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Research Article

Assessment of Acute and Sub-acute toxicity effects of methanol extract of leaves of *Solanum dasyphyllum* in an experimental mouse model.

***Oyinloye O.E.[1], Murtala A.A.[2], Oladoja F.A.[1], Okunye O.L.[3], Ogunjimi L.O.[2], Alabi A.O.[2], Aderinola A.A.[2] and Sule A.S.[4]**

[1]*Olabisi Onabanjo University, Faculty of Pharmacy, Department of Pharmacology and Toxicology, Sagamu Campus, Ogun State.*
[2]*Olabisi Onabanjo University, Obafemi Awolowo College of Health Sciences, Faculty of Basic Medical Sciences Department of Pharmacology and Therapeutics, Sagamu Campus, Ogun State.*
[3]*Olabisi Onabanjo University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Sagamu Campus, Ogun State .*
[4]*Olabisi Onabanjo University, Faculty of Pharmacy, Department of Clinical Pharmacy and Biopharmacy, Sagamu Campus, Ogun State*

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Abstract
Solanum dasyphyllum leaves are used to treat a range of diseases in ethnomedicine. However, there are few reports on its toxicity. The goal of this investigation was to determine the acute and sub-acute toxicity of methanol extract of leaves of *Solanum dasyphyllum* (MESd) in Swiss mice. Dried MESd was extracted with 80% methanol. The MESd was given orally to mice at a single graded doses (1.25, 2.5, 5, and 10 g/kg) and monitored for 14 days in an acute toxicity test. In the sub-acute toxicity study for 28 days, the animals received oral graded doses (125, 250, 500, and 1000 mg/kg) of MESd while the control group received tween-80 (1%). Body weights were measured at 2-day intervals. At the conclusion of the study, liver function tests, lipid profiles, and histology were determined. The LD_{50} was determined to be 5.62 g/kg. Except at 5 and 10 g/kg, MESd was relatively non-toxic, with no mortality or obvious symptoms of toxicity. In acute toxicity, the extract significantly ($p<0.05$) reduced food and water consumption in all the treatment groups relative to the control. Body weight in surviving mice in acute toxicity study increased, after the initial reduction in weights. In the sub-acute toxicity, the extract showed no significant increase in liver enzymes, while significant increase was observed in HDL at 500 and 1000 mg/kg. The histology of the liver and kidney showed no substantial changes, except in animals that received 1000 mg/kg MESd. Methanol extract of leaves of *Solanum dasyphyllum* appears to be safe in mice in lower doses. However, high dose for extended period appears to be associated with toxicity.

Key Words: *Solanum dasyphyllum,* Toxicity, Methanol, Safety, Mice

INTRODUCTION

In developing areas, medicinal plants are used as a natural substitute for pharmaceuticals (Ankur *et al*., 2010). Around 80% of the world's population, mostly in Africa and other developing countries, is still reliant on traditional or herbal medicine for disease treatment (Oreagba *et al*., 2011). Over 80% of people in Nigeria rely on traditional medicine, according to estimates (Chukuma *et al*., 2015). Plants produce a variety of metabolites, some of which are beneficial to humans and animals, while others are potentially poisonous (Kale *et al*., 2019).

Furthermore, several human cases of plant poisoning have been reported as a result of plant mis-identification, ingestion of wrong parts of edible plants, ingestion of certain plants parts in the wrong season and eating of plants that are edible to some but poisonous to others (Fred-Jaiyesimi and Ajibesin, 2012). When toxic plants are consumed in little or moderate quantities, they create chemical compounds that might cause negative reactions in people and animals. When ingested or in touch with humans, poisonous plants frequently have negative consequences. They stimulate or cause sadness, tremors, convulsions, paralysis, and strange

**Author for Correspondence:* Tel: +2348039196240

E-mail:*dapobuk2003@yahoo.com and oyinloye.oladapo@oouagoiwoye.edu.ng*

behaviours by affecting the body system in a variety of ways (Botha, 2008).

It has been demonstrated that contemporary medications can be beneficial at one dose but hazardous at another (Sharif *et al.,* 2015). Prior to their administration in humans, toxicological profiles of herbs thought to be beneficial to mankind must be completed (Moreira *et al*., 2019). To examine the harmful effect of new items or drugs, acute and sub-acute toxicity studies are routinely conducted. The first stage is to conduct an acute toxicity test to find out if a product has any negative side effects within 14 days of a single dose treatment (Rhiouani *et al.,* 2008; Bhardwaj and Gupta, 2012). To determine the median lethal dosage (LD_{50}) for a particular harmful chemical in rats or mice, acute toxicity tests are commonly conducted by oral routes (Gadanya *et al.,* 2011). Sub-acute toxicity tests are conducted on rodents, dogs or monkeys on a regular basis (Bhardwaj and Gupta, 2012; Gandhare *et al.,* 2013).

In both humans and animals, toxic substances primarily attack the liver and kidneys (Dybing *et al*., 2002; Saad *et al*., 2006). Consequently, these two organs are useful in determining the harmful effects of herbal or pharmaceutical medications (Bello *et al*., 2016). The liver is the primary organ for hazardous compounds because of its first contact with foreign products in the intestine before entering the blood stream, while the kidneys are exposed to toxins in the blood stream. Both organs are involved in the processes that lead to the body's removal of poisons and waste products (Rhiouani *et al*., 2008; Samuel *et al.,* 2012). Despite the fact that toxins are being detoxified by the liver, some can still harm the body if the liver's health and function is compromised (Ravikumar *et al.,* 2012). In animal models, the liver function test performed would aid in recognizing harmful consequences and, if generalized, would allow for a better understanding of its safety (Ravikumar *et al*., 2012).

Solanum dasyphyllum is a plant that has a wide range of medicinal use for a variety of diseases. It is rich in phenols and flavonoids, the leaves of *Solanum dasyphyllum* are consumed as vegetables, used as antibacterial, pain killers, laxatives and relief swelling (Burkil, 1985). Ingestion of the root of *Solanum dasyphyllum* (Nigeria/Yoruba name-Bobo awodi and Guinea-bissau/ Guinea-Bissau- Fula-pulaar) has been reported to cause death in domestic animals (Fred-Jaiyesimi and Ajibesin, 2012). However, information on the toxicity of the edible part of the plant, the leaves is limited. There is need for scientific information on the toxicity profile of *Solanum dasyphyllum* leaves. Thus this study assessed the acute and sub-acute toxicity effects of methanol leaf extract of *Solanum dasyphyllum* in an experimental mouse model.

MATERIALS AND METHODS

Collection of plant material: *Solanum dasyphyllum* leaves were obtained in Akungba Akoko, Ondo State, Nigeria, and identified at Forestry Research Institute of Nigeria (FRIN), Ibadan. The plant was then authenticated at the FRIN with the voucher number F.H.I 109799. The voucher specimen was deposited in the Herbarium, Department of Pharmacognosy, University of Ibadan, Ibadan.

Preparation of crude extract: Fresh leaves of *Solanum dasyphyllum* were air-dried and pulverised. Three kilogram (3 kg) of dried powder plant material was weighed and steeped for three days at room temperature in 15 L of 80% methanol. The mixture was stirred at regular interval. The mixture was filtered through muslin cloth and concentrated in vacuo using a rotary evaporator (Buchi Rotavapor -124) at 40°C, the extract was stored at 40C.

Experimental Animals: The animal house of the College of Medicine, University of Ibadan, Ibadan, Nigeria, provided Swiss mice of both sexes weighing 20 to 25 g for the study. For 7 days in the laboratory, the animals were kept in cages with wood shavings to acclimate. They were given unlimited access to water and food. The study followed International and local ethics in the use and care of animal (University of Ibadan Animal Use and Care Research Ethics Committee (ACUREC), (UI-ACUREC/APP/2016/021).

Determination of the acute and sub-acute toxicity of MESd

Determination of acute toxicity of MESd: The Behrens and Karber (1983) method was followed in conducting the acute toxicity study of MESd in mice. For this experiment, thirty (30) mice weighing 25-30 g were employed. Body weights of animals were determined during the acclimation phase. To assess acute toxicity, overnight-fasted mice were divided into five groups (n=6). The first group received 1% Tween-80 (T-80) vehicle orally, while the MESd treated groups received doses of 1.25, 2.5, 5 and 10 g/kg. Sluggishness, hostility, sensitivity to stimuli (tail pinch, noise), social interactions, altering posture, fore- stretching, and breathing difficulties, as well as faeces and mortality, were all examined for 3 hours. The animals were provided food and water *ad libitum* once behavioral and autonomic alterations were observed. Within 48 hours of extract administration, dead animals in each group were recorded, and an LD_{50} value was calculated using Behrens and Karber's formula (1983). The animals that survived were examined every day for 14 days. Body weight, food, and water consumption of mice were recorded.

$LD_{50}=LD_{100} - \Sigma (ab)/n$ (Behrens and Karber, 1983)

LD_{50} - 50% mortality dose, LD_{100} - 100% mortality dose, n - number of animals in each group, a -The difference between two consecutive doses, b -The arithmetic mean of the deaths from two consecutive doses.

Determination of sub-acute toxicity of MESd: Thirty mice were distributed into five groups (n=6). The control group received vehicle (1% Tween-80) while the remaining four groups of mice were given MESd at doses of 125, 250, 500, and 1000 mg/kg. Treatment was given orally for 28 days. One-fifth of the LD_{50} was employed as the highest dose for the sub-acute toxicity study. Throughout the 28-day experiment, all animals were examined twice daily for signs of morbidity and mortality. Body weights were recorded at the beginning, then every two days during the period of the study.

Preparation of serum samples and organs: The animals were sacrificed under ether anesthesia 24 hours after receiving the last dosage of *S. dasyphyllum* methanol extract. Blood samples collected by cardiac puncture were placed in

plain tubes and left to coagulate. After centrifuging the clotted blood samples at 3000 rpm for 15 minutes, the serum samples aspirated were used to determine the liver function tests and lipid profiles. The liver and kidney samples were excised, rinsed in normal saline, blotted on filter paper and fixed in 10% formalin for histological assessment.

Biochemical analysis: Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP), total cholesterol, triglycerides, Low density and High-Density Lipoprotein were determined in serum, using commercial kit. Lipid profiles and liver function kits were purchased from Randox Laboratories Ltd. in the United Kingdom, and Vital Diagnostic Spb Ltd in Russia, respectively.

Histological analysis: Each group's liver and kidney tissues fixed in 10% formalin were embedded in paraffin. A light microscope was used to view 5-6 mm sections stained with Haematoxylin and Eosin (H&E) (Olympus CH02). Differences between the liver and kidney of the control and treated mice were noted.

Statistical analysis: The values were presented in the form of mean ± standard error of the mean (SEM). The statistical analysis included one-way analysis of variance (ANOVA) and Tukey multiple comparison tests. P values < 0.05 were considered statistically significant.

RESULTS

Table 1:Acute toxicity effects of methanol extract of *Solanum dasyphyllum* in Swiss mice

Treatment (g/kg)	Effect		
	D/T	Mortality latency (h)	Symptoms of Toxicity
1% Tween 80 (0.0)	0/6	-	None
MESd 1.25	0/6	-	None
MESd 2.5	0/6	-	None
MESd 5	3/6	>6, <7	Piloerection, trembling, fore-stretching, breathing difficulty, constant changing position, immobility.
MESd 10	6/6	>5, <48	Piloerection, trembling, stretching, diarrhoea, fore-stretching, gasping, trembling, asthenia, constant changing position, immobility.

The LD_{50} value was 5.62 g/kg; D/T-Dead/Treated mice

Acute toxicity

Behavioural observations and mortality patterns: The median lethal dose value of MESd was found to be 5.62 g/kg. There were behavioural (changing position, fore-stretching and breathing difficulty) and autonomic changes (gasping, trembling, salivation, defeacation and urination) in the animals administered with MESd (5 and 10 g/kg). All the animals treated with dose of 10 g/kg MESd died between 5 to 48 hours (Table 1). Fifty percent mortality was recorded in the groups that received 5 g/kg MESd within 6 hours. The apparent cause of death was convulsion and respiratory distress.

Effect of MESd on Food, water consumption and body weight in mice: A significant dose dependent reduction in food and water consumption was observed in mice in all treatment groups compared to the control, in the first week. However, improvement was noted in the second week in the surviving animals treated with 1.25, 2.5 g/kg MESd (Figure 1-2). The result revealed weight gain in the surviving animals treated with 1.25, 2.5 g/kg MESd, after the initial weight loss. This weight gain was pronounced on the 8th day of the experiment (Figure 3)

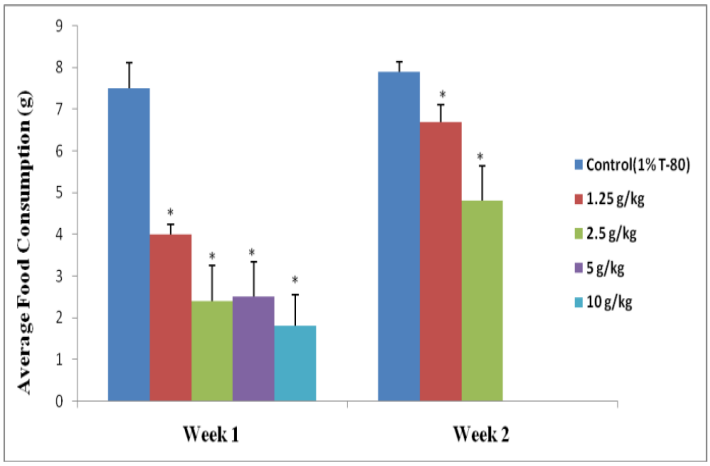

Figure 1:
The effect of acute toxicity of MESd on food consumption

*P< 0.05 treatment compared with control

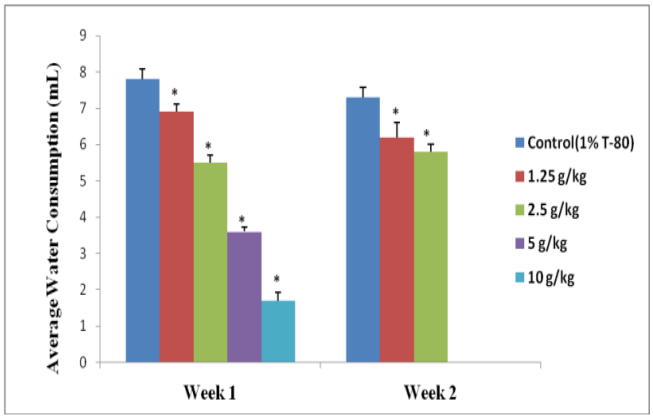

Figure 2:
Effect of acute toxicity of MESd on water consumption

*P< 0.05 treatment groups compared with control

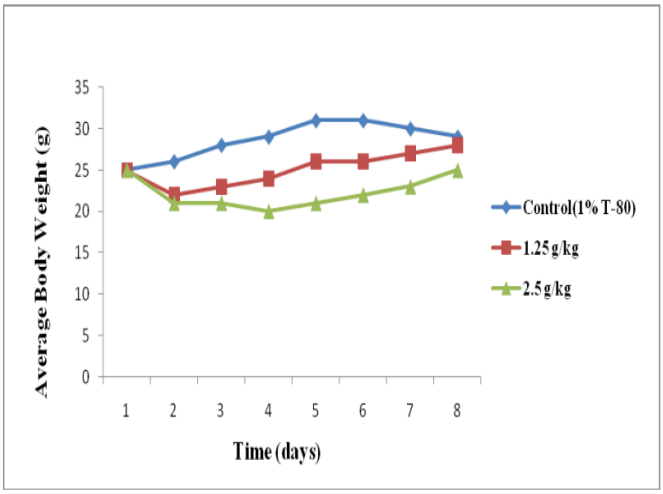

Figure 3:
Effect of MESd on average body weight in acute toxicity

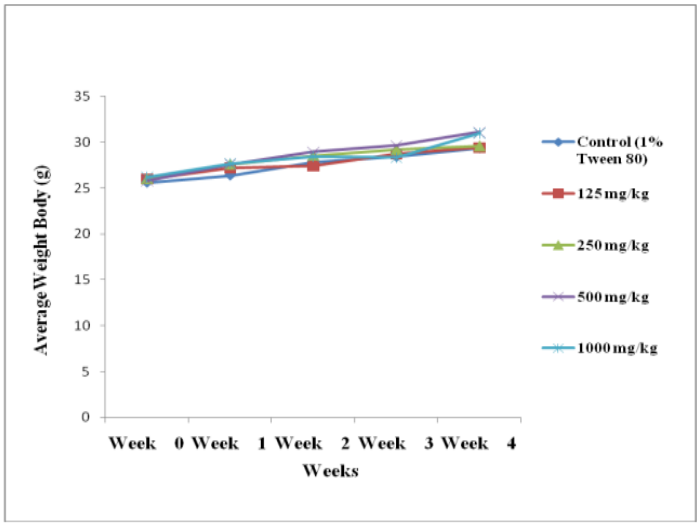

Figure 4:
Effect of MESd on average body weight in sub-acute toxicity

Sub-acute toxicity

Effect of MESd on body weight: Both the control and MESd treated groups showed a dose-dependent increase in body weights on a weekly basis, throughout the period of the study (Figure 4).

Effect of MESd on the lipid profiles and liver enzyme activity in sub-acute toxicity test: Treatment with MESd revealed a dose-dependent rise in lipid profiles at all dose levels (125, 250,500, and 1000 mg/kg), with a significant increase ($p<0.05$) in HDL in groups administered with 500 and 1000 mg/kg dose of MESd compared with the control (Table 2). There was no significant increase in serum ALT, AST and ALP levels in all groups treated with MESd in comparison with the control group (Table 3)

Table 2:
Effects of sub-acute administration of MESdon lipid profiles in mice

Lipid profiles	Control (1% T-80)	MESd 125 (mg/kg)	MESd 250 (mg/kg)	MESd 500 (mg/kg)	MESd 1000 (mg/kg)
Cholesterol (mg/dL)	35.3±0.8	36.2±2.6	38.9±1.3	39.5±2.4	40.1±1.2
Triglyceride (mg/dL)	16.9±5.5	19.1±1.9	20.2±0.2	23.21±1.4	25.2±0.1
HDL (mg/dL)	27.5±1.1	30.1±2.2	40.0±1.3	52.3±1.1*	64.2±0.4*
LDL (mg/dL)	17.9±0.3	20.6±1.2	20.8±0.1	21.3±0.8	22.9±1.1

N = 5, *p<0.05 = treatment groups compared with control, T-80 = Tween 80, HDL = High density lipoprotein, LDL = Low density lipoprotein.

Table 3:
Effects of sub-acute administration of MESd on liver enzymes in mice

Liver Function Test	Control (1% T-80)	MESd 125 (mg/kg)	MESd 250 (mg/kg)	MESd 500 (mg/kg)	MESd 1000 (mg/kg)
ALP (IU/L)	32.3±0.1	35.2±0.6	36.0±0.6	36.1±0.3	38.4±1.4
AST (IU/L)	33.2±1.2	37.1±1.4	37.4±2.1	37.5±1.4	38.1±1.2
ALT (IU/L)	40.3±0.1	41.2±0.2	41.6±0.2	42.2±0.2	43.1±1.5

T-80 = Tween 80, ALP = Alkaline phosphatase, AST = Aspartate transaminase and ALT = Alanine transaminase

Effect of MESd on Liver and kidney histology in sub-acute toxicity: Histological findings showed that the liver and kidney of the MESd (125, 250 and 500 mg/kg) treated group appeared normal and healthy with no observable lesion when compared with the control (Figure 7 & 8). Hepatic parenchyma and hepatocyte structures of groups treated with MESd 125, 250 and 500 mg/kg doses of MESd were fully preserved (Figure 7). The cortex of MESd 125, 250 and 500 mg/kg group were presented with regular distributed glomeruli and fine Bowman capsule with no inflammatory reaction (Figure 8). The liver and kidney histology of group treated with 1000 mg/kg dose of MESd showed cytoplasmic degeneration and some aggregation and infiltration of inflammatory cells (Figure 7 & 8)

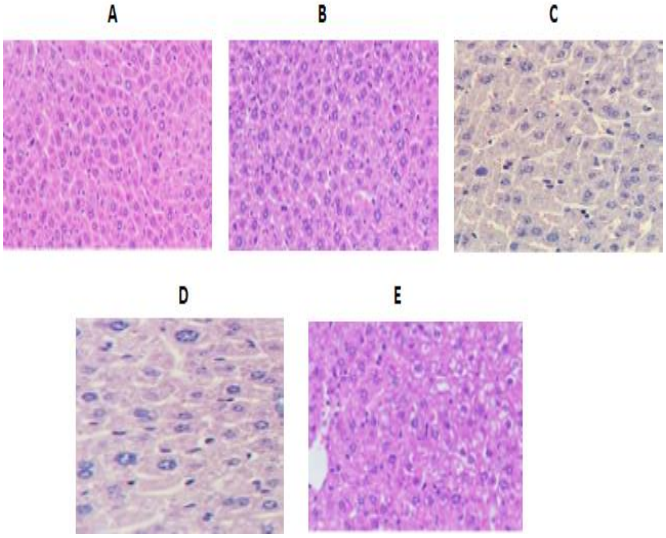

Figure 7:
Histology of the liver of control mice and mice subjected to graded doses of MESd for 28 days, HE x400
MESd – Methanol extract of *Solanum dasyphyllum*, A - control (1% T-80), B-E - 125 -1000 mg/kg MESd,

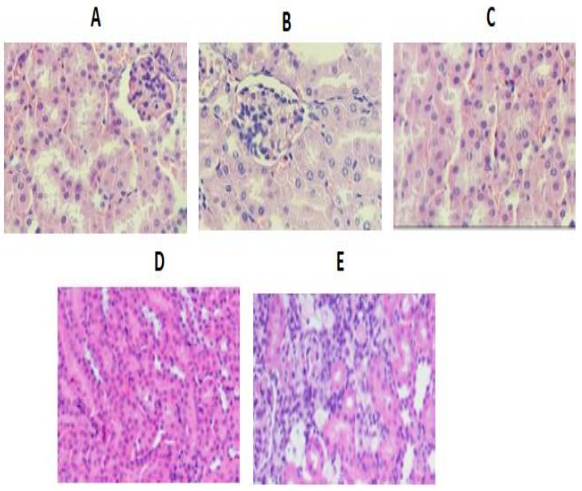

Figure 8:
Histology of the kidney of control mice and mice subjected to graded doses of MESd for 28 days, HE x400
MESd – Methanol extract of *Solanum dasyphyllum*, A – control (1% T-80), B-E - 125 -1000 mg/kg MESd,

DISCUSSION

In addition to therapeutic values of *Solanum dasyphyllum*, that have been documented (Adewunmi *et al.*, 2020) there is need for evaluation of its safety. Safety evaluation is very important in order to explore the plant for clinical use and to develop new products. Thus this study evaluated the toxicity potential of the leaves of *S. dasyphyllum*. The LD_{50} value of the methanol extract of leaves of *Solanum dasyphyllum* is 5.62 g/kg, with doses of 5 g/kg and 10 g/kg resulted in 50% and 100% fatality within 48 hours, respectively. The leaves and fruits of similar specie of Solanum, *S. macrocarpon* produced LD_{50} greater than or equal to 5000 mg/kg (Dougnon *et al*., 2013). It appears this plant is toxic in the acute study involving large doses. According to the OECD guideline, LD_{50} of extract greater than 5000 mg/kg suggest that the extract belongs to category 5 of the Global Harmonisation System of Chemical Substances considered as fairly toxic substance (Ngueguim *et al.*, 2015).

In the acute toxicity testing, there was a significant reduction in food and water consumption in all the treatment groups compared to the control group. Although, by the second week food and water consumption increased in the surviving mice that received the 2 lower doses. The initial weight loss of the mice could be attributed to anorexia and changes in carbohydrate, protein, or fat metabolism, which could have been influenced by extract administration (Ghelani *et al*., 2016). Food and water are essential for life and are required for the growth and development of all organisms (Ashafa *et al*., 2015). Reduction in food and water consumption observed in this acute toxicity testing might be indicators of toxicity because there was corresponding decrease in the body weight in surviving mice. This is because body weight is one of the first critical signs of toxicity (Sireatawong *et al*., 2008). Interestingly, in sub-acute toxicity testing, there was no weight loss in mice administered with the plant extract. This might be because the highest dose used for sub-acute toxicity is 10 times lower than the highest dose used in the acute toxicity testing. Both the control and MESd treated groups showed a dose-dependent increase in body weights on a weekly basis, throughout the period of the study. However, Abdel-Aziz *et al.* (2011) reported, no weight change observed in the experimental animals treated with *Solanum indicum* another specie of Solanum.

Twenty eight days administration of methanol extract of *S. dasyphyllum* in mice has no significant effect on liver enzymes (ALT, AST, and ALP), indicating that daily oral administration of the MESd might not be hepatotoxic at doses tested. In contrast, a report of significant increase in the liver enzymes after daily oral administration of another specie of Solanum, *Solanum erianthum* has been documented (Uzoekwe *et al*., 2021). It appears *S. dasyphyllum* at low doses is safer than other species. Total cholesterol, triglycerides, low-density lipoprotein, and high-density lipoprotein cholesterol measurements might provide valuable insight into lipid metabolism and the heart's proclivity for atherosclerosis and other cardiovascular disorders (Bhardwaj

and Gupta, 2012). The results revealed a non-significant increase in total cholesterol, triglycerides, and low-density lipoprotein cholesterol parameters in the treated mice, but a significant increase in good cholesterol (high density lipoprotein cholesterol) parameter at the two higher doses. Increase in the HDL-C level suggests that there was continuous export of excess cholesterol to the liver for excretion into the bile, thereby reducing the risk of atherosclerosis or coronary artery (Tchankou Leudeu *et al*., 2009).

The histological studies of both the liver and kidney of the experimental animals appeared to be normal in lower doses. The group treated with the highest dose (1000 mg/kg) had cytoplasmic degeneration and considerable aggregation of inflammatory cells in the liver, but no infiltration of inflammatory cells around the deformed glomerular tubules in the kidney. Overall, the methanol extract of *S. dasyphyllum* appeared to be safe at a daily dose ≤500 mg/kg over a 28-day period. The findings of this investigation provide valuable information on the acute and sub-acute oral toxicity profiles of methanol extract of *Solanum dasyphyllum* leaves which may be beneficial in future in vivo and clinical studies.

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