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Acute and sub-acute toxicity studies of acetone extract of *Sclerocarya birrea* stem bark in Wistar rats

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Abstract

Sclerocarya birrea (S. birrea) is a tropical plant used traditionally in the management of a variety of ailments. Many reports have been made scientific literatures suggesting its therapeutic usefulness. The present study was therefore carried out to evaluate the safety of acetone extract of S. birrea by acute and sub-acute toxicity studies. Acute and sub-acute toxicity studies were conducted in Wistar rats using Organization for Economic Co-operation and Development (OECD) 425 and 407 guidelines respectively. In the acute toxicity study, rats were administered a single dose of 2,000 mg/kg and 5,000 mg/kg orally and then observed individually for the first four hours, then over a period of 24 hours and at least once daily for 14 days. In the sub-acute toxicity studies, S. birrea extract was given orally at doses of 500 mg/kg and 1,000 mg/kg body weight daily for 28 days. General behaviour, adverse effects and mortality were observed throughout the experimental period. Body weight, organ weight, haematological and biochemical parameters as well as histopathological changes were evaluated. The limit doses of 2,000 mg/kg and 5,000 mg/kg did not cause any mortality or signs of acute toxicity in the rats tested during the observation period. Though significant increase ($P < 0.05$) was observed in weights of the liver and

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kidney as well as AST and ALP levels indicative of hepatotoxicity. In sub-acute toxicity tests, the results did not show any treatment related abnormalities in terms of haematological and biochemical parameters. There were no significant differences (P > 0.05) in body and organ weight, as well as histopathological changes in the liver and kidney between the control and treated groups. These results indicated that the oral lethal dose of acetone extract is more than 5,000 mg/kg but the non-observed-adverse-effect level of the extract is suggested to be 1,000 mg/kg per day for 28 days.

Keywords: Acute, Hepatotoxicity, *Sclerocarya birrea,* Sub-acute, Toxicity.

INTRODUCTION

It is estimated that approximately one quarter of synthetic drugs contain plant extracts or active ingredients obtained from or modeled on plant substances (Tripathi and Tripathi, 2003, Samy *et al*., 2007). In recent times there is an increasing awareness and interest in medicinal plants and their preparations commonly known as herbal medicines (Steve *et al*., 2009). The major hindrance to the use of traditional herbal preparations is the lack of scientific and clinical data in support of better understanding of the safety and efficacy of the plants (Sathya *et al*., 2009)

Sclerocarya birrea (A. Rich) Hochst., subspecies Caffra (family:Anacardiaceae) is an important food, commercial, cultural and ethnomedicinal plant in Africa (Ojewole *et al*., 2010). The tree is commonly found in semi-arid deciduous and savanna regions of sub-Saharan Africa (Borochov-Neori *et al.,* 2008). In Nigeria, the plant is commonly known as danya (Hausa), kemaa (Kanuri) and edere (fula-fulfulde). It is also known as maroola-plum (English) or marula (South Africa) (GRIN, 2018). The stem-bark, roots and leaves have been reported to possess medicinal and other properties in addition to the nutritional values of the fruit and seeds of the plant (Ojewole, 2004). *Sclerocarya birrea* is widely used in traditional medicine in Africa against hypertension, stomach or gastro-enteritis, cough and antihyperglycemia (Dimo *et al.,* 2007). It has also been reported to have a large number of therapeutic properties and pharmacological activities (Ojewole *et al.,* 2010). These include antifungal activities Runyoro *et al.* (2006), antihelminthic activity (McGaw *et al.,* 2007), antiplasmodial activity (Gathirwa *et al..* 2008), anti-inflammatory activity (Ojewole 2003a), hypoglycemic activity (Dimo *et al.,* 2007), anticonvulsant activity (Ojewole, 2006a), anti-hypertensive activity (Ojewole, 2006b), anti-bacterial activity (Eloff, 2001) and antioxidant activity (Masoko *et al.,* 2008). Even though *S. birrea* is an important source of nutrients and phytocompounds that play roles in protecting against a variety of ailments. There is no record in the medical literatures on the toxicity study of the acetone extract of its stem bark. The present study was therefore carried out to evaluate the safety of acetone extract of *S. birrea* by acute and sub-acute toxicity studies in Wistar rats.

MATERIALS AND METHODS

Collection and identification of plant material

Pieces of fresh stem bark of *S. birrea* were harvested from the Federal Polytechnic Staff Quarters, Bauchi, Bauchi State, Nigeria. This was identified and authenticated at the Department of Biological Sciences, Ahmadu Bello University, Zaria Herbarium with a voucher specimen number of 1071.

Figure 1: *S. birrea* plant www.bing.com)

Preparation of plant extract

The fresh stem-bark of *S. birrea* was air dried in a shaded area, minced and powdered using laboratory mortar. 1 kg of stem back powder was extracted in 2.5 litres of acetone (JDH) using a Soxhlet extractor. This was filtered using a Whatman's filter paper (24 cm). The filtrate was evaporated using a hot water bath and a total yield of 141.7 g (14.17% percentage yield) was obtained. Different doses of the extract were dissolved in distilled water each day and administered immediately.

Experimental animals

Thirty adult Wistar rats of both sexes (average weight of 120 g per group) were obtained from the Department of Human Anatomy, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. They were kept in plastic cages and maintained under laboratory conditions of temperature and light with free access to food (standard pellet diet, Grand Cereal Ltd, Jos Pleateau State) and water. The experimental animals were acclimatized for two weeks after which they were divided into six groups of five animals each. The experimental protocol was in accordance with the OECD guidelines and care of the experimental animals was done according to the Ahmadu Bello University, Zaria, Nigeria, animal use and care guideline.

Acute toxicity study

Acute toxicity studies of acetone extract of *S. birrea* stem bark was carried out in Wistar rats using Organization for Economic Co-operation and Development (OECD) guideline 425 (OECD 425, 2008). In this study, all rats received a single oral dose of the test sample. Rats in Group I received 1 ml/kg of distilled water while doses of 2,000 mg/kg and 5,000 mg/kg of the extract

were given using oral gavage to rats of Group II and Group III respectively. All the rats were observed for general behavioral changes, symptoms of toxicity and mortality after treatment for the first four (critical) hours, then over a period of 24 hours, thereafter daily for 14 days.

Sub-Acute Toxicity Studies

This study (28-day repeated oral toxicity study) was carried out according to OECD 407guidelines (OECD 407, 2008). Fifteen rats were used for the study. Rats were divided into three groups of 5 animals each. Group I received 1 ml/kg distilled water and served as a control group whereas Group II and Group III received 500 mg/kg and 1,000 mg/kg of extract respectively orally via gavage. All the groups of rats were observed twice daily for mortality and morbidity till the completion of the experiment. All the animals were observed for clinical signs and the time of onset, duration of these symptoms, if any were recorded. Body weights of the rats in all groups were recorded once before the start of dosing, once weekly during the treatment period and finally on the day of sacrifice. At the end of the experiment(on 29th day), experimental animals were humanely killed under chloroform anaesthesia and blood collected via cardiac puncture into heparinized and non-heparinized tubes for hematological and biochemical analyses.

Haematological parameters

The heparinized blood was used for the analysis of hematological parameters such as hemoglobin (Hb), packed cell volume (PCV) and white blood cell (WBC) count were measured using an automated hematology analyzer (PE 6000).

Biochemical Parameters

The serum was separated from non-heparinized blood and the serum biochemical parameters including total cholesterol, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN), triglycerides, total cholesterol, albumin, bilirubin and total protein were analyzed using a digital colorimeter AC-14.

Histopathology study

The liver and kidney were collected from all the animals for histopathological study. The collected organs were weighed and preserved in 10 % neutral buffered formalin, trimmed and a 5 µ thickness of tissue sections were stained with haematoxylin and eosin for histopathological study.

Statistical Analysis

IBM statistical package for social sciences (SPSS) 20.0 software was used to analyze the data. Results obtained from the weight of organs, haematological and biochemical parameters were expressed as mean ± standard error of mean (SEM). This was analyzed using One-Way analysis of variance (ANOVA) followed by Dunnett's post hoc test to check for significant difference between groups. Values of $p<0.05$ was considered as significant.

RESULTS

Acute Toxicity Studies

In the toxicity study, oral administration of the acetone extract of *S. birrea* at 2,000 mg/kg and 5,000 mg/kg did not produce any deaths or clinical signs of toxicity in the rats, and LD50 value of *S. birrea* was found to be greater than 5,000 mg/kg. On the other hand, a significant decrease in body weight ($p<0.05$) was observed at both 2,000 mg/kg and 5,000 mg/kg as compared to the control group (Fig 2).A significant increase in the weight of the liver was also observed in both extract treated groups i.e. both dosages as compared to the control while a significant increase in the weight of the kidney was observed at 5,000 mg/kg dose only as compared to the control group (Table 1).

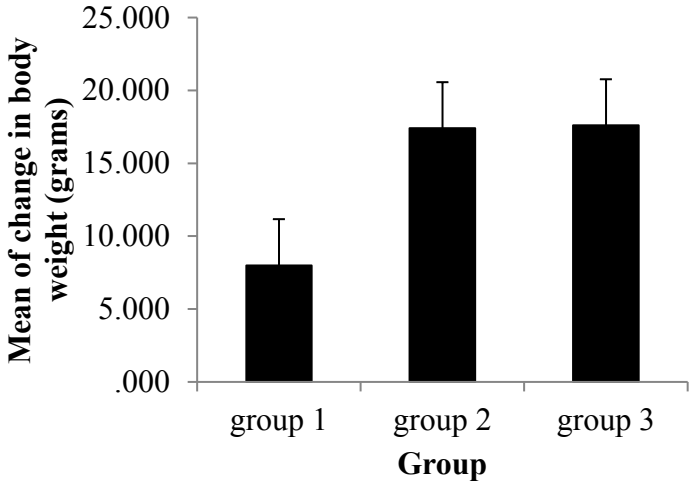

Figure 2: Change in body weight following acute oral administration of acetone extract of *S. birrea* stem bark in Wistar rats

Table 1: Change in organ weight following acute oral administration of acetone extract of *S. birrea* stem bark

Group	Liver	Kidney
Group 1 (control)	5.40±0.24	1.00±0.00
Group 2 (2,000 mg/kg)	6.80±0.37*	1.60±0.24
Group 3 (5,000 mg/kg)	6.80±0.37*	2.20±0.20*

Following One-Way ANOVA and Dunnett's post hoc test, *P<0.05

In the haematological study, significant increase was observed in the levels of WBC at both 2,000 mg/kg and 5,000 mg/kg dosage while a significant decrease was observed in the levels of PCV and Hb respectively at 5,000 mg/kg dosage only as compared to the control group (Table 2).

Table 2: Effect of acute administration of *S. birrea* extract on PCV, WBC and Hb levels

Group	PCV (%)	WBC(10^3/µL)	Hb(g/dL)
Group 1 (control)	42.40±1.69	7.26±0.55	12.52±0.52
Group 2 (2,000 mg/kg)	37.50±7.69	14.40±3.40*	11.20±2.28
Group 3 (5,000 mg/kg)	35.20±0.97*	14.08±1.56*	10.22±0.17*

Following One-Way ANOVA and Dunnett's post hoc test, *P<0.05

Results obtained from studies on the biochemical parameters of both the liver function test and kidney function test revealed that there was significant increase in the levels of AST following oral administration of acetone extract of *S. birrea* at both 2,000 mg/kg and 5,000 mg/kg dosages respectively while a significant increase was observed in the level of ALT at 5,000 mg/kg dose only. Furthermore, a significant increase in the level of K^+ was also observed at 2,000 mg/kg dosage only as compared to the control group as indicated in Table 3 and Table 4. No significant changes were observed in the levels of the other parameters tested.

Table 3: Effect of acute administration of *S. birrea* extract on biochemical parameters

Parameters	Group 1	Group 2	Group 3
AST (U/L)	89.20±4.87	123.80±8.95*	132.40±18.93*
ALT (U/L)	31.80±3.09	40.00±1.87	50.60±5.9*
ALP (U/L)	61.40±4.91	65.20±5.14	68.40±1.50
FBS (mmol/L)	6.14±0.65	4.74±1.43	5.46±1.45
TP (g/dL)	5.10±0.61	5.90±0.39	4.26±0.26
BIL (mg/dL)	8.00±0.71	8.60±0.87	9.20±0.58
ALB (g/dL)	3.40±0.20	2.90±0.24	3.60±0.52
UREA (mg/dL)	6.66±0.56	6.80±0.19	7.58±0.53
CHOL (mg/dL)	97.88±7.27	91.26±5.97	101.56±8.00
TRIG (mg/dL)	84.44±12.68	72.82±9.45	74.06±5.32
HDL (mg/dL)	42.34±3.63	41.72±1.92	54.76±7.10

Following One-Way ANOVA and Dunnett's post hoc test, *P<0.05

Table 4: Effect of acute administration of *S. birrea* extract on electrolyte levels

Parameter	Group 1	Group 2	Group 3
Na^+ (mmol/L)	130.20±7.21	138.80±2.39	141.40±3.63
K^+ (mmol/L)	6.64±0.27	10.26±0.69*	7.70±0.55
Cl^- (mmol/L)	105.20±7.73	106.00±3.54	108.40±4.11
HPO_4 (mmol/L)	17.60±1.17	19.40±1.21	17.00±2.00
Ca^{2+} (mmol/L)	6.18±0.33	7.46±0.69	7.22±0.81

Following One-Way ANOVA and Dunnett's post hoc test, *P<0.05

Results obtained from the histopathological analysis of the liver following acute oral administration of acetone extract of *S. birrea* stem bark showed loss of hepatocellular boundaries, vast areas of necrosis and vacuolation in the hepatocytes at 5,000 mg/kg indicating widespread hepatotoxicity as compared to the 2,000 mg/kg dosage group which showed areas of hepatocellular degeneration. On the other hand, the sections obtained from the kidney

showed areas of mild tubular degeneration at both 2,000 mg/kg and 5,000 mg/kg as seen in Fig. 3.

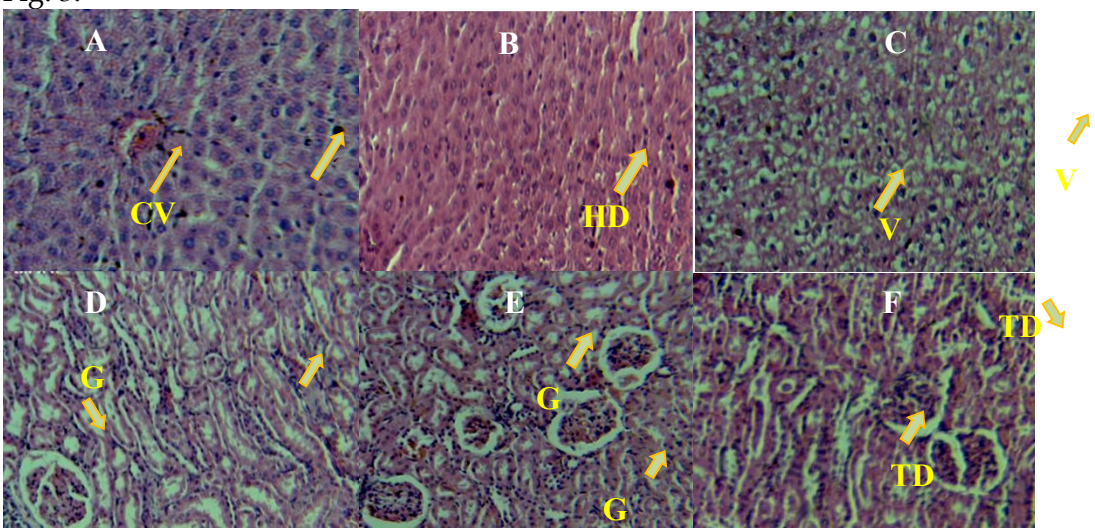

Figure 3: Photomicrographs of the liver (A= control, B=2,000 mg/kg, C=5,000 mg/kg) following acute oral administration of *S. birrea* stem bark extract in Wistar rats showing areas of hepatocellular degeneration (HD), vacuolation (V) and necrosis (N). Photomicrographs of the kidney (D=control, E=2,000 mg/kg, F=5,000 mg/kg) showing areas of glumerular (GD) and tubular (TD) degenaration respectively.

Sub-acute study

There were no treatment related toxicity signs and mortality observed in rats treated with 500 mg/kg and 1,000 mg/kg orally during the 4 weeks of treatment. No significant differences in body weight were observed between the initial and final body weight of the rats treated with *S. birrea* and control rats (Fig 4). Of the organs collected, a significant difference was observed only in the liver at 1,000 mg/kg dose (Table 5).

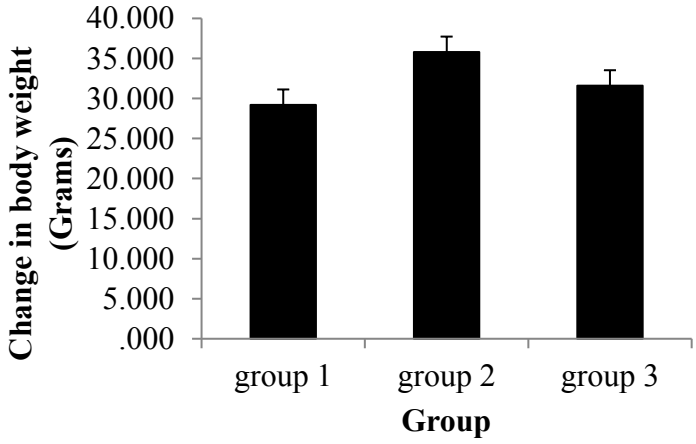

Figure 4: change in body weight

Table 5: Change in organ weight

Group	Liver	Kidney
Group 1 (control)	5.40±0.24	1.00±0.00
Group 2 (500 mg/kg)	6.00±0.32	1.00±0.00
Group 3 (1,000 mg/kg)	7.00±0.55*	1.80±0.37

Following One-Way ANOVA and Dunnett's post hoc test, *P<0.05

Results from the haematological analysis showed that there were no significant changes in levels of PCV, WBC and Hb during the experimental period (Table 6). Similarly, no significant changes were observed in the levels of parameters tested for both the liver and kidney function tests carried out as summarized in Table 7 and Table 8.

Table 6: Effect of sub-acute administration of *S. birrea* on PCV, WBC and Hb

Group	PCV	WBC	Hb
Group 1 (control)	38.00±2.35	10.78±0.50	11.50±0.54
Group 2 (500 mg/kg)	40.40±1.63	14.28±1.32	11.68±0.61
Group 3 (1000 mg/kg)	37.80±0.80	14.14±1.40	10.84±0.75

Table 7: Effect of sub-acute administration of *S. birrea* on biochemical parameters

Parameter	Group 1	Group 2	Group 3
AST (U/L)	63.40±16.86	90.20±3.92	85.40±4.59
ALT (U/L)	35.00±9.67	53.4±5.16	43.40±4.13
ALP (U/L)	56.20±14.31	75.40±5.80	76.00±5.61
FBS (mmol/dL)	3.86±1.70	3.44±0.90	4.68±1.18
TP (g/dL)	3.12±0.80	4.26±0.26	5.02±0.34
BIL (mg/dL)	5.80±1.56	7.00±1.79	7.60±0.68
ALB (g/dL)	2.38±0.65	2.64±0.69	2.78±0.18
UREA (mg/dL)	4.80±1.21	6.50±0.30	6.36±0.54
CHOL (mg/dL)	58.12±14.91	64.16±17.87	101.12±13.80
TRIG (mg/dL)	52.70±15.62	53.48±16.25	77.72±5.99
HDL (mg/dL)	35.72±15.03	41.08±11.86	50.42±6.31

Table 8: Effect of sub-acute administration of *S. birrea* on electrolyte levels

Parameter	Group 1	Group 2	Group 3
Na^+ (mmol/L)	96.80±4.12	110.40±8.09	113.20±5.90
K^+ (mmol/L)	7.40±0.85	7.62±0.60	6.72±0.64
Cl^- (mmol/L)	106.80±4.85	123.20±11.36	87.6±22.34
HPO_4 (mmol/L)	18.00±2.00	23.20±2.35	20.00±5.30
Ca^{2+} (mmol/L)	6.52±0.69	6.84±0.69	6.60±0.46

Results obtained from the histopathological analysis of the liver following sub-acute oral administration of acetone extract of *S. birrea* stem bark showed loss of hepatocellular boundaries, widespread necrosis and vacuolation in the hepatocytes at 1,000 mg/kg indicating widespread hepatotoxicity as compared to the 500 mg/kg dosage group which showed mild hepatocellular degeneration. On the other hand, the sections obtained from the kidney showed areas of mild tubular degeneration at both 2,000 mg/kg and 5,000 mg/kg.

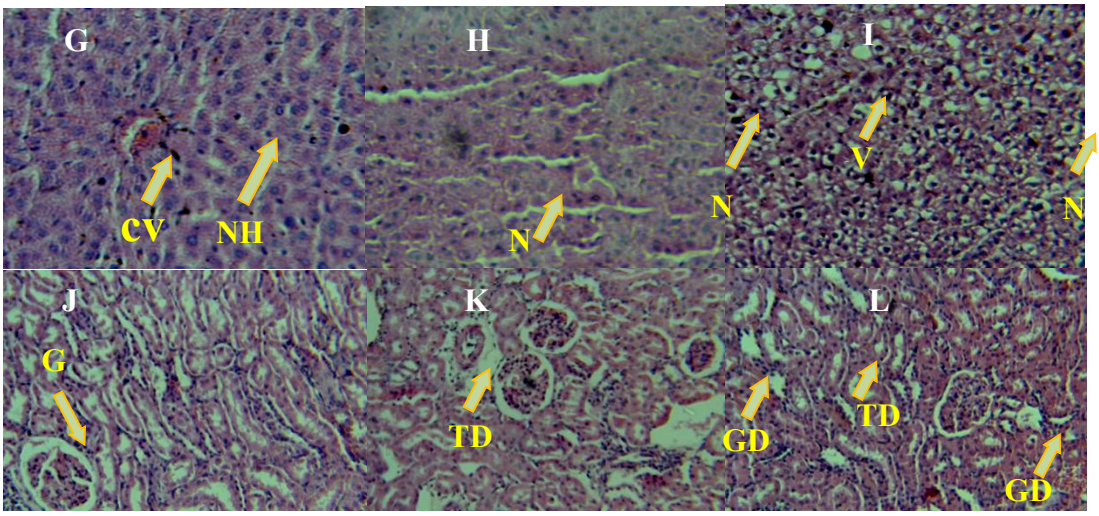

Figure 4: Photomicrographs of the liver (G= control, H=500 mg/kg, I=1,000 mg/kg) following sub-acute oral administration of *S. birrea* stem bark extract in Wistar rats showing areas of vacuolation (V) and necrosis (N). Photomicrographs of the kidney (J=control, K=500 mg/kg, L=1,000 mg/kg) showing areas of mild glumerular (GD) and tubular (TD) degenaration respectively. N=normal hepatocytes, CV=central vein

DISCUSSION

Herbal medicine also known as traditional or natural medicine has maintained a greater popularity all over the developing world particularly in countries like Nigeria and its use is rapidly on the increase (Jimoh *et al*., 2013). Due to their natural origin, these plants are usually considered safe for human use. However, various reports suggest the potential risks involved with the use of such remedies (Jordan *et al.,* 2010). *S. birrea* is a medicinal plant having vast nutritional and therapeutic potential and inspite of its potential and long history of use, there is limited report in the medical literature on its toxicological profile as well as adverse effect. Hence, the present study was carried out to evaluate the acute and sub-acute toxicity of the acetone extract of *S. birrea* stem bark.

In the present study, no mortality was observed in the acute toxicity study at both 2,000 mg/kg and 5,000 mg/kg dosages indicating that the LD50 of the acetone extract of *S. birrea* stem bark is greater than 5,000 mg/kg. Similarly, no mortality was observed in the 28 day oral sub-acute study at both 500 mg/kg and 1,000 mg/kg dosages.

Body weight and organ weight changes following toxicity testing serve as an indicator of adverse side effects of chemicals and drugs (Prasanth *et al*., 2014). The results of the acute toxicity study showed significant decrease in weight following treatment at both 2,000 mg/kg and 5,000 mg/kg dosages suggesting that the extract had adverse effects on body weight at these dosages. This decrease in body weight may be attributed to loss of appetite as evidenced by reduced food intake by the experimental animals.

The organ weight is an important indicator of the physiological and pathological status of animals and this is fundamental in confirming whether the organs were exposed to drug or

chemical injury or not. Some of the major organs affected by metabolic reaction caused by toxicants include the liver, kidney, spleen, heart and lungs (Dybing *et al*., 2002). A significant increase in the weight of the liver was observed in both 2,000 mg/kg and 5,000 mg/kg dosages of the acute toxicity study as compared to the control group while a significant increase in kidney weight was observed at only 5,000 mg/kg as compared to the control. This indicates an adverse pathological effect of *S. birrea* extract on these organs. On the other hand, a significant increase in weight of the liver was observed only at 1,000 mg/kg in the 28-day sub-acute study as compared to the control while no significant weight change was observed in the kidney. Thus, suggesting that the extract didn't have any adverse effect on the kidney following the 28 days treatment at both 500 mg/kg and 1,000 mg/kg.

Haematological indices have been shown to have a higher predictive value for toxicity (Oshilonya *et al*., 2015). In the haematological study, a significant decrease was observed in the levels of PCV and Hb at 5,000 mg/kg as compared to the control in the acute toxicity study indicating the presence of drug induced anaemia. Anaemia following the administration of an agent can be as a result of lysis of the red blood cells or inhibition of blood synthesis by the active constituents of the extract. A decrease in haematological parameters has been established in experimental animals to be strongly associated with anaemia (Oshilonya *et al*., 2015). Phytochemical analysis i.e. analysis of the secondary metabolites of the acetone extract of *S. birrea* indicates the presence of alkaloids (Mohammed *et al*., 2015) and it has been established that one of the toxic effect of alkaloids in animals is anaemia (Olayemi *et al*., 2010). Furthermore, the significant increase in the levels of WBC at both 2,000 mg/kg and 5,000 mg/kg dosages respectively as compared to the control group in the acute study could be attributed to the presence of glycosides (Mohammed *et al*., 2015) which are compounds having anti-inflammatory properties and hence, having vital effects on the inflammatory process of some pathological states such as bacterial infection, malaria and liver diseases (Antai *et al*., 2009). The 28 day sub-acute study showed a decrease in both PCV and Hb levels as well as an increase in WBC levels though these changes were not to significant levels indicating that the extract at 500 mg/kg and 1,000 mg/kg did not have any adverse effect on these blood parameters.

The liver enzymes ALT and AST are present in high concentrations in normal hepatocytes and these enzymes leak into circulation when hepatocytes or their cell membranes are damaged (Das and Vasudevan, 2005; Ahsan *et al*., 2009). It has also been reported that ALT is more specific for liver damage as compared to AST as it is found almost exclusively in the liver (Dufour *et al*., 2000). These enzymes have also been shown to be good indicators of liver function and are hence used as biomarkers to conclude the probable toxicity of drugs and xenobiotics (Rahman *et al*., 2000). The significant increase in the levels of AST and ALT at 5,000 mg/kg in the acute toxicity study indicates that *S. birrea* acetone extract at this dosage was injurious to the hepatocytes thus, confirming the presence of liver injury. On the other hand, results from the 28 days sub-acute toxicity study did not show any significant change in the levels of AST and ALT in the extract treated groups as compared to the control group indicating that the extract at 500 mg/kg and 1,000 mg/kg did not affect liver function or metabolism. No significant changes were observed in the levels of the other biochemical parameters tested indicating that the extract did not have any adverse effect on kidney function (creatinine and

urea), synthetic function of the liver (total protein) and heart (cholesterol, triglycerides, LDL and HDL) in both the acute and sub-acute toxicity study at the administered dosages. Furthermore, no significant changes were observed in the levels of electrolytes in both the acute and 28 days sub-acute study indicating that the extract did not have any adverse effect in the kidney's function in maintaining homeostasis. Though a significant increase was observed in the levels of K^+, the reason for this increase still remains unknown.

The results obtained from the histopathological study showing areas of loss of hepatic cellular boundaries, necrosis and vacuolation in the acute toxicity study at 5,000 mg/kg dose confirming the presence of liver damage as evidenced by the increase observed in the levels of AST and ALT from the biochemical study. Thus, suggesting that *S. birrea* acetone extract had adverse pathological effect on the liver at 5,000 mg/kg though it didn't cause any mortality in the experimental animals. Similarly, these hepatocellular changes were also observed in the sub-acute toxicity study at 1,000 mg/kg indicating the presence of liver injury though no significant changes were observed in the biochemical parameters assayed. Histopathological analysis of the kidney showed areas of tubular degeneration at 5,000 mg/kg in the acute toxicity study as compared to the mild changes observed in the sub-acute toxicity study indicating insignificant damage to the kidneys thus, corroborating results obtained from the biochemical analysis of Na^+, K^+, and Ca^{2+} where no significant changes was observed in their levels.

In conclusion, the oral lethal dose of acetone extract of *S. birrea* stem bark is higher than 5,000 mg/kg as it didn't cause any mortality in the experimental animals but the non-observed-adverse-effect level of the extract is suggested to be 500 mg/kg per day for 28 days as at this dosage, no haematological, biochemical or pathological changes were observed in the experimental animals.

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