Trends in
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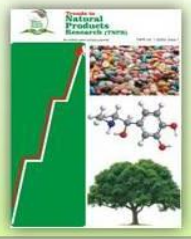

Evaluation of the acute and sub-acute toxicity of methanol leaf extract of *Stylochaeton warneckei* Engl., (Araceae) in Wistar rats.

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Keywords *Stylochaeton warneckei*, Acute toxicity, Sub-acute toxicity, Biochemical, Haematological parameters.

Abstract: *Stylochaeton warneckei* is used by herbalists to treat diabetes, bacterial infections, and other ailments. There is limited scientific evidence regarding its safety profile despite the wide local usage. This study was designed to assess the acute and sub-acute toxicity of the methanol leaf extract of *Stylochaeton warneckei* in rodents. The acute toxicity of the extract was estimated in mice while the sub-acute toxicity investigations were conducted in male Wistar rats at three dose levels (200, 400, and 800 mg/kg). At the end of the treatment period, blood samples were collected and vital organs harvested and weighed. Gross observations were made and the liver was subjected to Histopathological investigations. At doses up to 5000 mg/kg, the extract was not lethal to the mice. In the sub-acute toxicity investigations, administration of the extract for 28 days did not significantly ($p > 0.05$) change the organ weights. The haematological and biochemical parameters were not significantly ($p > 0.05$), altered by the extract. Histopathological investigation of the liver revealed normal histology of hepatocytes, and liver parenchyma, in the control as well as that of the treatment groups. The results of these studies showed that the methanol leaf extract of *Stylochaeton warneckei* is relatively safe and pose no toxicity concern in short- and medium-term usage.

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INTRODUCTION

Instinct and experience were relied on in the olden days in the use of medicinal plants for the treatment of diseases (Alozie, 2020). It is estimated that about 80 % of the world's population access plant preparations and herbal medicines to meet health needs. Medicinal plants have been sources of raw materials for modern medicine even as it is for traditional medicines (Biljana, 2012). As plant-based remedies are of natural origin, it is misleading to consider them as generally safe, even when they are having proven efficacy against many diseases. Toxic reactions and, sometimes, severe life-threatening outcomes have been documented with the use of some medicinal plants (*Taofeeq et al*., 2010). Plants and their products that are employed medicinally for the treatment of various diseases must be assessed and evaluated for possible toxic potentials (loubna*, et al*., 2020). Usually, toxicity tests in rodents are used to determine the safety and possible deleterious effects of plant extracts. The safe dose range of plant extracts to be administered is also established by toxicity testing (Falya *et al*., 2020). Medicinal plants contain various mixtures of different phytochemicals that may act individually or in synergy to improve health (Monach *et al*., 2004). Such biological active principle includes alkaloids, tannins, saponins, glycosides, fatty oils, volatile oil, etc. Despite the numerous medicinal relevance of plants, some of their secondary metabolites are toxic (Okoro, *et al*., 2011).

Stylochaeton warneckei (S. warneckei*)* belongs to the family Araceae. It is endemic to Africa and it is geographically distributed from West Tropical Africa to Sudan (Bogner, 2011). The plant is erect on a vertical rhizome, 10-15 cm thick, with numerous adventitious roots in clumps, 5 mm thick. The female flowers are separated from the male flowers by a smooth space of 5 mm (Hesse *et al.,* 2001). Some species of *Stylochaeton* are used by the traditional healers of the Limpopo province, South Africa to treat rhinitis. *Stylochaeton natalensis,* specie of the genera *Stylochaeton*, is used in the treatment of sinusitis, sore throat, and related symptoms (Semenya and Maroyi, 2018). Some species of *Stylochaeton* are used as traditional leafy vegetables in Benin (Dansi, *et al*., 2009). Preparation of the root of *Stylochaeton* species when combined with preparation of the bark of *Anogeissus leiocarpa* is used to treat haemorrhoids. Some species of Stylochaeton are used to treat asthma and related symptoms by Bapedi traditional healers in Limpopo province, South Africa (Maroyi and Semenya, 2018). The widespread use of Stylochaeton in traditional medicine motivated this study aimed at assessing the acute and sub-acute toxicity of *Stylochaeton warneckei* methanol leaf extract in rodents.

Materials and Methods

Collection and authentication of plant material

Fresh leaves of *Stylochaeton warneckei,* was sourced from local farmers at Obollo Afor, Enugu State, Nigeria in 2022. The plant material, *Stylochaeton warneckei* (CEDD/16289) was identified and authenticated by Mr. Alfred Ozioko a Staff of the Bio-resource Development and Conversion Program (BDCP) Laboratory, at Obe Echara in Nsukka, Enugu State.

Animals

Twenty healthy male Wistar rats (100-160 g) and twelve male mice (11-30 g) were used in this study. The animals were obtained from the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. The animals were housed in metal cages and were allowed free access to standard rodent pellets and potable water throughout the experiment. The University of Nigeria, Faculty of Pharmaceutical Sciences Research Ethics Committee gave approval to this study with the approval number FPSRA/UNN/23/0053.

Extraction of the plant extract

The leaves were air-dried for five days and milled to a fine powder. One (1) kg of the milled leaves was extracted with 3 L of methanol by cold maceration for 72h. A 226.3g of the extract was obtained after the filtrate was concentrated using a rotary evaporator.

Acute toxicity study

The median lethal dose (LD_{50}) of the test extract was estimated in mice by the oral route using the method described by Lorke (1983). Briefly, the tests involved two phases. The first phase involved the determination of the toxic range. The mice were placed in 3 groups (n = 3) and the methanol extract (10, 100, and 1,000 mg/kg) was administered orally. The mice were observed for 24 h for lethality. The death pattern in the first phase determined the doses used for the second phase. In the second phase, 3 different doses (1600, 2900, 5000 mg/kg) of the tests extract were administered (per os) as predetermined in the earlier phase of the study. The animals were observed for lethality and

signs of acute intoxication for the next 24 h. The LD_{50} was calculated as the geometric mean of the highest nonlethal dose and the least toxic dose.

Sub-acute toxicity study

Rats were acclimatized for 7 days, during which they were fed and allowed access to potable water *ad libitum*. Thereafter, the rats were randomized into four groups (n=5). The first group served as the control group and received vehicle (10 ml/kg distilled water /rat, p.o.) once daily for 28 days. The second group was administered the extract (200 mg/kg, p.o) once daily for 28 days. The third group received the extract (400 mg/kg, p.o) once daily for 28 days while the fourth group was given the extract (800 mg/kg, p.o) orally once daily for 28 days. All the groups were observed daily for mortality and morbidity throughout the experimental period.

Effect of treatment on body weight

The weight of the individual rats in each group was recorded before the experiment, weekly throughout the experiment, and finally on the day of sacrifice.

Blood sample collection

On day 28, the rats were fasted overnight after which they were anaesthetized and blood samples collected through a retro-orbital puncture. Blood samples were collected using an EDTA bottle for Haematological analysis and a non-EDTA bottle for biochemical analysis.

Isolation of vital organs

The animals were euthanized at the end of the 28th day and the essential organs (heart, liver, lungs, spleen, and kidney) were harvested and weighed. The relative organ weight was determined and the organs were subjected to histological study.

Measurement of Haematological parameters

Haematological parameters such as packed cell volume (PCV), haemoglobin (Hb) concentration (cyanmethemoglobin method), red blood cell (RBC) count, white blood cell (WBC) count, differential white blood cell (WBC) count, platelet count was assessed using the standard laboratory methods (Cheesbrough,2006).

Biochemical Assay

Biochemical parameters such as serum total protein, albumin; alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) (Liver enzymes) were analyzed using the related kits (Randox Laboratory Ltd. Co. Antrim, United Kingdom).

Histopathological investigations

The liver tissues were subjected to Histopathological studies. The tissues were quickly fixed in neutral buffered formalin until processed for analysis. The tissues were fixed and sectioned to a thickness of 3-5µm stained and observed under a light microscope at x200 and x400 for histological changes (Slaoui and Fiette, 2011).

Statistical analysis

Data were analyzed using one-way ANOVA and Tukey HSD multiple post hoc comparison and expressed as mean ± standard error of mean. Difference in mean was considered significant at $p > 0.05$.

RESULTS

Acute Toxicity Test

In the acute toxicity tests in mice, methanol leaf extract administered orally at doses up to 5000 mg/kg body weight had no lethal effect or any sign of acute intoxication in the mice after a 24-h observation period. The LD_{50} is therefore greater than 5000 mg/kg.

Effect of the Extract on body Weight

Daily administration of the extract at doses of 200, 400, 800 mg/kg did not cause significant ($p > 0.05$) difference in the body weight of rats when compared to the control (Table 1). However, it is observed that the animals in all the groups gained weight with each succeeding week.

Table 1: Effect of the Extract on the Weight (grams) of the Animals

Group	Week 0	Week 1	Week 2	Week 3	Final week
Negative Control	109.62±1.68	122.30±6.02	141.34±9.59	160.03±12.79	166.06±14.32
Extract (200 mg/kg)	114.72±12.09	128.±10.75	150.23±19.19	161.09±10.23	166.52±14.29
(400 mg/kg)	117.30±3.05	131.67±3.71	152.66±4.93	176.00±14.24	181.36±23.70
(800 mg/kg)	119.38±1.53	132.34±1.59	154.96±7.21	168.71±14.37	189.45±11.59

Result expressed as mean ± std. deviation, n=5.

Effect of Extract on relative organ weight

Daily oral administration of the extract at (200, 400, 800 mg/kg) had no significant ($p > 0.05$) effects on the relative organ weights of the lungs, liver, kidney, heart and spleen when compared to those of the control group (Table 2).

Table 2: Effect of Extract on Relative Organ Weight

Group	liver	kidney	Heart	Spleen	Lungs
Negative Control	0.034±0.036	0.007±0.008	0.004±0.001	0.007±0.000	0.007±0.01
Extract (200 mg/kg)	0.032±0.056	0.007±0.007	0.004±0.000	0.007±0.002	0.008±0.001
(400 mg/kg)	0.028±0.003	0.006±0.001	0.004±0.001	0.005±0.001	0.007±0.001
(800 mg/kg)	0.033±0.003	0.007±0.001	0.004±0.000	0.009±0.003	0.008±0.001

Result expressed as mean ± std. deviation, n=5.

Effect of Extract on haematological parameters

The extract (200, 400, 800 mg/kg) did not significantly ($p > 0.05$) affect the packed cell volume, red blood cells, haemoglobin, total white blood cell, platelets, neutrophils, lymphocytes, monocytes, eosinophils, and basophil (Table 3).

Table 3: Effect of Extract on Hematological parameters

Parameters	Negative Control	Extract (200 mg/kg)	Extract (400 mg/kg)	Extract (800 mg/kg)
Hb(g/dL)	13.04±0.34	12.88±1.07	12.88±1.17	12.93±0.80
PCV (%)	42.00±6.90	43.00±5.57	41.60±6.19	42.35±5.49
RBC ($x10^{12}$/L)	5.29±1.53	5.20±1.41	4.97±1.75	5.23±1.29
Total WBC($x10^9$/L)	8.88±4.03	8.00±3.65	8.84±4.95	8.46±4.50
Platelets($x10^9$/L)	383.60±25.04	376.80±45.02	372.80±36.40	376.20±56.29
Neutrophils (%)	24.20±5.89	25.20±6.87	23.40±8.14	24.40±9.04
Lymphocytes (%)	71.20±8.79	65.60±10.11	66.60±10.11	66.80±5.81
Monocytes (%)	5.80±3.56	4.00±2.35	4.60±2.41	5.20±3.83
Eosinophils (%)	0.66±0.47	0.90±0.73	0.20±0.28	0.20±0.28
Basophils (%)	0.20±0.45	0.00±0.00	0.20±0.045	0.20±0.45

Result expressed as mean ± std. deviation, n=5. Hb – Haemoglobin., PCV- Packed cell volume, RBC-Red blood cell, WBC- White blood ce

Effect of Extract on Serum Biochemical Parameters

The experiment revealed no significant ($p > 0.05$) change in the biochemical parameters of the extract treated groups when compared with the control group (Table 4). The biochemical parameters examined were: alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum total proteins and albumin.

Table 4: Effect of Extract on Serum Biochemical Parameters

Group	AST(IU/L)	ALT (IU/L)	ALP(IU/L)	TP(IU/L)	ALB(IU/L)
Negative Control	149.67±56.12	34.96±11.18	199.30±63.32	7.06±0.79	3.78±0.59
Extract (200 mg/kg)	142.84±21.74	34.62±2.66	198.97±78.83	7.20±0.59	3.82±0.58
(400 mg/kg)	152.84±22.82	36.15±19.73	194.26±53.34	6.76±0.38	3.89±0.68
(800 mg/kg)	139.82±27.35	36.44±3.89	192.24±59.36	6.80±0.56	3.54±0.54

Result expressed as mean ± std. deviation, n=5., AST – Aspartate Aminotransferase, ALB- Albumin
ALT – Alkaline Aminotransferase, TP- Total protein, ALP – Alkaline Phosphates

Effect of Extract on liver histology

Group A: Control

There is normal histology of the liver parenchyma and the hepatic portal area. There is evidence of a normal structure of the peri-portal region. The portal tract comprised normal hepatic portal vein (PV), hepatic arteries (HA) and bile ducts (BD) surrounded by a well-defined limiting membrane. There is the presence of normal hepatocytes with deeply eosinophilic cytoplasm (large white arrow). Normal sinusoidal spaces (black arrow head) with scanty presence of RBCs (small white arrow) in the portal vessels and sinusoidal spaces. There is scanty presence of inflammatory cells (small black arrow) in the peri-portal region and the sinusoids (Fig 1).

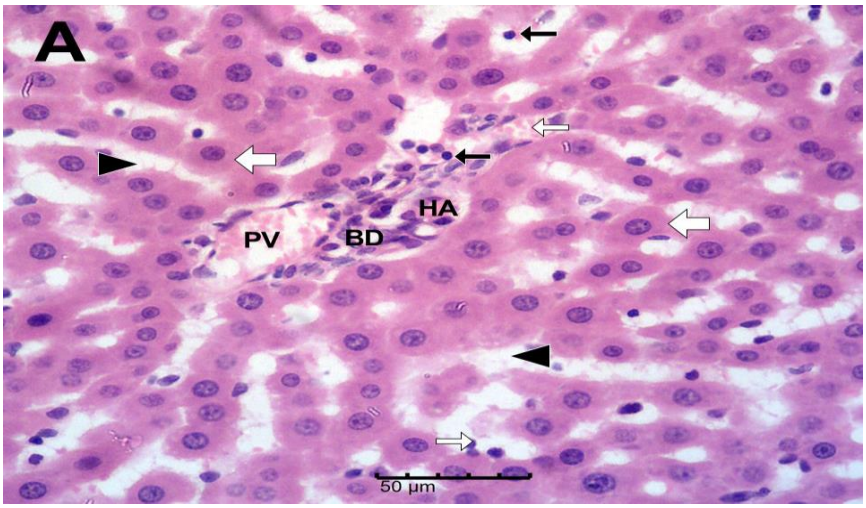

Figure 1: H&E-stained sections of rat liver, Hepatic Portal area (group A) (scale bar = 50µm).

Group B: Extract (200 mg/kg)

There is normal histology of the liver parenchyma and the hepatic portal area. There is evidence of a normal structure of the peri-portal region. There is the presence of normal hepatocytes with deeply eosinophilic cytoplasm (large white arrow). There are normal sinusoidal spaces (black arrow head) with scanty presence of RBCs (small white arrow) in the portal vessels and sinusoidal spaces. There is scanty presence of inflammatory cells (small black arrow) in the peri-portal region and the sinusoids (Fig 2).

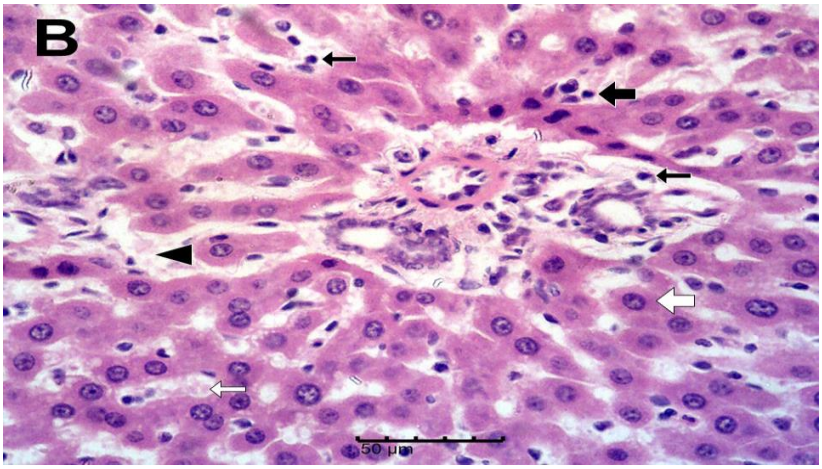

Figure 2: H&E-stained sections of rat liver, Hepatic Portal area (group B) (scale bar = 50µm).

Group C: Extract (400 mg/kg)

There is normal histology of the liver parenchyma and the hepatic portal area. There is evidence of a normal structure of the peri-portal region. There is the presence of normal hepatocytes with deeply eosinophilic cytoplasm (large white arrow). Normal sinusoidal spaces (black arrow head) with scanty presence of RBCs (small white arrow) in the portal vessels and sinusoidal spaces. There is scanty presence of inflammatory cells (small black arrow) in the peri-portal region and the sinusoids (Fig 3).

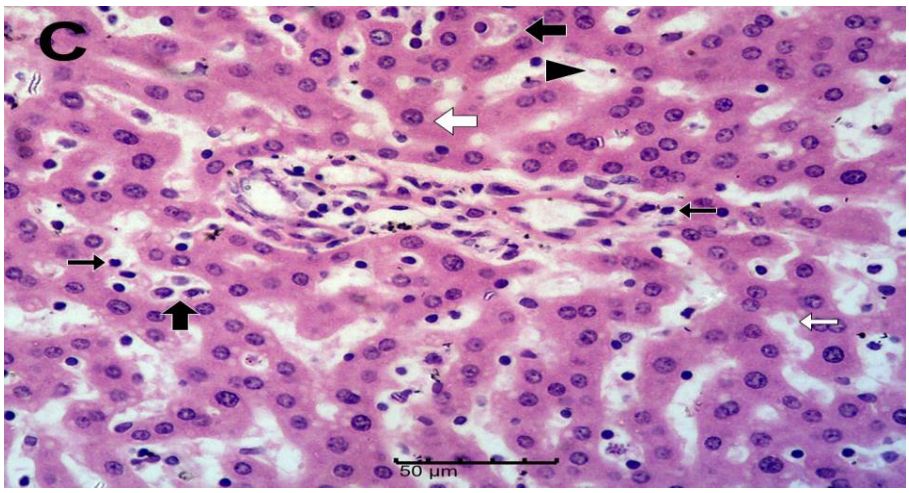

Figure 3: H&E-stained sections of rat liver, Hepatic Portal area (group C) (scale bar = 50µm).

Group D: Extract (800 mg/kg)

There is normal histology of the liver parenchyma and the hepatic portal area. There is the evidence of a normal structure of the peri-portal region. There the presence of normal hepatocytes with deeply eosinophilic cytoplasm (large white arrow). There are normal sinusoidal spaces (black arrow head) with scanty presence of RBCs (small white arrow) in the portal vessels and sinusoidal spaces. There is scanty presence of inflammatory cells (small black arrow) in the peri-portal region and the sinusoids (Fig 4).

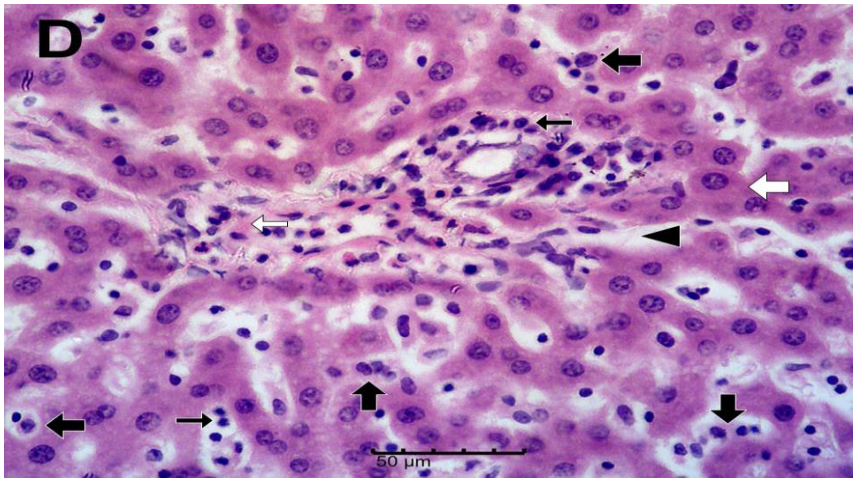

Figure 4: H&E-stained sections of the rat liver, hepatic portal area (group D) (scale bar = 50µm).

DISCUSSION

The acute toxicity is the preliminary step employed in the toxicological examination of known and unknown substances as well as unused plant extract (Falya *et al*., 2020). The acute toxicity (LD_{50}) study result indicates that the methanol leaf extract of *S. warneckei* is safe and non-toxic at 5000 mg/kg body weight since there was no mortality or any observable behavioural changes in all the test groups. There was no hypertrophy of the organs as was seen from the results of the relative organ weight however, the appreciable weight gain by the animals may be due to hypertrophy (Berinyuy,*et al*., 2015). The usefulness of the information from the haematological parameters serves to explain the adverse effects of substances on blood as well as the blood-related functions of agents (Berinyuy,*et al*., 2015). Oral administration of *S. warneckei* did not cause any RBC destruction, not anti-thrombopoietin and did not affect haematopoiesis indicating the absence of anaemia and other hematological disorders (Bashir *et al*., 2015, Mishra and Tandon, 2012).

Measurements of enzyme markers in tissues help in the assessment of safety and/or toxicity (Adeyemi and Akanji, 2010). The results on the activity of the liver enzyme markers suggest that oral administration of *S. warneckei* did not cause tissue damage, and did not compromise the integrity of the hepatocellular membrane. Hence, no necrosis of hepatocytes erythrocytes occurred (Macfarlane *et al*., 2000; Akanji *et al*., 1993). The liver is the largest organ in the body. It plays important role in metabolism and biotransformation. In the study, the histopathological evaluation of the liver after 28 days' post administration of the extract, showed normal sections of the liver. From the histological study it is evident that the methanol leaf extract of *S. warneckei* has no deleterious effects on the liver (A) (Irma *et al*., 2021). The Liver is an important organ

for the biotransformation of xenobiotic and drugs and is also susceptible to damage from toxic test drugs. Biochemical analyses of hepatic function parameters are done to evaluate the possible alterations in hepatic functions caused by the test samples. Haematological parameters as well as tissue histology are assessed to determine the toxicity of the plant extract (Bouhrim, *et al*., 2018; Lubna, *et al*., 2020).

CONCLUSION

This study shows that the methanol leaf extract of *S. warneckei* can be considered safe in animal model experiment as there are no deaths as well as toxicities in organ damage associated with its use. However, further investigations such as chronic studies and other specialized toxicological (mutagenicity, teratogenicity and carcinogenicity) studies are needed to ascertain its prolonged safety in humans.

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