rats (male and female) in the control group showed normal glomerular tufts and variably-sized normal renal tubules lined by cuboidal to low columnar cells having round nuclei and moderately eosinophilic cytoplasm (Figure 4). The male rats administered 250 mg/kg of *P. falcisepla* extract showed healthy glomeruli and renal tubules while female rats from same group showed altered renal glomeruli and dilated distal tubules (Figure 4). Normal glomerular tufts and variably-sized renal tubules were observed in sections of kidney of both male and female rats administered 500 mg/kg of the extract (Figure 5). Photomicrographs of male rats administered 1000 mg/kg of *P. falcisepla* extract showed normal glomeruli while that of female rat showed necrosis with oedema exudate (Figure 5).

Table 5. Effect of *P. falcisepala* on hematological parameters in the sub-acute study

Parameters	Groups			
	Control	250 mg/kg	500 mg/kg	1000 mg/kg
WBC (x 10^9/L)	7.18 ± 1.08	5.75 ± 0.84	6.48 ± 0.57	5.98 ± 0.53
RBC (x 10^{12}/L)	7.53 ± 0.70	5.82 ± 1.22	8.00 ± 0.35	7.65 ± 0.45
MCV (fL)	59.53 ± 0.93	59.28 ± 1.84	61.45 ± 0.63	60.75 ± 1.68
MCT (%)	44.85 ± 4.41	34.73 ± 7.72	49.13 ± 2.45	46.5 ± 3.35
HGB (g/dL)	13.25 ± 1.18	10.98 ± 2.26	14.65 ± 0.53	13.6 ± 0.91
MCH (pg)	17.6 ± 0.37	18.85 ± 0.89	18.28 ± 0.21	17.68 ± 0.23
MCHC (g/dL)	29.63 ± 0.43	31.88 ± 0.86	29.83 ± 0.53	29.25 ± 0.48
PLT (x 10^9/L)	615.5 ± 164.5	470.5 ± 117.76	726.75 ± 91.16	670.75 ± 62.14
PDW (fL)	15.53 ± 0.32	16.03 ± 0.11	15.73 ± 0.14	15.90 ± 0.27
MPV (fL)	6.88 ± 0.20	7.03 ± 0.20	7.20 ± 0.16	7.13 ± 0.24
PCT (%)	0.43 ± 0.12	0.33 ± 0.08	0.52 ± 0.06	0.48 ± 0.05
Lymph (%)	43.98 ± 13.54	48.6 ± 12.41	28.48 ± 3.93	41.25 ± 14.70
Gran (%)	33.1 ± 7.89	27.63 ± 7.01	36.1 ± 1.85	34.75 ± 8.78
Mid (%)	22.93 ± 6.41	23.78 ± 6.03	35.43 ± 2.48	24.00 ± 6.15

Values are presented as mean ± SEM. No statistically significant difference ($p > 0.05$) between treated and control groups.

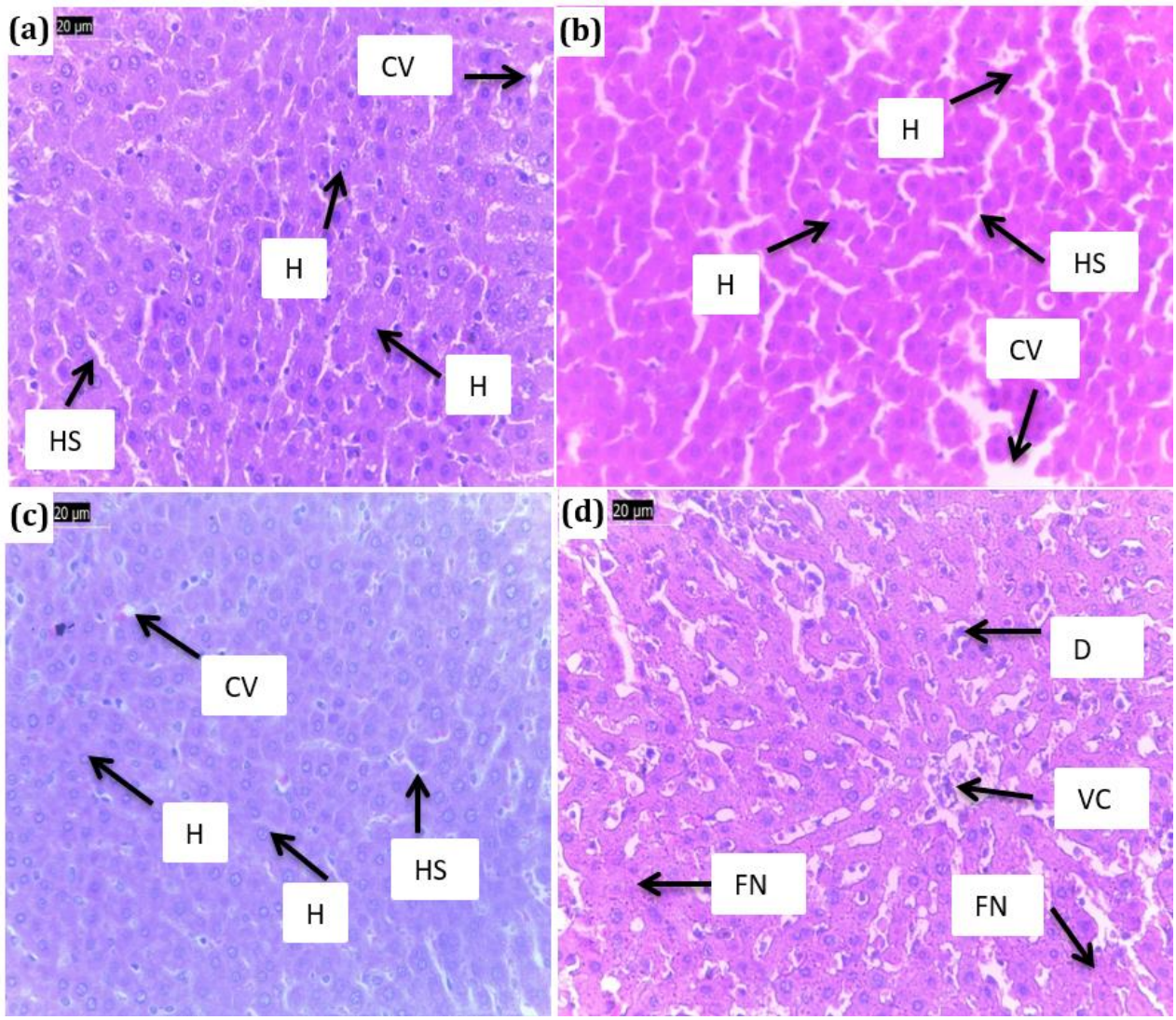

Figure 2. Liver section of male **(a)** and female **(b)** rats in control group, showing normal hepatocytes (H), central vein (CV) and numerous hepatic sinusoids (HS). Liver section of rats administered 250 mg/kg of *P. falcisepala* extract shows normal hepatocytes (H), hepatic sinusoids (HS) and central vein (CV) in male **(c)** while female **(d)** shows focal necrosis (FN), dilation of lobules (D) and vascular congestion (VC). (× 20)

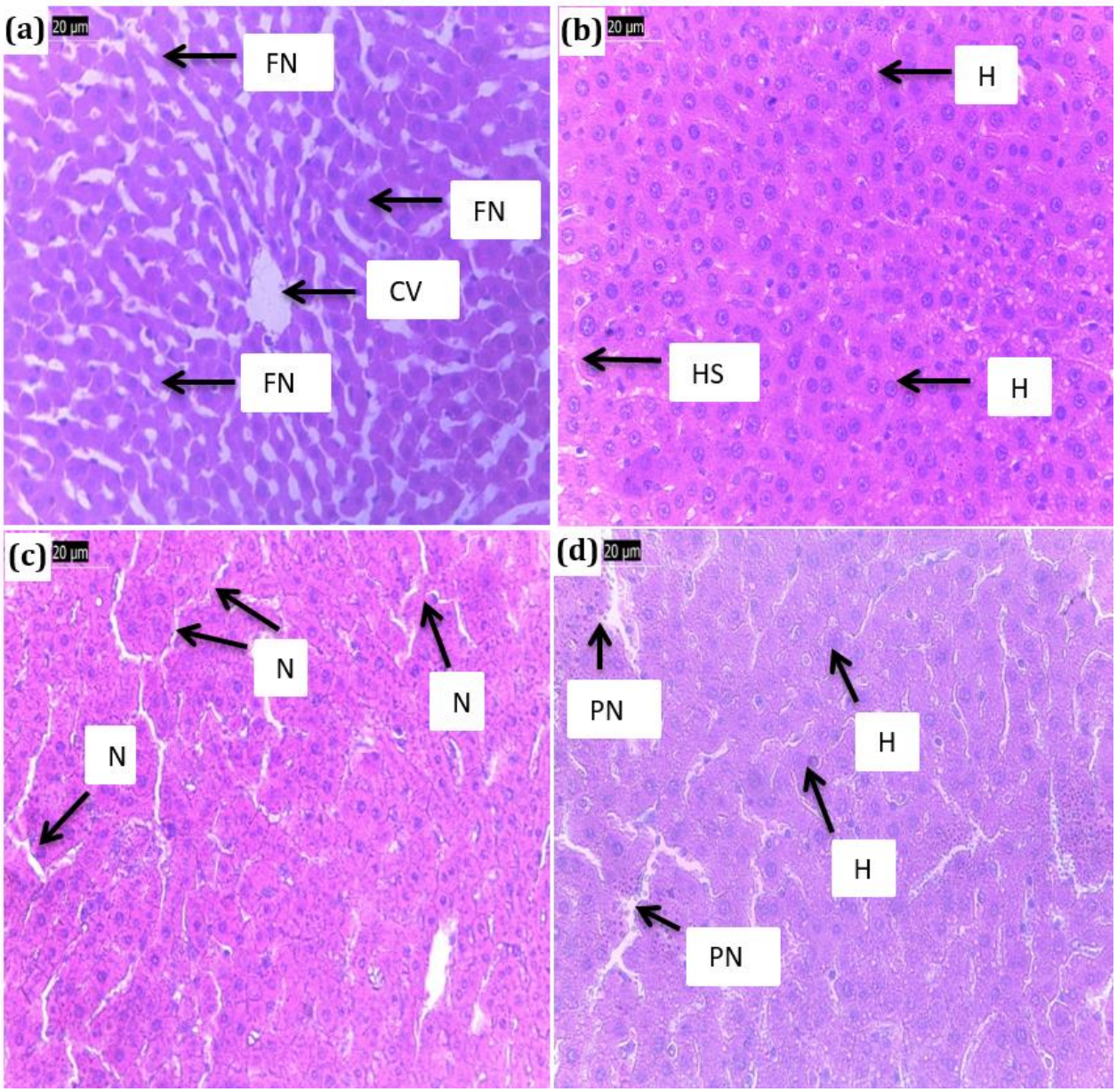

Figure 3. Liver section of male rat **(a)** administered 500 mg/kg of extract shows focal necrosis (FN) of the hepatocytes and tubules and central vein (CV). Female rat **(b)** shows normal hepatocytes (H) and hepatic sinusoids (HS). Liver section of rats administered 1000 mg/kg show zonal necrosis (N) in male rat **(c)** and periportal necrosis (PN) with normal hepatocytes (H) in female rat **(d)** (× 20)

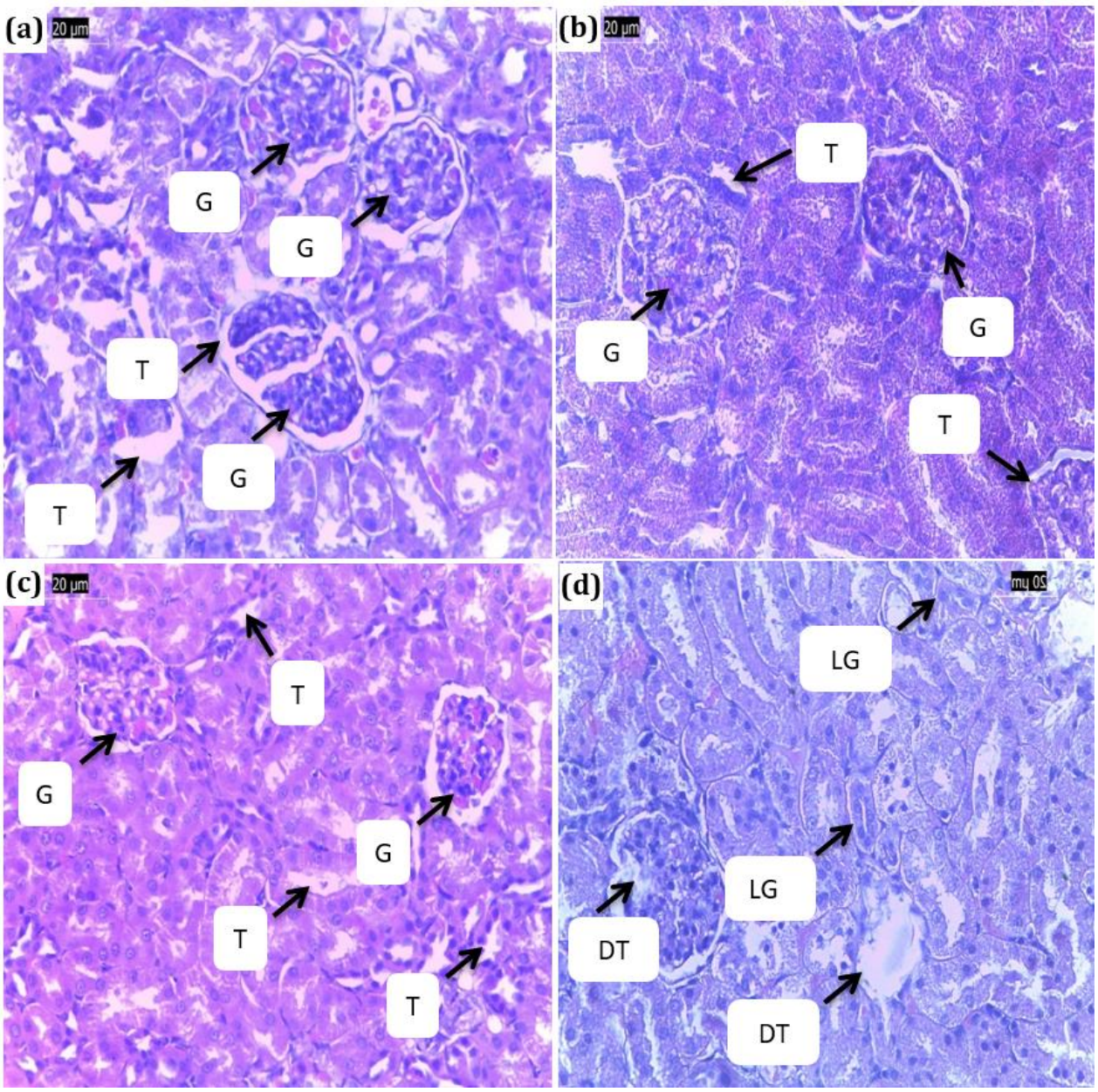

Figure 4. Section of kidney of male **(a)** and female **(b)** rats in control group show normal glomeruli (G) and renal tubules (T). Section of kidney of rats administered 250 mg/kg of *P. falscisepala* extract; male rat **(c)** shows normal glomeruli (G) and tubules (T) while female rat **(d)** shows slight lobulation of renal glomeruli (LG) and dilated distal tubules (DT). (× 20)

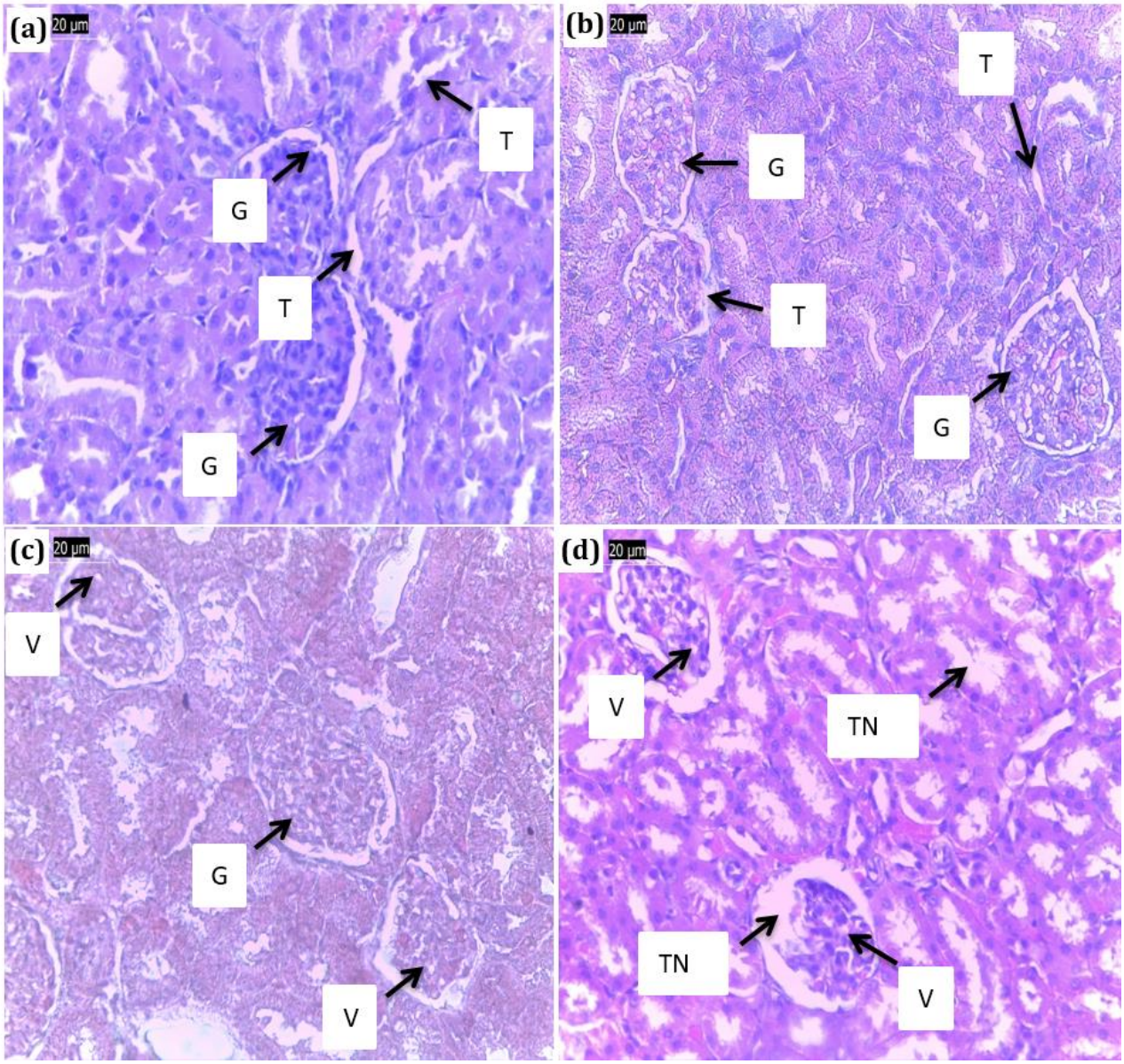

Figure 5. Section of kidney of male (a) and female (b) rats administered 500 mg/kg of *P. falscisepala* extract, showing normal renal glomeruli (G) and tubules (T) in both sex. Section of kidney of rats administered 1000 mg/kg show slight dilation, partial vacuolization (V) and normal glomeruli (G) in male rat (c); tubular necrosis (TN), vacuolization (V) and oedema exudate are shown in female rat (d). (× 20)

DISCUSSION

Many traditional herbal preparations often find their way for use in humans without the empirical knowledge of their pharmacological properties and safety. This could inadvertently worsen the health of the individuals who receive them. Preparations containing *P. falcisepala* are used by humans for treating many conditions, including rheumatic pain, wound fever, and diabetes, despite the paucity of data about their safety and efficacy. Therefore, there is need to properly elucidate the safety properties of *P. falcisepala.* In a recent study (Usman *et al*., 2020), the acute toxicity property of whole plant of *P. falcisepala* was investigated; no toxic effect was observed after oral administration of a single dose of 5000 mg/kg of the plant extract. Acute toxicity data are of limited clinical and practical applications (Alaribe *et al*., 2022); application of medicinal remedies

often requires repeated dosing over a period of days. Thus, this study was conducted to investigate the sub-acute toxicity of whole plant of *P. falcisepala* on daily dosing for 28 days. Sub-acute study could provide information on dosage regimens, target organ toxicity, and identify cumulative adverse or toxic effects that could occur on prolonged administration, even at very low doses (Abotsi *et al*., 2011; Porwal *et al*., 2017). Given that *P. falcisepala* has an LD_{50} greater than 5000 mg/kg (Usman *et al*., 2020), in this study, extract of *P. falcisepala* was evaluated in rats at doses of 250, 500 and 1000 mg/kg/day.

The phytochemical classes detected in whole plant of *P. falcisepala* include, phenols, flavonoids, alkaloids and terpenes. The results were consistent with those previously reported (Oladipupo *et al*., 2021) for methanol extracts of leaf and stem of *P. falcisepala*. The presence of these phytochemicals could be responsible for its reported pharmacologic properties, which include anti-inflammatory, alpha amylase and alpha glucosidase inhibitory, antimitotic and cytotoxic activities (Abiodun *et al*., 2018; Oladipupo *et al*., 2021; Usman *et al*., 2020).

The daily administration of oral doses of 250, 500 and 1000 mg/kg of *P. falcisepala* extract over 28 days period to Wistar rats did not show any behavioral changes or deaths. Overall, the doses did not induce significant differential weight gain or loss in the experimental animals compared to the control, but there were differential weight changes across all the time points in all the groups, both experimental and control.

The haematopoietic system is one of the most sensitive systems to toxic effects of phytomedicines and is an important index of physiological and pathological status in man and animals (Adeneye *et al*., 2006; Diallo *et al*., 2008). The results showed that *P. falcisepala* extract did not cause any significant effect on all the haematological parameters of the treated rats. This suggests that *P. falcisepala* may be non-toxic to circulating white and red blood cells and platelets and may not interfere with hematopoietic system when consumed. Similarly, *P. falcisepala* extract did not induce significant alteration in hepatic parameters at the end of the 28 days treatment. Renal parameters such as creatinine and urea levels, as well as serum electrolyte levels are important biochemical markers of renal function (Obidah *et al*., 2009). *P. falcisepala* extract did not cause any significant changes to renal parameters and levels of sodium, potassium, and chloride ions in treated animals compared to control group. The normal levels of these markers could indicate that *P. falcisepala* did not alter the integrity and function of renal system in the treated animals.

Changes in lipid profile could provide information on the effect of the test substance on lipid metabolism and consequential health effects. At 250 and 500 mg/kg doses, *P. falcisepala* extract altered the levels of VLDL, LDL, TG and TC compared to the control group. However, the levels were within the reference ranges in albino rats previously reported (Ihedioha *et al*., 2013; Ononogbu, 1988). At the highest dose, 1000 mg/kg, no significant alteration in lipid profile was caused by the plant extract.

Organ weight changes are widely accepted as a critical indicator of substance-induced changes to organs; however, comparison of organ weight between test and control groups of animals have their own limitations in toxicological assessment in animal models (Michael *et al*., 2007). They are usually limited by the differences in the body weight between groups, hence, relative organ weights of animals is a more useful measure of toxicity (Bailey *et al*., 2004). The liver and kidneys are vital organs in the body responsible for detoxification and excretion processes and are often targets for toxic substances. The liver and kidneys are important in toxicity studies as a result of their being sensitive to harmful substances and changes to their weights can be used to predict toxicity (Michael *et al*., 2007). Similarly, changes in weight of testes or ovaries and heart could be indicative of reproductive and cardio- toxicities (Michael *et al*., 2007; Woldemeskel, 2017). Alterations in weight of lungs do not have much toxicity importance as a result of the lungs' limited role in detoxification process in the body (Greaves, 2011; Sellers *et al*., 2007). The comparisons of relative organ weights of control and experimental groups in this study did not show any statistically significant differences for ovaries, testes, heart and kidneys in the animals. Significant reductions in weight of lungs was observed in both male and female animals treated with 500 and 1000 mg/kg of the extract. Female rats treated with same doses also showed significant decrease in weights of liver.

Toxicity-related changes in weights of kidneys and liver are often accompanied by corresponding histopathological findings. Histopathological examinations revealed the presence of hepatic sinusoids, vascular congestion and abnormal hepatocytes in the liver following treatment with 500 mg/kg of the extract. These effects were more pronounced at 1000 mg/kg of extract. Meanwhile, normal renal features were observed in the animals following treatment with different doses of *P. falcisepala* extract; except female rats treated with 1000 mg/kg of extract, which showed abnormal glomeruli with oedema exudate and tubular necrosis. The toxic effects of *P. falcisepala* extract on kidneys and liver could be due to one or more of the phytochemicals present in the extract. Phytochemical investigation indicated the presence of appreciable amount of tannins in *P. falcisepala* extract. A large intake of tannins could cause deleterious effects to the kidneys and liver (Bajaj, 1988; Yamasaki *et al*., 2002).

CONCLUSION

This study provides scientific data on the sub-acute oral toxicological profile of extract of whole plant of *P. falcisepala*. The findings showed that daily administration of oral doses of *P. falcisepala* extract up to 1000 mg/kg for 28 days did not cause any behavioral changes or deaths. Generally, the plant extract did not alter body weights, haematological parameters, hepatic parameters, renal parameters and serum levels of sodium, potassium, and chloride ions. Changes were observed in lipid profile and histology of kidneys and liver of rats treated with the plant extract, particularly at the highest dose, 1000 mg/kg. These findings suggest that extract of *P. falcisepala* could be safely applied for its medicinal properties at low to medium doses. However, sub-acute administration of doses up to 1000 mg/kg/day could pose deleterious risk to the liver and kidneys.

Conflict of interest

The authors have no conflict of interest to declare.

Author contribution

SIO was involved in conceptualization, sample preparation, data collection and analysis, as well as manuscript preparation. OAR was involved in conceptualization, study design and implementation, sample preparation, data analysis and manuscript preparation. ASC was involved in conceptualization, project design/implementation, supervision and administration, data analysis and manuscript preparation. All authors read and approved the final draft of the manuscript.

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