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Evaluation of acute and sub-acute toxicity potentials of methanol leaf extract of *Hyptis suaveolens* (L) Poit in mice and Wister rats

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ABSTRACT: The present study was aimed at evaluating the acute and sub-acute toxicity potentials of methanol extract of the leaves of *Hyptis suaveolens* using mice and Wister rats. Using the modified Lorke' method for the acute toxicity investigation, twelve mice received single doses of 10, 100 and 1000 mg/kg body weight respectively in the first phase. Absence of death led to the second phase, using different mice, and 5000, 7500 and 10,000 mg/Kg doses of the extract respectively was administered to one (1) mouse each. Gross toxicological symptoms and possible death in the animals were monitored for 14 days to ascertain the oral median lethal dose (LD_{50}) of the extract. In the sub-acute investigation, the rats were given oral doses of 250, 500 and 750 mg/Kg/day for 28 consecutive days. Then, haematological and biochemical analyses were carried out. The kidneys, livers, spleens, lungs and hearts of the rats were subjected to histological studies. Results obtained showed that there were no signs of toxicity up to an acute maximum dose of 10,000 mg/Kg. Sub-acute treatment did not significantly alter animal body weights, organ-to-body weight ratios, haematological and biochemical parameters ($p<0.05$). However, the levels of monocyte were significantly increased and immunoglogbin decreased at a dose of 750 mg/kg. The concentration of alkaline phosphatase (ALP) level was also significantly increased while creatinine was decreased ($p<0.001$) at a dose of 500 and 750 mg/Kg/day, when compared with the control. Histological evaluations of the studied organs in the treated rats showed no changes when compared to the control. However, the liver showed normal hepatocytes with feature of necrosis while the lungs showed dilated alveoli spaces, desquamated cells and acute inflammation. At the treatment doses (250 - 750 mg/Kg), the stomach revealed atrophy of gastric mucosa and gastric glands while destruction of inflammatory cells from necrosis was observed in the spleen at 750 mg/Kg only. Conclusively, the methanol leaf extract of *Hyptis suaveolens* is relatively safe sub-acutely at low doses while high doses should be discouraged.

Keywords: *Hyptis suaveolens,* methanol extract, mice and rats, toxicity.

INTRODUCTION

All through the ages from the very beginning of human existence, and even up till today, man has been using plants in traditional medicine in different forms; traditionally prepared concoctions or in the form of pure active principles to manage or prevent various diseases (Parandin and Haeri-Rohani, 2010; Shakya, 2016).The use of these medicinal plants globally is becoming popular due to low cost, affordability, acceptability and perhaps, low toxicity (reduced side effect) (Ozolua *et al*., 2009). Leaves, flowers, stems, roots, seeds, fruit, and bark of medicinal plants can all be constituents of herbal medicines. More so, some of the modern pharmaceuticals currently in use today for the treatment of various ailments or diseases are products of plants and plant-based

medicines (Mozhiyarasi and Anuradha, 2016).

The therapeutic activity of these plants is due to the fact that many of these medicinal plants possesses phytochemicals constituents that are essentially responsible for producing definitive physiological and pharmacological actions on the human body. Some of which are alkaloids, tannins, flavonoids and phenolic compounds (Akinmoladun *et al*, 2007; Ojo *et al*., 2013). These chemical constituents function alone or in conjunction with one another to produce the desired pharmacological effects (Parasuraman *et al*., 2014).

The African continent is one of the continents endowed with the riche biodiversity globally with plants used as herbs, food and for therapeutic purposes (Iwu, 1994). One of such important traditional medicinal plants is *Hyptis suaveolens* (L) Poit, belonging to the family, Lamiaceae. It is commonly called bush mint, bush tea, pignut (Prasanna and Koppula, 2012). It is also known as daddoya-ta-daji in Hausa; efiri in Yoruba; nchuanwu in Ibo; and tanmotswangi-eba in Nupe (Ngozi *et al*., 2014). Originally native to tropical America, *Hyptis suaveolens* is considered a weed worldwide (Chukwujekwu *et al*., 2005), and a very common plant found along roadsides and farmsteads in different parts of the world mainly in the tropics and subtropics. It has been reported to be found in French Guiana, Brazil, Venezuela, Ecuador in Southern America; United States in North America; Bangladesh, China and India in Asia; Benin, Kenya, Nigeria, Sudan, and Cameroon in Africa (Ngozi *et al*., 2014).

The stem of the plant is four-angled, velvety, thick, having long hairs and small erect glandular dots while the leaves are simple, opposite and ovate, about 2.5 to 10 cm long. The leaves are often purple, tinged particularly on the margin. The flowers are auxiliary with long stalk, hairy calyx and about 4 mm long. They are often dark purple and glandular while the corolla is two-lipped, mauve with dark purple lines at the base of the broad two-lobed upper lip. The seeds are flat and mucilaginous (Chukwujekwu *et al*., 2005; Danmalam *et al*., 2009; Ngozi *et al*., 2014).

Different parts of the plant have been used by traditional healers in the treatment of various ailments and disease conditions. In the northern part of Nigeria, a decoction of the leaves is used for treating boils, eczema and diabetes mellitus (Danmalam *et al*., 2009; Ngozi *et al*., 2014). Crushed leaves are applied on the forehead to treat headaches. Infusion made from the leaves and the inflorescence is used as stimulant, carminative, diuretic and antipyretic (Arnason, 1995; Ngozi *et al*., 2014) while a decoction of the whole plant is also used to alleviate diarrhoea and various kidney ailments (Ngozi *et al*., 2014).

Nutritionally, it has been reported in literature that *Hyptis suaveolens* contain some basic food nutrients such as protein, carbohydrates, fats and fibre and phytonutrients such as alkaloids, tannins, saponins, flavonoids and terpenoids (Raizada, 2006; Edeoga *et al*., 2006; Ngozi *et al*., 2014). The presence of these bioactive constituents makes the plant to possess antioxidant, anti-inflammatory, antimicrobial, anti-diarrhoeal, anthelmintic, and anti-diabetic, anticancerous, wound-healing and insecticidal properties (Ngozi *et al*., 2014). Also, it has been reported that the plant is rich in some mineral elements like potassium (K), calcium (Ca), magnesium (Mg), nitrogen (N), sodium (Na) and phosphorus (P) (Ngozi *et al*., 2014).

Ethnomedicinally, different parts of the plant are used by traditional healers of different tribes or communities to treat various ailments and diseases conditions. In Nigeria for example, it is used to treat respiratory tract infections, colds, pain, fever, cramps, and diabetes mellitus and skin diseases. Seeds extract of the plant, is a remedy for menorrhagia, leucorrhoea and temporary male sterility (Prashantkumar and Vidyasagar, 2006). Crushed leaves are applied on the forehead to treat headaches while infusion made from the leaves and the inflorescence is used as stimulant, carminative, diuretic and antipyretic (Ngozi *et al*., 2014). A decoction of the whole plant is also used to alleviate diarrhoea and various kidney ailments. However, no much information is available about the biosafety of the plant. Therefore, having found no much toxicological report on the methanol extract of *H. suaveolens*, this experiment was designed to assess its acute and sub-acute safety profiles in mice and Wister rats.

MATERIALS AND METHODS

Experimental animals

Mice and rats of both sexes weighing between 18-22 and 155-230 g, respectively were used for this study. The rats and mice were obtained from the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria, and were housed in plastic cages in the Animal House of the Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria, where they were used for the experiment. Animals were kept in well aerated cages where bedding was replaced each day, at a room temperature of about 26–27°C and 12 hours light/dark cycle. The animals (mice and rats) were allowed two weeks acclimatization, fed with standard commercially available rat pellets (Pelletised grower feed, Vital feed Ltd, Jos, Nigeria) and tap water *ad libitum* before they were randomly grouped. All experimental animals were handled in line with institutional guidelines for the use of animals in research (Pub No. 85-23, revised 1985; Ozolua *et al*., 2009).

Plant collection and extraction

Fresh leaves of *Hyptis suaveolens* were harvested from road side along Capitol, University of Benin, Ugbowo

Campus, Ovia North East local government area of Edo State, Benin City, Nigeria. The leaves were identified and authenticated by a taxonomist at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, and a voucher specimen reference number UBH-L6171 was then deposited at the herbarium for future reference.

Leaves from the plants were shredded off the stem and washed with clean water to remove dust and debris and was then air dried at room temperature until a constant weight was attained. The dried leaves were crushed to fine powder using mechanical grinder and stored in an airtight amber bottle. The fine powder was macerated in 3L of 99.9% methanol in a clean, airtight container for 3 days with occasional shaking (Ozolua and Uwaya, 2013; Ugwah-Oguejiofor *et al*., 2019). The mixture was filtered through wire gauze and a porous sieve. The filtrate was recovered and concentrated to dryness in a 40ºC water bath to obtain the extract before being stored in a clean glass container in the refrigerator until use. The weight of the concentrate was determined, and the % yield was computed.

Acute toxicity studies

The acute toxicity study (LD_{50}) for the methanol leaf extract of *H. suaveolens* in mice was carried out orally using the modified Lorke method (Lorke, 1983) at single doses as described by Usman *et al*. (2016). Nine mice were divided into three groups of three mice each in the first phase. The first group received the extract orally at a dose of 10 mg/Kg; group 2 received the extract at a dose of 100 mg/Kg, while the last group received the extract at the dose of 1000mg/Kg body weight respectively. Animals were observed for general signs and symptoms of toxicity including mortality over a period of 24 hours. Absence of death in the first phase led to the second phase. In the second phase using three different mice, doses of 5000, 7500 and 10,000 mg/Kg body weight respectively of the methanol leaf extract of *Hyptis suaveolens* were administered to one mouse each. The control mice were given distilled water. After the administration of the extract, animals were observed for death and symptoms of toxicity within three days in the first instance and then for 30 min each day for another eleven days (Ozolua *et al*., 2010). Gross toxicological symptoms were monitored and the LD_{50} was calculated as the square root of the geometrical mean of highest non-lethal dose and the lowest lethal dose (Lorke, 1983; Danmalam *et al*., 2009).

Experimental design

From the outcome of the acute toxicity study, where no death was observed at 10,000 mg/Kg, three doses of the methanol leaf extract of *Hyptis suaveolens*; 250, 500 and 750 mg/Kg were selected for the sub-chronic test (Bautista *et al*., 2004; Ozolua and Uwaya, 2013).Twenty (20) rats were randomly distributed into four groups of five rats each (n=5) with their initial body weight measured. Groups II, III and IV rats were treated daily with 250, 500 and 750 mg/Kg of the methanol leaf extract of *Hyptis suaveolens* while group I received 1 ml of normal saline (the vehicle of administration) daily for 28 days using orogastric tube.

At the end of the experimental phase, all animals were fasted overnight and thereafter the final body weight was taken before being euthanized by halothane anaesthesia. After euthanasia, blood was obtained via the abdominal aorta with a 5 ml syringe (Monoject pharmaceutical LTD, Nigeria) into labelled plain bottles without anticoagulant for the biochemical assay and EDTA bottles for the haematological analysis.

Hematological analysis

Blood samples were obtained via the abdominal aorta with a 5 ml syringe into EDTA bottles, and the content was mixed by gentle rolling of the bottle for haematological assays. The hematological analyses were performed using an automated hematological analyzer (ABX Pentra XL 80, France).

Biochemical analysis

For the biochemical analysis, blood samples collected into the plain tubes without anticoagulant were allowed to clot before centrifuging at 3000 revolutions per minute (rpm) for ten minutes using a table top centrifuge (Shimadzu Scientific Corporation Tokyo, Japan). The clear sera were carefully separated from the blood cells by the use of sterile pasteur pipettes into another set of clear labeled plain bottles that was used for the biochemical assay. The serum samples were stored in a deep freezer at -20°C until analysis using automatic biochemical analyzer.

Body weight measurement

Body weight of all experimental animals was taken by using digital electronic balance before commencing the first oral administration and then weekly till last day of oral administration of the extract.

Statistical analysis

The results were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by

Turkey's multiple comparison test to evaluate significant differences between groups. Results were considered to be significant at $p<0.05$. All statistical analyses were performed using Graph Pad Prism V. 6 Software Inc., USA.

RESULTS AND DISCUSSION

The result of the acute toxicity test for the methanol leaf extract of *Hyptis suaveolens* indicates no death after 24 hours of administration of the various doses of the extract (10, 100 and 1000 mg/Kg body weight) in the first phase, and in the second phase using different rats (5000, 7500, and 10,000 mg/Kg body weight).

The determination of acute oral toxicity (LD_{50}) has remained a useful tool in the evaluation and safety assessment of toxicity of substances (Ozolua and Uwaya, 2013). This is usually a preliminary step. Prior to the use of drugs on humans, testing for toxicity is very crucial in the screening of new drugs or compounds (Enenebeaku *et al*., 2021a). These tests are usually carried out on experimental animals (Cunny *et al*., 2009). Acute toxicity test is a short term evaluation of potential hazards by a test substance (Monosson, 2013). Results obtained from these studies give way for dose determination in animal studies.

In the present study, the experimental mice were treated with single dose each of 10–10,000 mg/kg b. wt. of the extract. Toxicity and mortality were not recorded within 24 hours and subsequently for 14 days in both phases of the study for methanol extracts of the leaves of *H. suaveolens*. Physical signs of toxicity; such as paw licking, salivation, stretching, and weakness were not recorded thus the LD_{50} was estimated to be ≥10,000 mg kg^{-1} b.wt. This is so, since the maximum dose of 10 g/kg did not cause death or any other gross toxicological symptoms in the mice of treatment groups. Absence of mortality in the test animals indicated that the extracts were not an acute toxins (Salawu *et al*., 2009).

In toxicity studies, one of the sensitive indices used in the assessment of toxicity of substances in animals or humans after exposure to toxic substances is body weight (Debelo *et al*., 2015). In this study, treatment with methanol leaf extract of *H. suaveolens* had no effect on the body weight of the animals after 28 days administration of the extract (Table 1). There was a progressive increase in general body weight of the treated and control animals. This indicates a positive health status of the animals (Tousson *et al*., 2011). Again, organ-to-body weight changes are sensitive indicators of toxicity, effects on enzymes, physiologic disturbances and target organ injury (Michael *et al*., 2007). An increase in organ-to-body weight changes suggests the occurrence of hypertrophy while a decrease suggests necrosis in the target organ (Teo *et al*., 2002). These ratios obtained from this study were not significantly altered by sub-acute treatment of the animals (Table 2).

From results obtained in this study, the extract did not cause any significant change in the haematological parameters evaluated in the treated animals compared with the control except for Immunoglogbulin and monocyte (Table 3). At a dose of 750 mg/kg, a significant increase was observed in monocyte ($6.82\pm1.19^{*aab}$) and a significant decrease in immunoglogbin (72.40 ± 6.97). Monocytes are a type of white blood cells that mediate immune response to foreign substances (Pearce *et al*., 2013). The increase in monocytes suggests possible immuno-stimulatory effect of the extract.

Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) values have been identified as indicators of cellular injury caused by exposure to toxic substances (Devaki *et al*., 2012; Usman *et al*., 2016). However, AST is not specific for the liver only but is also located in other organs such as heart, brain, kidney and skeletal muscle, while ALT is found predominantly in the liver and it helps to specifically ascertain the integrity of hepatocellular membrane. Alkaline phosphatase (ALP) is a marker of cholestasis (Yang *et al*., 2014). The results showed that the methanol leaf extract of *H. suaveolens* did not significantly increase the activities of liver function enzymes (AST, ALT and ALP) in the serum of test animals in comparison to the controls. This indicates that the extract did not cause any significant toxic effect or hepatic damage on the liver. This could be linked to the incessantly used of the plant by locals in traditional medicine. However, ALP level was significantly increased at the dose of 500 and 750 mg/kg/day. This may indicate possible injury to internal organs at that dose.

Albumin and total protein are some of the markers of liver dysfunction. Albumin transports bilirubin and other substances in blood (Ogbe *et al*., 2010; Enenebeaku *et al*., 2021b). Total protein is a marker of the synthetic function of the liver and serves as a guide in accessing the severity of liver damage (Iweala *et al*., 2011; Enenebeaku *et al*., 2021b). Significant increase in serum total protein is an indication of tissue damage while decrease suggests hepatic toxicity (Gatsing *et al*., 2015; Enenebeaku *et al*., 2021b). Results from this study showed that the methanol leaf extract of *H. suaveolens* did not cause any significant change to the activities of the albumin, total protein and bilirubin. The non-significant difference in the levels of these biochemical parameters in the treated animals when compared to the controls could suggests that the methanol extract may not interfere significantly with the metabolism of these biochemical parameters.

The effect of the of methanol leaf extract of *H. suaveolens* on the kidney function was also analyzed by estimating the levels of urea, uric acid, creatinine, sodium, chloride and potassium (Table 5). The evaluation of these parameters plays a significant role in knowing the synthetic and excretory roles of the kidney (Chiamaka *et al*., 2019; Yusuf *et al*., 2020).

Findings from this study revealed that the extract of *H.*

Table 1. Body mean weight of rats fed the methanol leaf extract of *H. suaveolens.*

Parameters	Control	250 mg/kg	500 mg/kg	750 mg/kg
Week 0	152.0 ±8.15	134.0 ± 32.23	149.0 ± 8.72	164.0 ± 8.86
Week 1	176.0 ± 1.87	192.0 ± 14.46	172.0 ± 9.95	168.0 ± 20.53
Week 2	182.0 ±4.89	212.0 ± 19.85	174.0 ± 10.65	180.0 ± 20.00
Week 3	194.0 ± 4.85	220.0 ± 16.81	189.0 ± 10.05	194.0 ± 27.04

Values are presented as Mean ± SEM, n=5. p>0.05: Not significantly different from control group. In this study, the weights of the animals were not affected after 28 days of administration of *H. suaveolens.*

Table 2. Effect of methanol leaf extract of *H. suaveolens* on organ body weight ratios of the rats.

Parameters	Control	250 mg/kg	500 mg/kg	750 mg/kg
Liver to body weight ratio	0.031±0.0009	0.03±0.002	0.04±0.002	0.03±0.002*abb
Spleen to body weight ratio	0.003±0.0003	0.004±0.0002	0.004 ± 0.0003	0.004±0.0003
Lungs to body weight ratio	0.008±0.0008	0.25 ± 0.22	0.024 ± 0.02	0.02 ± 0.01
Heart to body weight ratio	0.004±0.0003	0.003±0.00008	0.004 ± 0.0006	0.004±0.0004
Kidney to body weight ratio	0.006±0.0001	0.006±0.0002	0.0064±0.0007	0.0064±0.0003
Stomach to body weight ratio	0.008±0.001	0.008±0.0007	0.01 ± 0.001	0.02 ± 0.01

Values are presented as Mean ± SEM, n=5. p>0.05: Not significantly different from control group. In this study, the organ body weight ratios of the animals were not affected after 28 days of administration of *H. suaveolens.*

Table 3. Mean values for Haematology in Albino rats administered methanol leaf extract of *H. suaveolens.*

Parameters	Control	250 mg/kg	500 mg/kg	750 mg/kg
WBC (10^3/µl)	10.62±1.41	15.08±1.38	13.68±1.06	19.20±3.55
IGM (10^3/µl)	93.18±2.02	94.00±1.16	89.10±2.26	72.40±6.97
MON (10^3/µl)	2.68 ± 0.86	3.40 ± 0.47	4.30±0.25[q]	6.82±1.19*[aab]
GRAN (10^3/µl)	5.16±1.83	2.60±0.76	6.60±2.34	20.78±5.78
HGB (g/dl)	7.71±2.83	15.30±0.59	11.93±0.86	10.05±1.75
HCT (%)	26.92±10.35	45.90±1.81	35.75±2.57	30.18±5.24
PLT (10^3/µl)	650.8±138.6	928.4±72.53	975.0±69.05	881.0±62.55
MCV (µM3)	72.06±5.23	64.90±1.34	68.15±2.83	73.88±4.54
MCH (pg)	47.78±20.95	19.28±1.94	21.25±2.31	37.03±11.78
MCHC (g/dl)	33.42±0.37	32.02±0.45	32.32±0.36	33. 48±0.53
RBC (g/dl)	4.17±1.73	7.09±0.31	5.31±0.45	3.56±0.69

Data are presented as mean ± S.E.M, n = 5. p>0.05: Not significantly different from control group. WBC= White blood Cell; LYM= Lmphocyte; MON= Monocyte; GRAN= Granulocyte; HGB= Heamoglobin; HCT= Heamatocrit; PLT= Platelet; MCV= Mean Corpuscular Volume; MCH= Mean Corpuscular Heamoglobin; MCHC= Mean Corpuscular Heamoglobin Concentration.

Table 4. Mean values for liver function in albino rats administered methanol leaf extract of *H. suaveolens*

Parameters	Control	250 mg/kg	500 mg/kg	750 mg/kg
AST ((mg/l))	68.03±18.70	90.35±21.48	73.19±27.16	70.06±32.64
ALT (mmol/l)	58.20±19.61	51.00±13.70	55.25±22.45	70.50±11.93
ALP (mmol/l)	0.37±0.02	0.33±0.02	0.48±0.01	0.24±0.06
ALB (g/dl)	2.25±0.94	2.43±1.04	3.84±1.17	2.73±0.87
T. B (mmol/l)	1.60 ± 0.56	1.19 ± 0.18	0.86 ± 0.23	0.46 ± 0.05
D.B (mmol/l)	1.53±0.52	0.23±0.58	0.25 ± 0.11	1.22±0.12
T.P (mg/d)	33.0±0.76	32.95±0.99	31.77±1.47	36.67±3.07

Data are presented as mean ± S.E.M, n = 5. p>0.05: Not significantly different from control group. ALP =Alkaline phosphate; ALT= Alanine-transaminase; AST= Aspartate-transaminase; ALB=Albumin; TB= Total bilirubin; DB= Direct bilirubin; TP= Total Protein.

Table 5. Mean values for kidney function in albino rats administered methanol leaf extract of *H. suaveolens*.

Parameters	Control	250mg/kg	500 mg/kg	(750 mg/kg
Urea (mg/l)	14.90±2.45	16.64±3.17	16.55±2.70	11.36±2.73
Sodium (mmol/l)	46.92±11.00	55.82±4.10	49.49±10.29	41.61±5.67
Potassium (mmol/l)	11.34±3.57	7.36±1.34	9.12±1.36	11.12±2.13
UricAcid (mg/l)	16.97±3.04	20.96±2.87	11.76±1.42	11.40±1.31
Chloride (mmol/l)	5.308±0.49	6.00±0.09	6.02±0.16	6.14±0.12
Createnine (mg/l)	0.11±0.03	0.19±0.07	0.17 ± 0.04	0.10±0.03***aaabbb

Data are presented as mean ± S.E.M, n = 5. p>0.05: Not significantly different from control group.

Table 6. Histopatological effects of daily oral treatment with methanol leaf extract of *H. suaveolens*.

Parameters	Heart	Liver	Kidney	Lungs	Stomach	Spleen
Control	Normal myocytes	Normal hepatocytes	Normal glomerulus and tubules	Normal patent airway with alveoloi sac	Normal gastric mucosa and gastric glands	Normal splenic tissue with inflammatory cells
250 mg/Kg	Normal myocytes	Hepatocytes with feature of necrosis	Normal glomerulus and tubules	Thickened interstitial membrane	Atrophy of gastric epithelium and gastric glands	Normal splenic tissue with inflammatory cells
500 mg/Kg	Normal myocytes	Hepatocytes with feature of necrosis	Normal glomerulus and tubules	Desquamated cells and acute inflammation	Erosion of gastric mucosa	Normal splenic tissue with inflammatory cells
750 mg/Kg	Normal myocytes	Hepatocytes with feature of necrosis	Normal glomerulus and acute tubular necrosis	Desquamated cells and acute inflammation	Atrophy of gastric mucosa and gastric glands	Destruction of inflammatory cells from necrosis

Slide for each organ from two different animals in a group were prepared and viewed with an Leica® microscope DM500 at x 100 magnification with eosin (E) and hematoxylin (H) as stains.

suaveolens did not alter the kidney function significantly. The only change observed was in the case of creatinine at the dose of 500 and 750 mg/kg/day where a highly significant decrease ($p<0.001$) was observed when compared to the control animals. Creatinine is the catabolic waste products of creatinine phosphate which is used by the skeletal muscle, during muscle contraction. It is a metabolite of muscle–creatinine, whose amount in serum is proportional to the body's muscle mass. It is an index of glomerular function (Treasure, 2003), and thus It is excreted exclusively through the kidney. Hence, elevated levels of creatinine will usually indicate diminished renal function (Umar *et al*., 2019). The significant ($p< 0.001$) decrease in creatinine following repeated oral administration of the extract is a possible indication that the functional capacity of the kidney might not be compromised.

Urea is a byproduct from the breakdown of protein. About 90% of urea produced is excreted through the kidney (Walmsley *et al*., 2010). Impaired balance of nitrogen coupled with lowered protein synthesis leads to increased concentration of urea and uric acid in serum (Umar *et al*., 2019) which indicates progressive renal damage. However, the study revealed a non-significant change in the levels of urea and uric acid. This therefore indicates that the continuous oral administration of the extract could not impair kidney function. There was also a non-significant effect in the levels of chloride, potassium and sodium concentrations in rats following oral administrations of extracts at all doses tested, suggesting that the normal functioning of kidney tubules as regard to these electrolytes was preserved (Umar *et al*., 2019).

Histological studies of the hearts, spleens, kidneys, livers and lungs in the treated rats showed no changes when compared to the control (Table 6). However, the liver showed normal hepatocytes with feature of necrosis while the lungs showed dilated alveoli spaces, desquamated cells and acute inflammation. At doses of 250 -750 mg/Kg, the histology of the stomach revealed atrophy of gastric mucosa and gastric glands while destruction of inflammatory cells from necrosis was observed in the spleen at 750 mg/Kg.

Conclusion

The acute toxicity (LD_{50}) result of the methanol leaf extract of *H. suaveolens* showed there was no mortality observed

and no obvious toxicological signs at 10,000 mg/kg body Weight. This has shown that the oral LD_{50} is greater than 10,000 mg/Kg and is generally considered to be safe. However, prolonged administration has revealed that it has the potentials of causing mild toxicity to the liver, kidney, and the other organs studied in rats and these high doses may not be safe in humans. Based on the findings of the present study, it is therefore recommended that a detailed chronic toxicity treatment be carried out on the plant to conclude the toxicity profile.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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