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Article · September 2018

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European Journal of Medicinal Plants

25(1): 1-11, 2018; Article no.EJMP.43613
ISSN: 2231-0894, NLM ID: 101583475

Evaluation of Toxicity of *Strychnos henningsii* (Gilg) (Loganiaceae) Leaves and Root Aqueous Extracts in Mice

Edward Kiprotich Tirop[1], Naomi Njogu Maina[1*], John Kagira Maina[2], Peter Kariuki Njenga[3], Esther Magiri[4] and Victoria Wambui Ngumi[3]

[1]Department of Biochemistry, College of Health Sciences, Jomo Kenyatta University of Agriculture and Technology, P.O.Box 62000-00200 Nairobi, Kenya.
[2]Department of Animal Sciences, Faculty of Agriculture, Jomo Kenyatta University of Agriculture and Technology, P.O.Box 62000-00200 Nairobi, Kenya.
[3]Department of Botany, College of Pure and Applied Sciences, Jomo Kenyatta University of Agriculture and Technology, P.O.Box 62000-00200 Nairobi, Kenya.
[4]Co-operative University of Kenya, P.O.Box 24814-00502 Karen-Nairobi, Kenya.

Authors' contributions

This work was carried out in collaboration between all authors. The research idea was conceived by authors VWN and NM. Author VMV sourced for the research funds. Authors NM and EKT designed the experimental plan. Author PK collected the plant samples and identification. Author EKT undertook experimental work under the guidance of authors NM, EM and JMK. JMK was responsible for animal welfare and care during experimentation. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2018/43613
Editor(s):
(1) Dr. Marcello Iriti, Professor, Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Kouadio Bene, University Nangui Abrogoua, Côte d'Ivoire.
(2) Sandro Rostelato Ferreira, Universidade Paulista, Brazil.
(3) Andrew S. Lamm, University of Technology, Jamaica.
Complete Peer review History: http://www.sciencedomain.org/review-history/26230

Original Research Article

Received 9th June 2018
Accepted 29th August 2018
Published 13th September 2018

ABSTRACT

Aim: *Strychnos henningsii* (Gilg) (Loganiaceae) has been used for treatment of various health conditions such as gastrointestinal complications, rheumatism and snake bites. However, the safety of extracts from *S. henningsii* has not been evaluated. This study was therefore carried out to evaluate the *in vivo* toxicity of *S. henningsii* leaves and root extracts with the view of determining if their use poses health risk.

**Corresponding author: E-mail: nmaina@jkuat.ac.ke, nmail@hotmail.com;*

Methodology: Acute and sub-acute toxicity was conducted in accordance with OECD guidelines. Swiss white mice were randomly selected and divided into 24 groups (n = 6) for acute (single administration) and sub-acute toxicity (28 days administration) studies. The mice were fasted overnight and graded doses of the aqueous extracts were orally administered to the tested groups at a dosage ranging from 75 to 2500 mg/kg. The control groups were orally administered with plain water. Clinical signs, mortality, fasting glucose levels, alanine transaminases, blood urea nitrogen and haemoglobin were evaluated. At the end of study, organs were harvested and processed for histopathology.

Results: In all the groups, there were no mortalities. The clinical signs which were noted in mice administered with extracts ranging from 750 mg/kg and above included: dullness, raised skin fur, staggering, reduction in locomotion and in food consumption, and mucoid stool. There were no significant (P>0.05) changes in body weight and the levels of fasting glucose, haemoglobin, blood urea nitrogen and alanine transaminases in the studied mice. Histopathology evaluation of organs of mice from sub-acute toxicity study administered dosages from 750 mg/kg showed mucoid enteritis and exfoliation. The loss of hepatolobular arrangement, periportal infiltration with inflammatory exudate in the liver and congestion of renal blood vessels was observed at a dosage of 2,500 mg/kg of root extract.

Conclusion: The study showed no mortality up to dose of 2,500 mg/kg. In addition sub-acute administration of dosages up to 750 mg/kg had no effect on the biochemical and hematological parameters. However, at histopathology dosages above 750 mg/kg bwt showed intestinal, hepatic and renal pathological alterations. The study recommends that the plant extracts may be safe at dosages below 750 mg/kg bwt.

Keywords: Strychnos henningsii; hematology; histopathology; mice; aqueous extracts; toxicity.

1. INTRODUCTION

Medicinal plants are used for the treatment of various human and livestock health disorders worldwide [1]. The demand globally for herbal products is not only large, but it is steadily growing [2]. In developing countries, 80% of the population depends on traditional treatments which form a critical component of the health system [3,4].

Strychnos henningsii (Loganiaceae) is an endangered plant species, which used to be found in many countries in the world and is considered a multipurpose tree whose main function is treatment of gastrointestinal complications [5]. The roots, stems, bark, leaves and fruits of the plant are prepared in a number of ways depending on the condition being treated, though the most often used is the bark [6]. In East Africa, root, stem and bark extracts are used to prepare meat and milk soups among various communities. The Maasai, Kikuyu and Kamba communities use it for treatment of; painful joints, fitness and general body pains [7]. Further, the soup from the plant is used as an aphrodisiac and for treatment of nausea, colic and syphilis [2]. The fruits of *S. henningsii* are used by Mbeere people of Kenya to flavor traditional beer [7]. Other medicinal uses of the plant include treatment of rheumatism and snake bites [5]. The bark is used by herbalists as purgative [6], while some of its alkaloids phytochemical compounds have muscle relaxing effects [2]. In livestock, the ground bark has been used as antiseptic and for treatment of cattle's wound [5].

S. henningsii (Gilg), has been reported to have antimicrobial activity against *Escherichia coli, Staphylococcus aureas* and *Bacillus subtilis* [8]. The mono and bis-indole alkaloids isolated from the plant have high antiplasmodial activities [9]. Some of the phytochemicals found on stem and bark extracts include alkaloids, saponins, tannins, flavanoids, phenols, terpenes, steriods and tannins [10].

In vitro and *in vivo* studies of the extracts from the plant possess antioxidant, anti-inflammatory, analgelsic and antispasmodic activities [11,12]. Indeed, new antispasmodic and antinociceptive drugs have been developed from the plant [11]. The plant bark and fruits have poisonous bitter alkaloid [10]. The alkaloids extracted from *S. henningsii* bark extracts induced convulsions and paralysis on mice [13]. The scientific literature has limited information of the toxic effect of *S. henningsii* extracts. Therefore, this study sought to evaluate the toxicity of *S. henningsii* leaves and root aqueous extracts with the view of determining if their use poses health risk to the users.

2. MATERIALS AND METHODS

2.1 Plant Collection and Extraction

Samples of *S. henningsii* (leaves and root) were collected from Kabiruini forest (on latitude 0°23'0"S and longitude 36°57'0"E) in Nyeri County, Central Kenya. Authenticity of the plant was confirmed by a taxonomist at the Department of Botany, Jomo Kenyatta University of Agriculture and Technology (JKUAT) Kenya. It was given voucher specimen number (*S. henningsii* 001/2014) and deposited at the herbarium. Root barks were peeled and cut into small pieces. The leaves and root were shade-dried at ambient temperature then ground using a plant mill into powder form. This was then stored in sterilized sealed containers at room temperature (25°C).

Extraction was done as previously described [14,15]. Briefly, 100 g of the powdered *S. henningsii* leaves and root material were boiled in 1 L of distilled water at 98°C for 2 hrs. After cooling, the extracts was then decanted into a 1 litre clean dry conical flask and filtered (Whatman® filter paper no 1 Whatman International Ltd Maidstone, England) under vacuum pump. Decantation and filtration processes were repeated until the sample became clear. The filtrate was centrifuged at 3000 rpm for 5 minutes and the supernate obtained were 400 ml of leaves and 500 ml of root extracts. The supernate was quickly frozen at -40°C and dried for 48 h using a freeze dryer (Alpha 1-4 LD Plus (Christ, Germany) to give yield of 13.25 g and 12.6 g of dry root and leaves extracts, respectively. The human dosages were prepared from dry extracts as prescribed by the herbalist (750 mg/kg dose). The mouse equivalent doses were calculated using the formula described previously [16]. The resulting extracts were reconstituted in distilled water to give doses of 75, 500, 750, 1000, 2000 and 2500 mg/kg body weight which were used in this study. The samples were stored in fridge at 4°C before use in subsequent bioassay.

2.2 Phytochemical Screening

Preliminary phytochemical analysis of the aqueous extracts involved determination of; alkaloids, anthraquinones, saponins, sterols, triterpenes, tannins and flavonoids using standard methods as described [17].

2.3 Laboratory Animals

The animals used in this study were 144 Swiss white mice (male and female) that weighted 20-25g. They were obtained from Institute of Primate Research (IPR), Kenya and were left to acclimatize for one week before the start of experiment. They were housed in medium size aluminum cages (5x12.5x6.5inches) that consisted of 6 mice per group. The aluminum cages were placed in a well ventilated house at 23±1°C and humidity of 40-45%. The mice were provided with free access of water and pellets (Mice pellets®, Unga Feeds Ltd, Kenya).

2.4 Acute and Sub-acute Toxicity Trials

The acute toxicity was determined according to the previously described method and guidelines [18,19]. The 14 groups of 6 mice per group each were fasted overnight and graded doses of 75, 500, 750, 1000, 2000 and 2500 mg/kg of the leaves and root aqueous extracts were orally administered once using gastric gavage.

Sub-acute toxicity study was carried out as previously described [20]. Briefly, 10 groups of 6 mice per group were fasted overnight and the tested group received oral doses of 75, 250, 750 and 2500 mg/kg body weight of each plant crude extract daily for 28 days. The mice body weights were determined at day 0 and after every 5 days up to day 28 post treatment. The control groups were orally administered with water. The animals were then allowed free access to food and water. Mortality and any clinical signs of toxicity were noted. The observation period in acute and sub-acute toxicity was up to 14 and 28 days, respectively.

2.4.1 Determination of fasting glucose

The animals were fasted overnight at 0, 14 and 28 days. Tail prick blood was collected on test strips and glucose levels read immediately using a glucometer (expeeed™ Blood Glucose Monitoring System, Korea).

2.4.2 Collection of blood

The animals were sacrificed on the 29th day post administration of the extracts. All animals were sacrificed using carbon dioxide gas as a euthanasia agent as previously described by American Veterinary Medical Association (AVMA) guidelines issued in 2013 [21]. A flow rate of 10% to 30% volume/min carbon dioxide

gas was used in sacrificial because it is optimal for the humane euthanization of small rodents [21]. The blood (0.3 ml) was collected using a syringe and placed into heparinised eppendorf tube and used for determination of haemoglobin as previously described [22]. The remaining blood (0.7 ml) was placed in the other eppendorf tube, which was centrifuged (Mikro 200 Andreas Hettich GmbH, Germany) at a speed of 3,000 rpm for 10 mins to obtain plasma. The plasma was analysed for Alanine transaminases and blood urea nitrogen using Reflotron® plus biochemical analyzer (Roche Diagnostics GmbH, Germany) with compatible Reflotron test strips [22].

2.4.3 Histopathology examination

The liver, kidney and gastrointestinal tract were sampled and preserved in 10% buffer formaldehyde solution. These tissues were processed for histopathology using the previously described methods [23] and slides examined under microscope (Olympus, Japan).

2.5 Data Analysis

The data obtained on body weight, fasting glucose levels, hematological and biochemical parameters were analyzed using one way analysis of variance (ANOVA) followed by Dunnett post hoc test. The data was then expressed as means ± standard error of mean (S.E.M) of six replicates. Statistical analysis was conducted using IBM® SPSS Statistics Version 21 (International Business Machine Corporation, USA). The obtained values were considered significant at $P \leq 0.05$.

3. RESULTS

3.1 Phytochemicals

The aqueous extracts of *S. henningsii* leaves and root aqueous extracts contained tannins, alkaloids, flavonoids, flavonols, flavones, saponins, bound anthraquinones and trace amounts of sterols (Table 1).

3.2 Effects of Administration of Extracts of *S. henningsii* in Mice

3.2.1 Clinical observations

There were no mortalities in mice administered dosage of up to 2500 mg/kg. There were no clinical signs observed in mice administered single dosages up to 2500 mg/kg. There were also no clinical signs in mice that received oral doses of up to 250 mg/kg body weight of plant crude extract daily for 28 days. The changes in behavior that were observed in mice administered dosages of 750 and 2500 mg/kg in sub-acute toxicity are shown in Table 2.

In mice treated for 28 days, mucoid faeces of light green and purple colour were observed. It was deduced that the faeces colour arose from the colour of leaves or root extracts.

3.2.2 Body weight

All mice in the sub-acute toxicity study gained body weight. The only significant gain was noted in the mice that received 250 and 750 mg/kg of *S. henningsii* leaves aqueous extracts. The 750 mg/kg dosage showed significant increase from day 5 (P=0.014) to day 28 (P=0.008) while 250 mg/kg dosage showed significant gain in day 15 (P=0.039) (Table 3).

3.3 Fasting glucose Levels

The mean fasting glucose levels in control mice ranged from 6.783±0.32 mmol/L to 7.966±0.48 mmol/L throughout the study. The repeated administration of different of aqueous extracts did not (P>0.05) affect fasting glucose levels in mice during the study. The mean fasting glucose level of animals in all the groups treated with different doses of leaves extracts ranged between 6.733±0.17 mmol/L to 8.00±0.25 mmol/L and root extracts ranged between 6.433±0.37 mmol/L to 7.65±0.22 mmol/L.

3.4 Hematological Parameters

The mean haemoglobin (HGB) levels in control mice for leaves extracts study was 8.98±0.76 mmol/L and ranged from 5.75 to 12.40 mmol/L, while for the root extracts, it was 6.32±0.91 mmol/L (range = 3.61-8.90 mmol/L). There was insignificant change (P>0.05) in haemoglobin levels in mice in all the study groups of both extracts when compared to those of the controls. The mean levels of haemoglobin in the mice treated with different doses of root extracts ranged between 4.84±0.66 mmol/L to 5.91±0.49 mmol/L, while for the leaves extracts it ranged from 6.86±1.17 mmol/L to 8.98±0.76 mmol/L (Fig. 1).

3.5 Biochemical Parameters

The mean blood urea nitrogen (BUN) levels in control mice for leaves extracts studies was

7.89±0.91 mmol/L and ranged from 5.42 to 11.10 mmol/L, while for the root extracts it was 7.92±0.39 mmol/L (range = 6.49-9.13 mmol/L). There was insignificant change (P>0.05) in blood urea nitrogen levels in mice in all the study groups of both extracts compared to the controls.

The mean levels of blood urea nitrogen in the mice treated with different doses of leaves extracts ranged between 7.89±0.91 mmol/L to 9.16±1.01 mmol/L, while for the root extracts, it ranged from 7.84±0.53 mmol/L to 8.92±0.69 mmol/L (Fig. 2).

Table 1. Phytochemisty of *S. henningsii* leaves and root aqueous extracts

Class of compound	Root extracts	Leaves extracts
Tannins	+	+
Alkaloids	+	+
Sterols	+ (trace)	+ (trace)
Terpenoids	-	-
Flavonoids	+	+
Flavonols	+	+
Flavones	+	+
Saponins	+	+
Free Anthraquinones	-	-
Bound Anthraquinones	+	+

Key: + Positive (trace) slightly positive; - Negative

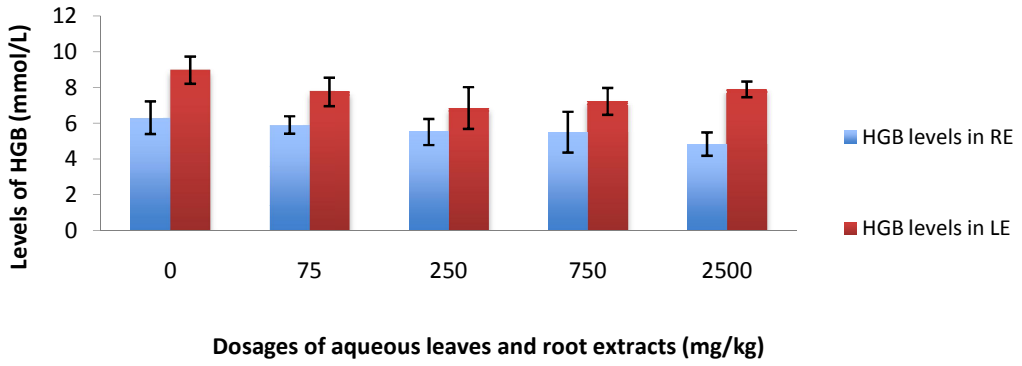


Fig. 1. Effects of dosages of *S. henningsii* aqueous leaves and root extracts on HGB in mice (n=6)

Note. RE=Root Extracts, LE=Leaves Extracts

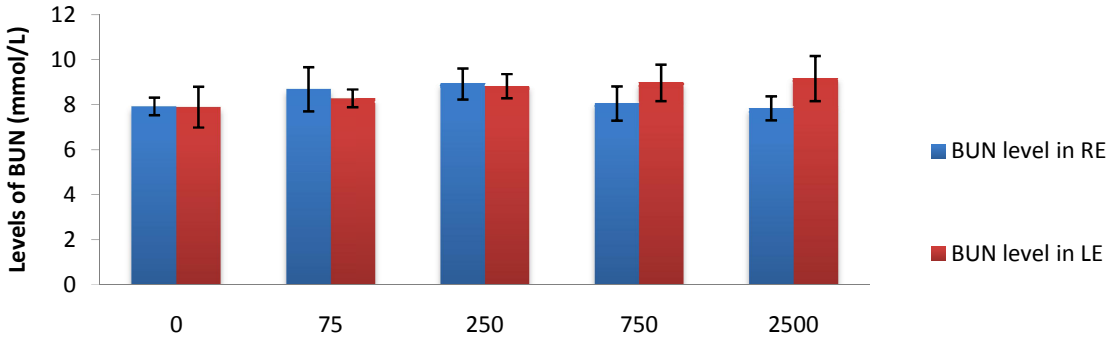


Fig. 2. Effects of dosages of *S. henningsii* aqueous leaves and root extracts on BUN in mice (n=6)

Note. RE=Root Extracts, LE= Leaves Extracts

Table 2. Clinical signs in mice administered various dosages of *S. henningsii* leaves and root aqueous extracts

Sub- acute toxicity				
Dosage (mg/kg) of RE and LE	**Mortality**	**Physical and behaviour changes**	**Time of onset***	**Time of offset***
Control	None	None	N/A	N/A
75	None	None	N/A	N/A
250	None	None	N/A	N/A
750	None	Raised skin fur, dullness, staggering and slight reduction in locomotion, reduction in food consumption	2 days	Up to 11 days-
2500	None	Raised skin fur, dullness, staggering, reduction in locomotion, reduction in food consumption	2 days	Up to 24 days

** = After drug adminstration; N/A = Not applicable; RE=Root extracts and LE=Leaves extracts*

Table 3. Effects of dosages of aqueous leaves and root extracts on body weight of mice (n=6)

Dosage (mg/kg)	**Body weight of mice (g)**											
	Day 0		**Day 5**		**Day 10**		**Day 15**		**Day 20**		**Day 28**	
	LE	**RE**	**LE**	**RE**	**LE**	**RE**	**LE**	**RE**	**LE**	**RE**	**LE**	**RE**
Control	28.45±1.30	24.47±0.79	31.70±1.54	27.69±1.09	32.54±1.46	28.11±1.03	33.21±1.38	28.76±1.09	33.58±1.39	29.14±1.02	33.91±1.43	29.29±0.99
75	27.20±1.94	26.93±0.68	28.63±2.06	29.21±1.28	29.29±2.21	29.57±1.23	29.76±2.19	29.46±1.29	30.35±2.21	29.78±1.34	30.57±2.20	30.17±1.49
250	25.86±1.36	29.02±1.57	26.22±1.37	31.41±1.82	26.48±1.34	31.51±1.70	26.81±1.33[a]	32.05±1.56	27.09±1.31	32.74±1.59	27.34±1.27	33.27±1.59
750	22.88±1.73	28.01±1.98	24.03±1.51[a]	31.22±2.45	24.43±1.62[a]	31.76±2.50	24.98±1.63[a]	32.57±2.21	24.89±2.13[a]	33.29±2.05	25.22±2.29[a]	34.25±1.96
2500	27.45±2.00	28.08±0.88	28.50±1.99	31.19±1.15	28.56±1.69	31.28±1.16	28.89±1.65	32.16±1.15	29.68±1.63	33.22±1.21	30.02±1.63	33.48±1.13

The data are expressed as Mean ± Standard Error of Mean (S.E.M). [a] Superscripted Items indicate significant differences for each group at a time period from versus the control values: P<0.05. Note, LE= Leaves aqueous extracts, RE= Root aqueous extracts.

The mean alanine transaminases (ALT) levels in control mice for leaves extracts studies was 79.33±6.95 U/L (range = 59.20-103.00 U/L) while mean ALT levels for the root extracts was 61.44±3.11 U/L (range = 39.10-86.70 U/L). There was insignificant change (*P*>0.05) in ALT levels. The mean levels of ALT in the mice treated with different doses of leaves extracts ranged between 59.68±6.74 U/L to 81.30±5.57 U/L, while for the root extracts it ranged from 39.98±7.64 U/L to 76.64±2.84 U/L (Fig. 3).

3.6 Histopathology Changes

3.6.1 Liver

The liver of mice in the control groups showed typical normal hepatolobular architecture. The livers of mice administered dosages of 750 mg/kg and below did not manifest any histopathological changes. However, livers of mice treated with root extracts of dosages 2500 mg/kg which had pathological alterations such

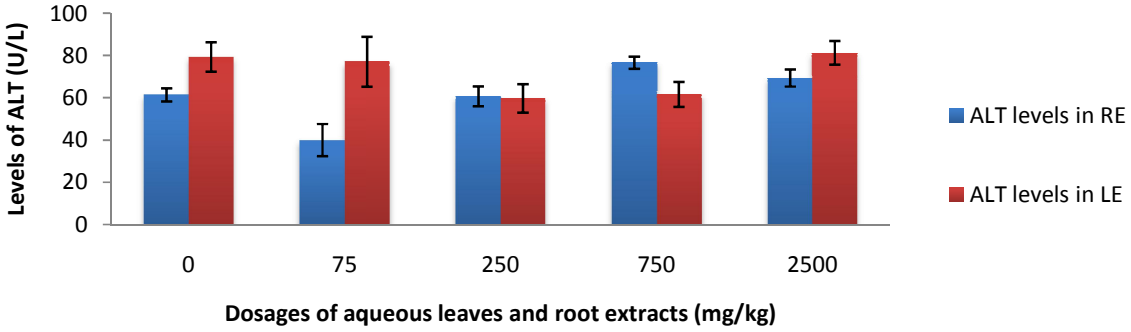
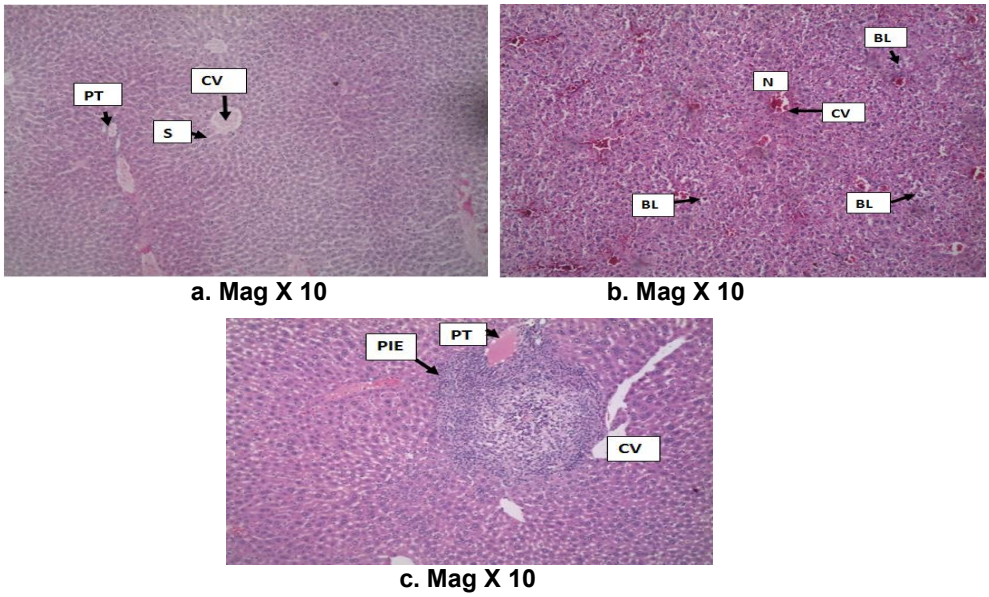


Fig. 3. Effects of dosages of *S. henningsii* aqueous leaves and root extracts on ALT levels in mice (n=6)

Note. LE= Leaves Extracts, RE=Root Extracts



a. Mag X 10 **b. Mag X 10**

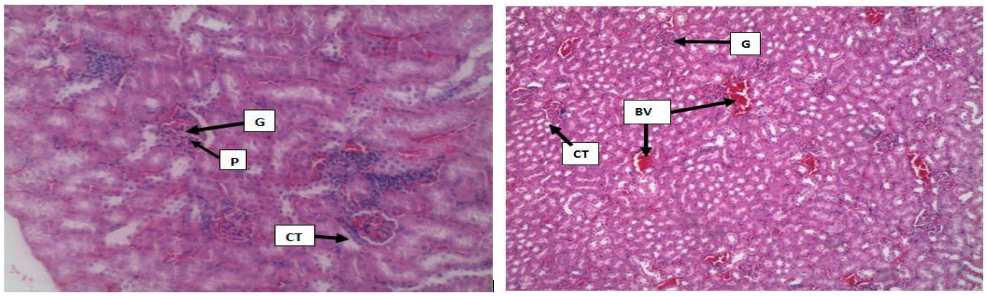
c. Mag X 10

Fig. 4. The photomicrograph of liver of control and treated mice

a. ***Control (Mag. X 10).*** *Normal structure of liver showing central vein **(CV)** with radiating cords of hepatocytes, regular sinusoids **(S)** arrangement around the central vein and portal triad **(PT)**.*

b. *Root extracts 2500 mg/kg. **(Mag. X 10).** Scale bar = 100 µm. Distorted architecture of the liver showing loss of regular hepatolobular arrangement. Ballooning and degeneration of the hepatocytes **(BL).** Pale necrotic areas **(N)** around the central vein **(CV).***

c. *Root extracts 750 mg/kg. **(Mag. X 10).** A portal triad **(PT)**. Central vein **(CV)**. Periportal infiltration with inflammatory exudate containing lymphocytes **(PIE).***

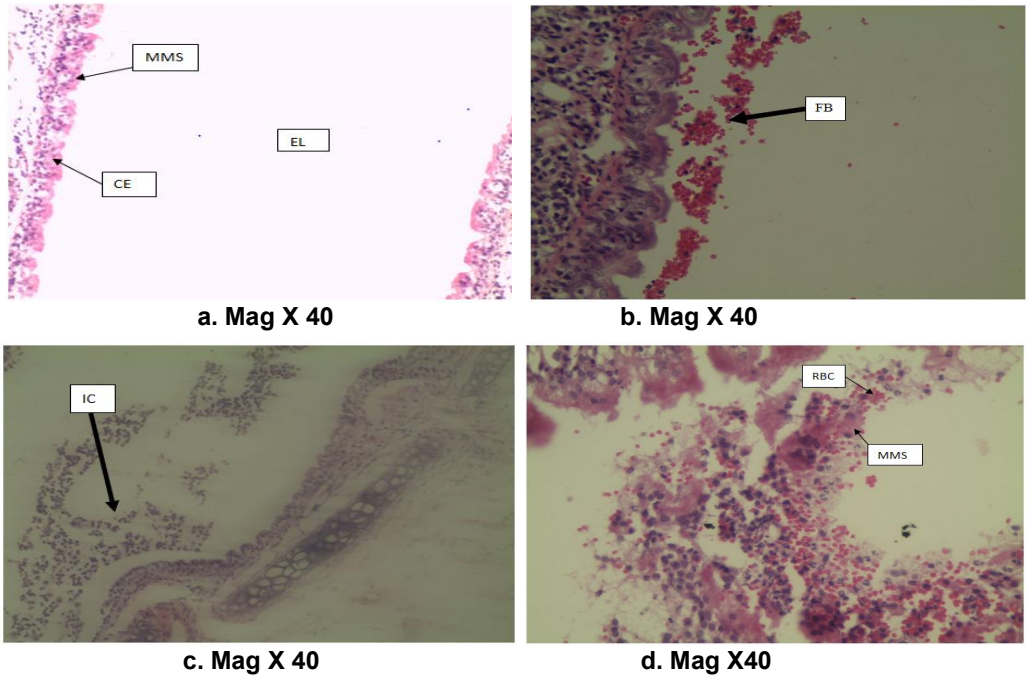


a. Mag X 10 **b. Mag X 10**

Fig. 5. The photomicrograph of kidney of control and mice administered with root extracts

a. ***Control (Mag. X 10).*** *Scale bar = 100µm. Normal structure showing glomerulus (**G**), podocytes (**P**) and convoluted tubules **(CT)**.*
b. *Root extracts 2500mg/kg. **(Mag. X 10).** Normal glomerular **(G)** and convoluted tubule **(CT)** structure. Blood vessels **(BV)** are congested.*

as; increase in number of kupfer cells, loss of regular hepatolobular arrangement, hepatocellular necrosis around central vein, ballooning and vacoulation of hepatocytes and a peri-vascular zone of mononuclear infiltration. The liver of mice treated with root extracts of dosage 750 and 2500 mg/kg also showed periportal infiltration with inflammatory exudate composed of mainly lymphocytes (Fig. 4).



a. Mag X 40 **b. Mag X 40**

c. Mag X 40 **d. Mag X40**

Fig. 6. The photomicrograph of intestines of the control and treated mice

a. *Control (Mag. X 40). Scale bar = 100µm. Columnar Epithelium (CE) in small intestine, coat of mucus on mucosal surface (MMS). Empty lumen (EL) No pathology.*
b. *Root extracts 2500 mg/kg **(Mag. X 40).** Severe inflammation in small intestine showing Frank Blood **(FB)** in intestinal lumen.*
c. *Root extracts, 750 mg/kg **(Mag. X 40).** Intestinal lumen in small intestine with debris containing inflammatory cells **(IC)** trapped in mucus.*
d. *Leaves extracts, 750 mg/kg. **(Mag. X 40**). Mucosal wall of large intestine heavily debrided and lumen contains copious amounts of mucus, inflammatory cells and Red blood cells **(RBC)** indicating intestinal hemorrhage.*

3.6.2 Kidney

The kidneys of mice in the treatment groups (75, 250 and 750 mg/kg) and the control showed normal typical structures of the kidney. However, blood vessels in mice administered 2500 mg/kg of the root extracts were congested (Fig. 5).

3.6.3 Intestines

The control mice showed normal histological structure of the small intestine. There was no pathology noted in mice from the treatment groups administered 75 and 250 mg/kg of the extracts. The mice administered with 2500 and 750 mg/kg (of both extracts) had inflammation characterized by copious amounts of mucus and exfoliation of the gastrointestinal tract epithelium. The inflammatory cells in lumen and mucous on the epithelial surface was dose related (Fig. 6).

4. DISCUSSION

In African traditional medicine, *S. henningsii* leaves and root aqueous extracts have been used to treat a variety of conditions including syphilis, snake bites, gastrointestinal complications (purgative), rheumatism, snake bites, malaria, diabetes mellitus and dysmenorrhoea [2]. The dosage used by Kenyans herbalists was 750 mg/kg/day [2]. Similar to a previous study [24], the current study showed that the extracts did not cause mortality in mice. However, oral administration of 1000 mg/kg for 28 days in a previous study resulted in rat mortality of 7% [24]. In the current study, weight increase was observed in all dosages used and this is similar to previous studies [24]. Weight increase was attributed to the effect of drug as well as normal growth of the mice.

Mucoid enteritis was consistently observed in the mice treated with dosages above 750mg/kg of the extracts. This shows that the extracts, at these dosages, caused chronic intestinal inflammation. Previous studies showed that phytochemicals can have direct effect on host intestinal epithelium which can cause enteritis [25]. Further, indirect effects of the extracts can cause changes in the microbial communities within the intestines leading to enteritis [25].

All the mice in treatment groups showed normoglycemia and this is similar to previous findings [26] which showed that oral administration of 100 and 200 mg/kg of an aqueous extracts and seed powder of *Strychnos potatorum* Linn for 90 days did not have any effect on glucose levels. These results indicate that *S. henningsii* aqueous leaves extracts does not change functioning of the pancreas and liver, organs involved in glucose metabolism. Contrary, the mice administered root extracts from dosage of 750 mg/kg had pathology of the liver indicating that *S. henningsii* aqueous root extracts are hepatotoxic.

The vital index in evaluating toxicity of plant extracts is the functions assessment of liver and kidney [27,28]. In this study, ALT serum levels were within normal ranges in mice fed up to 250 mg/kg of root extracts and up to 750 mg/kg of the leaves extracts. ALT serum levels increased at the dosage of 2500 mg/kg of leaves aqueous extract possibly due to hepatocellular injury observed in the current study. Similarly, ALT serum levels increased in mice administered dosages of 750 and 2500 mg/kg of root aqueous extracts, respectively. These ALT changes were further supported by histopathology of the liver at the dosages of 2500 mg/kg of leaves extracts and 750 and 2500 mg/kg of root extracts which revealed necrosis of portal triad of the liver. The histopathology findings of the liver corroborates with previous report [24], which observed a similar effect of aqueous stem bark extracts of *S. henningsii* in Wistar rats administered at the dosages of up to 1000mg/kg. According to previous study [29], ALT levels of more than 5 times the upper limit of normal range indicates a potentially serious active hepatocellular injury, while ALT levels of more than 15 times the upper limit of normal range indicate chronic liver disease. The liver is able to withstand moderate zonal or diffuse necrosis due to its ability to regenerate but severe intoxication can lead to liver failure.

The extracts did not interfere with function and integrity of the kidneys of the mice. The findings were in agreement with an earlier study [24] which showed insignificant changes on blood urea nitrogen levels with dosages up to 1000 mg/kg. The BUN test was further supported by lack of histopathological changes in kidneys of mice treated up to 750 mg/kg. However, there was a mild congestion of renal blood vessels in mice administered 2500 mg/kg of the root extracts, showing possible renal toxicity at that dosage.

In the current study, the levels of blood haemoglobin were not affected by administration of up to extracts up to 2500 mg/kg. This shows

that the extracts might not have any effect on erythropoiesis [30].

5. CONCLUSIONS AND RECOMMENDATION

The current acute toxicity study showed that up to 2500 mg/kg dosage, the aqueous extracts are safe. Sub-acute toxicity study of *S. henningsii* leaves and root aqueous extracts showed the extracts are relatively safe at dosages below 750 mg/kg bwt. Further study is recommended on isolation of the bioactive compounds of *S. henningsii* leaves and root aqueous extracts and evaluating their safety. Chronic toxicity tests may be important to further determine the long term effects of the extracts.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The study did not use human participants. The permission to use plant from Kabiruini forest was granted from the Kenya Forest Service in Nyeri County, Central Kenya. Animal experiments were performed in accredited facilities according to the revised animals (Scientific Procedures) Act 1986 in the United Kingdom (UK) and European governmental guidelines (2010/63/UE). Animal experiments were approved from the Animal care and use ethics committee of Institute of Primate Research (IPR), Kenya.

ACKNOWLEDGEMENT

Funded by Jomo Kenyatta University of Agriculture and Technology under its Research and Extension (RPE) program.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/26230