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TOXICOLOGICAL EVALUATION OF *Pistia stratioles* METHANOL LEAF EXTRACT

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ABSTRACT
Medicinal herbs have been used by humans for a long time without any proof of their toxicity. The goal of this study was to determine the toxicity profile of *Pistia stratioles*. Standard procedures were used to conduct phytochemical screening of *Pistia stratiotes* methanol leaves extract. The limit dose test was used to determine acute oral toxicity (LD_{50}), whereas graded doses (150, 300, 600, and 1200mg/kg) were given daily for 28 days in the sub-chronic toxicity research. The presence of flavonoids, saponins, anthraquinones, phlobatanins, alkaloids, and tannins was discovered in *P. stratioles*. The (LD_{50}) of *P. stratioles* methanol leaf extract (*PS*MLE) was determined to be larger than 3000mg/kg body weight, and there was no fatality up to 14 days of observation. *PS*MLE (150-1200mg/kg) treatment resulted in a substantial reduction ($p<0.05$) in Aspartate Amino Transferase and Alanine Amino Transferase in a 28-day oral toxicity trial. When compared to the control, there was no significant difference in (Alkaline Phosphate, Albumin, Total Protein, Direct Bilirubin, and Total Bilirubin) and renal function markers (Urea, Uric acid, and Creatinine). In addition, when the extract-treated groups were compared to the control, there was a significant ($p<0.05$) difference in Platelets and Mean Corpuscular Volume. Finally, the current finding strongly suggests that *P. stratioles* methanol leaf extract is effective. In albino rats, neither acute nor sub-chronic investigations revealed any major toxic effects. As a result, it should be deemed safe to take *P. stratioles* in an acceptable amount, especially at low doses, for its traditional use.

Keywords: Acute oral toxicity (LD_{50}), Phytochemicals, *Pistia stratiole*, Sub-chronic.

INTRODUCTION

As people become more interested in traditional medicine, they are evaluating the toxicity and dangers of the many herbal medications that are used to treat diseases and infections (Mensah *et al.*, 2019). They employ these in the therapy of diseases without considering the negative health consequences that may result.

A rise in the number of cases of acute renal failure in the country has been related to the use of herbal medicines without sufficient approval (Plana-Ripoll *et al.*, 2019). However, local people's usage of plants in the treatment of ailments without understanding their adverse effects may result in health problems later in life (Cock, 2015). Several toxicological problems associated to a history of taking traditional aristolochic acid-containing treatments among cancer patients have lately been discovered, according to Aydin *et al.*, (2017).

Pistia stratiotes is a common name for a species of *Pistia stratiotes*. The leaves can be up to 14cm long and have no stem, according to Arogundade and Adedeji (2020), and Araceae plant sections (usually

above ground) have higher macro element (Nitrogen, Phosphorous, and Potassium) content. Eczema, leprosy, ulcers, and piles are all treated with *Pistia stratiotes* leaves. Furthermore, there is a scarcity of empirical data on the safety and efficacy of herbal medicine. As a result, it's critical to investigate the safety and potency of *Pistia stratiotes* leaves in order to learn about their potential benefits to humanity (Mohamed *et a*l., 2011). The goal of this study was to provide a comprehensive toxicological assessment of *Pistia stratiotes* methanol leaves extract.

MATERIALS AND METHODS

Plant identification and collection

Pistia stratiotes were obtained in February 2019 from a local market in Ilorin, Nigeria, then identified and authenticated at the University of Ilorin's Department of Plant Biology. The Herbarium received a voucher specimen (UILH/002/1376) that was prepared and deposited.

Plant material extraction

Pistia stratiote leaves were air-dried in the shade. The dried leaves were pulverized into moderately coarse powder, the powdered leaves sample (100 g) was macerated in methanol for 72 hours. Using a rotary evaporator set to 45^0C the filtrate was evaporated to dryness (Aliyu and Sani, 2011). Weighed, tagged, and stored in an airtight bottle, the dried extract was collected.

Screening for phytochemicals

The *Pistia stratiotes* methanol leaf extract was screened for phytochemicals using conventional phytochemicals according to the procedure previously reported (Sofowora, 1993).

Animals used in research

In February 2019, thirty (30) albino rats (120-150g) of both sexes (males and females) were acquired and transferred to the Department of Biochemistry, Kebbi State University of Science and Technology, Aliero from the Animal House, Usmanu Danfodiyo University, Sokoto, Nigeria. They had unrestricted access to tap water and common animal feed. Before receiving the *Pistia stratiotes* methanol leaf extract, the experimental animal was given two weeks to acclimate.

Oral toxicity test (acute) (LD_{50})

The Organization for Economic and Cooperation Development's (OECD, 2001) limit test dose, up and down procedure was used. *Pistia stratiote* Methanol Leaf Extract (3000mg/kg body weight) was given to 5 rats in a single oral dose (one after the other) and studied for 72 hours. A control group of five rats was given distilled water. Toxic signs such as weakness, loss of appetite, trouble moving, noise sensitivity, and death were seen in the rats. The animals were then monitored for another 14 days to see if there were any signs of delayed toxicity.

Toxicology test on a sub-chronic basis

A sub-chronic toxicity test was carried out by the guidelines of the Organization for Economic Cooperation and Development (OECD, 2001). A total of 25 albino rats weighing 120-150g were randomly divided into five (5) groups of five (5) rats each and given the following treatments for 28 days: *PS*MLE (150mg/kg), *PS*MLE (300mg/kg), *PS*MLE (600mg/kg), and *PS*MLE (1200mg/kg). Group I: Normal Control (2ml/kg Distilled water), Group II: *PS*MLE (150mg/kg), Group III: *PS*MLE (300mg/kg), Group IV: *PS*MLE (600mg/kg), and Group V: *PS*MLE (1200mg/kg).

Each rat's body weight was measured weekly with a weighing balance. The animals were fasted overnight, anesthetized with chloroform vapor, dissected, and blood samples were obtained by heart puncture into heparinized and non-heparinized tubes for analysis after 28 days of daily therapy.

Toxicological markers

The blood samples collected at the end of the sub-chronic toxicity tests were analyzed for the following parameters: Aspartate aminotransferase (AST) (Reitman and Frankel, 1957), Alanine aminotransferase (ALT) (Reitman and Frankel, 1957), Alkaline phosphatase (ALP) (Rec, 1972), -glutamyltransferase (GGT) (Tietz, 1995), Lactate dehydrogenase (LH) (Rec, 1972), Total and Direct Bilirubin (Koch *et al*., 1982), Hematocrit (PCV), hemoglobin (Hb), red blood cell (RBC), mean cell volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell (WBC) lymphocytes, neutrophils, monocytes, basophils, and platelets (Jung and Parekh, 1970), electrolytes and creatinine (Slot, 1965), and automatic haematological analyzer (cell Dyn).

Analytical statistics

Graph Pad Instat software was used to analyze the data. One-way ANOVA was used, followed by Duncan multiple comparison tests. At ($p<0.05$), the data was significant.

RESULTS

Phytochemical analysis

Flavonoids, saponins, anthraquinones, phlabotannins, alkaloids, and tannins were among the phytochemical compounds discovered (Table 1).

Acute oral toxicity (LD_{50})

There was no mortality after an oral acute dose of *Pistia stratiote* Methanol Leave Extract (3000 mg/kg b.w), indicating that the LD_{50} of *Pistia stratiote* Methanol Leave Extract is greater than 3000 mg/kg bw.

The effect of *Pistia stratiote* Methanol leave extract on Albino Rats' mean body weight

From week 2 to week 4, albino rats treated with 150 - 1200mg/kg body weight showed a substantial ($P<0.05$) increase in mean body weight (Figure 1). When compared to week 0, there were no significant ($P>0.05$) variations in body weights in the control group.

Effects of *Pistia stratiote* Methanol leave extract on Liver function biomarkers in Serum

In comparison to the normal control, there was no significant ($p<0.05$) difference in Alkaline Phosphatase, Albumin, Direct Bilirubin, Total Protein, and Total Bilirubin after *PS*MLE treatment. In contrast, when animals given *PS*MLE (150-1200mg/kg) were compared to normal controls, Aspartate Amino Transferase and Alanine Amino Transferase showed a substantial ($p<0.05$) reduction (Table 2).

Renal function Indices and *Pistia stratiote* Methanol leave extract

When rats given 150mg/kg of *PS*MLE were compared to normal controls, the effect of *PS*MLE on renal function indices revealed a substantial ($p<0.05$) rise in Creatinine, Uric acid, Potassium, and Chlorine, as well as a decrease in Urea and Bicarbonate. When compared to normal control, *PS*MLE revealed no significant ($p<0.05$) difference in sodium at any dose (Table 3).

Hematological parameters affected by *Pistia stratiote* Methanol leave extract

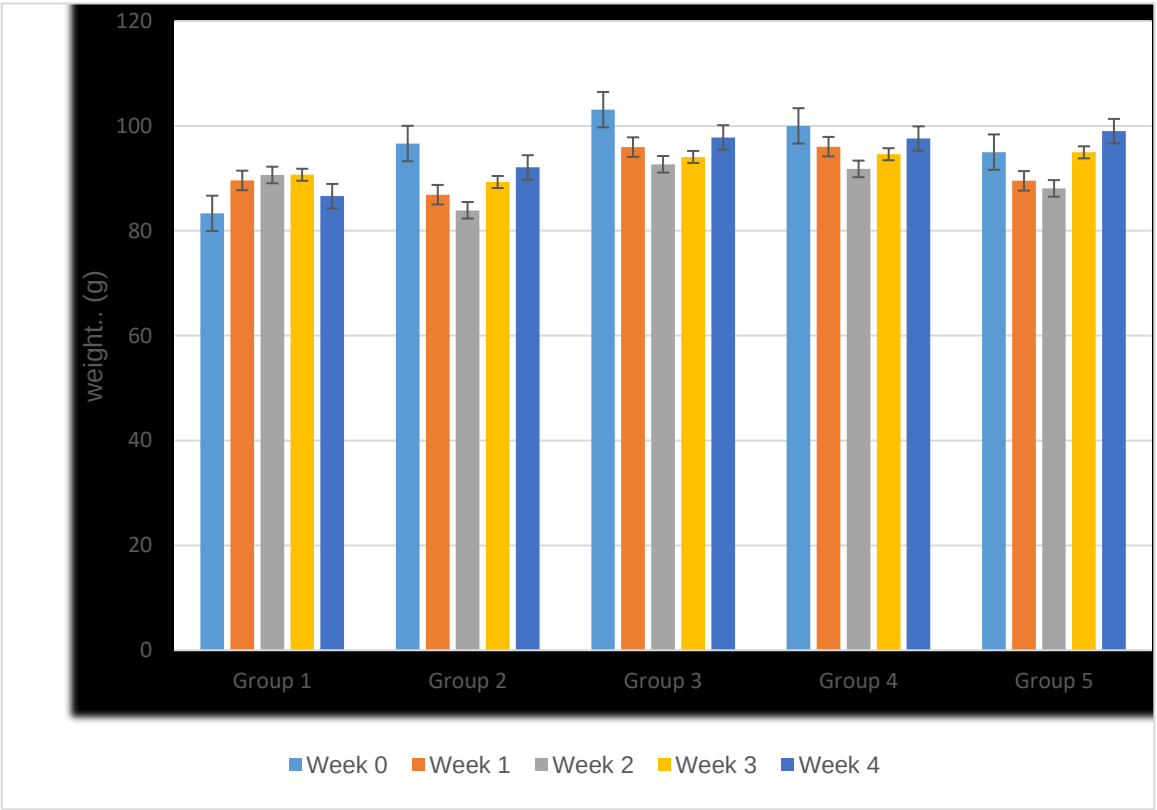
The effects of *Pistia stratiote* methanol leave extract on haematological parameters in rats during a sub-chronic toxicity assay revealed significant differences ($p<0.05$) in values of White Blood Count, Red Blood Count, Packed Count, Packed Cell Volume, and Haemoglobin in all *PS*MLE treated groups compared to the control. However, as the dose of *PS*MLE was increased, a dose-dependent decrease in Mean Cell Haemoglobin concentration, Mean Concentration Haemoglobin, Platelets, and Mean Corpuscular Volume was found in the *PS*MLE treated groups. In addition, when *PS*MLE treated, groups were compared to control groups, there was a significant ($p<0.05$) change in Platelets and Mean Corpuscular Volume (Table 4).

DISCUSSION

Preliminary phytochemical analysis helps in identifying the numerous chemical components found in plant extracts, allowing for quantitative evaluation and identification of pharmacologically active phytoconstituents. Alkaloids, tannins, anthraquinones, saponins, and flavonoids were found in this study, implying that the plant contains components that may be responsible for its medicinal effects. Experimental animal toxicology studies are commonly used to assess potential human health hazards.

Toxicity studies, according to Oakley *et al.*, (2018), analyze and x-ray the hazard and determine the risk level by studying the effect of exposure at various degrees. As a result, a substance with a toxicity of greater than 1000mg/kg body weight can be termed low toxicity and utilized safely (Partrick-iwuanyanwu *et al.*, 2012). According to the findings of this study, *Pistia stratiole* is safe and non-toxic when taken orally at 3000mg/kg body weight. A plant extract's cumulative toxic effect cannot be established just by acute toxicity (Aniagu *et al*., 2005). Subchronic or chronic toxicity studies are required to examine the safety profile of medicinal plants, according to Mohammadpour *et al.*, (2019).

As a result, it's clear that body weight change is an important indicator for assessing toxicity. Because the bodyweight of mice is used to determine compound-related effects on their growth, this is the case (Feng *et al.*, 2021). In a sub-chronic toxicity study, the *PS*MLE increased the rats' body weight (week 2-4). This could indicate that *PS*MLE had no effect on the feed utilization ratio of the animals. Burlacu *et al.*, (2019) found that Albizia chevalier crude aqueous root extract can increase body weight, which supports this claim.



re Figure 1: Albino rat body weights in a sub-chronic toxicity investigation

Table 1: Qualitative phytochemical constituents of *Pistra stratioles* Methaol leaves extract

PHYTOCHEMICALS	RESULTS
Alkaloids	+
Flavonoids	+
Tannins	+
Saponin	+
Anthraquinones	+
Phenol	-
Terpenes	-

KEY: + = Present, - = Not detected

Table 2: Effects of *Pistia stratiote* methanol leave extract (mg/kbw/day) on serum Biomarkers of liver function markers

Treatment (Doses)	AST (mg/dl)	ALT (mg/dl)	ALP (mg/dl)	ALB (G/l)	TP ((G/l)	DB(mg/dL)	TB (mg/dL)
Distled H_20 (2ml/kg)	144.6±2.80^{c}	41.6±2.16^{c}	26.8±2.24^{a}	53±2.48^{a}	80.6±3.53^{a}	0.424±0.06^{a}	0.68±0.06^{ab}
*PS*MLE (150mg/kg)	119.6±0.93^{b}	33.8±3.47b^{c}	25.8±1.16^{a}	48.8±3.29^{a}	77.4±2.87^{a}	0.256±0.04^{a}	0.76±0.07^{b}
*PS*MLE 300mg/kg)	118.2±2.75^{b}	36±1.95^{bc}	26.6±1.17^{a}	46.4±3.31^{a}	72±4.42^{a}	0.268±0.03^{a}	0.66±0.08^{ab}
*PS*MLE (600mg/kg)	102.6±14.12^{b}	30.4±1.81^{b}	31.6±3.39^{a}	47.6±2.16^{a}	75.6±1.36^{a}	0.216±0.03^{a}	0.54±0.07^{ab}
*PS*MLE (1200mg/kg)	55.8±3.48^{a}	21.4±3.33^{a}	27.4±2.80^{a}	46.2±2.13^{a}	79.2±1.69^{a}	0.354±0.06^{a}	0.52±0.05^{a}

The results are reported as mean standard error of the mean (n = 5 per group). For each parameter, test values with superscripts that differ from the control are substantially different (P0.05). not the same as the control *PS*MLE- *Pistia stratiote* methanol leaf extract, AST- Aspartate Amino Transferase, ALT- Alanine Amino Transferase, ALP- Alkaline Phosphatase, ALB- Albumin, TP- Total Protein, TB- Total Bilirubin, and DB- Direct Bilirubin

Table 3: Effects of *Pistia stratiote* methanol leaves extract on renal function indices

Treatment (Doses)	Cr (mg/dl)	U (mmol/l)	UA (mg/dl)	Na+ (mmol/l)	K+ (mmol/l)	Cl- (mmol/l)	HCO3- (mmol/l)
DistledH_20 (2ml/kg)	1.31± 0.06^{a}	7.64± 0.27^{b}	2.876± 0.1^{a}	137.024±2.28^{ab}	5.26± 0.33^{a}	90.74± 2.32^{a}	31.012± 2.35^{b}
*PS*MLE (150mg/kg)	1.47± 0.017^{b}	6.62± 0.22^{a}	3.51± 0.21^{b}	146.21± 5.97^{b}	5.57± 0.19^{ab}	92.51± 3.59^{a}	24.032± 2.13^{a}
*PS*MLE(300mg/kg)	1.65± 0.07^{c}	7.11± 0.21^{ab}	3.55± 0.20^{b}	139.65± 3.69^{b}	5.84 0.36^{ab}	106.16±4.06^{b}	24.81±0.87^{a}
*PS*MLE(600mg/kg)	1.41± 0.06^{ab}	6.93± 0.26^{ab}	2.67± 0.15^{a}	136.53±3.27^{ab}	6.68± 0.19^{b}	95.29±1.93^{ab}	22.94± 1.26^{a}
*PS*MLE(1200mg/kg)	1.37± 0.02^{ab}	6.94± 0.33^{ab}	2.59± 0.15^{a}	126.23± 2.18^{a}	6.48± 0.32^{b}	92.43± 4.62^{a}	24.76± 1.10^{a}

Values are presented as mean ± SM (n = 5) Test values carrying superscripts different from the control for each parameter are significantly different t (P<0.05) w hen compared to the control Cl−: Chloride ions, Na+: Sodium ions, HCO3: Bicarbonate ions (K+) Potassium ions. Cr-Creatinine, U-Urea, UA- Uric acid, *PS*ML E- *Pistia stratiote* methanol leave extract

Table 4: Effects of *Pistia stratiote* Methanol Leaves Extract on Hematological Parameters

Treatment (Doses)	WBC (x10^{9}/L)	RBC (x10^{12}/L)	HGB (g/dL)	PCV (%)	MCHC (g/dL)	MCH (pg)	PLT (x10^{9}/L)	MCV (fL)
DistilledH_20(2ml/kg)	9.16±0.48^{ab}	5.47±0.36^{a}	10.9±0.43^{a}	37.16±1.36^{a}	29.16±0.53^{b}	19.18±0.59^{a}	586.68±115.01^{b}	64.2±4.09^{c}
*PS*MLE (150mg/kg)	9.76±1.87^{b}	4.27±0.41^{a}	10.5±0.17^{a}	29.16±2.74^{a}	29.98±1.02^{b}	22.66±1.13^{b}	606.72±27.18^{b}	59.56±1.21^{c}
*PS*MLE (300mg/kg)	6.56±0.32^{a}	4.27±0.40^{a}	10.5±0.17^{a}	29.16±2.74^{a}	29.96±0.54^{b}	20.36±0.61^{ab}	539.54±16.33^{ab}	47.1±1.58^{ab}
*PS*MLE (600mg/kg)	6.56±0.32^{a}	4.27±0.40^{a}	10.5±0.16^{a}	29.16±2.74^{a}	22.78±1.64^{a}	47.64±1.13^{c}	452.82±17.68^{ab}	49.72±2.36^{b}
*PS*MLE (1200mg/kg)	6.56±0.32^{a}	4.27±0.40^{a}	10.5±0.17^{a}	29.16±2.73^{a}	24.76±1.29^{a}	48.26±0.68^{a}	413.28±5.18^{a}	42.3±1.08^{a}

Values are Mean ±SEM. Test values carrying superscripts different from the control for each parameter are different when compared to control (P<0.05) RBC-Red Blood Count, MCHC-Mean Cell Haemoglobin Concentration, MCH- Mean Cell Haemoglobin, MCV- Mean Corpuscular Volume, HGB- Haemoglobin, PCV-Packed Cell Volume, PLT- Platelets, WBC- White Blood, *PS*MLE-*Pistia stratiote* Methanol Leaf Extract

The liver is a main organ involved in drug metabolism and clearance, as well as the levels of numerous biomarkers that can help determine whether a medicine is hepatotoxic or hepatoprotective, according to Pingili *et al.,* (2019). High enzyme levels in the liver have been linked to liver necrosis and other illnesses that cause abnormal permeability of the liver cell membrane. Alanine Aminotransferase (ALT), Aspartate aminotransferases (AST), and Alkaline Phosphatase (ALP) are biochemical indicators of the liver that can be evaluated in the bloodstream and used as markers to determine the liver's functional capacity (Yakubu *et al.,* 2003).

The findings of this study showed that giving *PS*MLE for 28 days had no effect on liver indicators. As a result, a large amount of blood travels through *PS*MLE, and the toxins filtered are frequently concentrated in the renal tubules; the kidney is known to be particularly vulnerable to toxic substances (Al-Attar *et al.,* 2017). Blood concentrations of electrolytes, uric acid, and creatinine may potentially reveal information about the action of plant extract on the tubular and glomerular portions of the kidney (Ukwuani *et al*., 2012).

Increased serum uric acid, creatine, potassium, and chlorine levels in *PS*MLE-treated rats could be attributable to kidney-related variables, according to a recent study by Zhuang *et al.,* (2021). The haematopoietic system is a critical indicator of physiological and pathological situations in people and animals, as well as one of the most sensitive targets for hazardous substances (Mukinda *et al.,* 2017). The assessment of haematological parameters could be beneficial in determining the extract's toxicity in animal blood. A decrease in RBC, MCV, and MCHC indicates anemia.

Low WBC numbers, which include lymphocytes, monocytes, eosinophils, and platelets, may suggest a weakened immune system (Schluter *et al.,* 2020). *PS*MLE medication showed no effect on WBC, RBC, HGB, or PCV in both sexes of rats in the current study, but it did induce a dose-dependent decrease in MCHC, MCH, MCV, and PLT. As a result, the new research backs up prior results that plant extracts were used more frequently.

CONCLUSION

According to this discovery, 3000mg/kg body weight of *Pistia stratioles* is safe and non-toxic for oral administration. Toxicological examination of *Pistia stratiotes* methanol leaves extract is an excellent starting point for biological target-oriented drug discovery, and pharmacological characterization could lead to the development of new medications. More research is needed, however, to isolate and identify the active component in this plant that is responsible for these well-known toxicological assessments.

Competing interests declaration

The authors do not have any competing interests.

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