Research Article
Evaluation of the Acute and Sub chronic Toxicities of the Methanolic Stem Bark Extract of *Spathodea campanulata* (P. Beauv.) Bignoniaceae

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Abstract: *Spathodea campanulata* (*P. Beauv.*) *Bignoniacea* is widely used in Ugandan traditional medicine without having much knowledge on any toxicity data. This study carried out the toxicity profile of its methanolic stem bark extract (SCE) at both acute and sub-chronic levels. A single oral dose administration of SCE at the limit dose of study (5000 mg/kg) did not cause any observable toxic effect or mortality. However, a 90 day oral administration in Wistar rats (200, 400 and 800 mg/kg) resulted in an acceleration of bodyweight increase as indicated by the calculation of percentage bodyweight increments at the doses of study. It had no effect on the relative organ weights considered except an increase on the stomach at the 800 mg/kg dose. Among the biochemical parameters considered (ALT, AST ALP, GGT, creatinine and urea) the only significant ($p<0.05$) increase was observed with ALT at the 800 mg/kg level. The levels of WBC, RBC, RDW and HCT dropped significantly ($p<0.05$) at the 800 mg/kg and 400 mg/kg for WBC alone in the SCE-treated groups. There was however a significant elevation ($p<0.05$) of MCV, MCH, MCHC levels at 800 mg/kg in the treated animals compared to the control group. A histological analysis of the isolated organs (heart, testes, lungs, liver, stomach and kidneys) showed that the heart is the vulnerable organ with myocardial necrosis and hemorrhage occurring at the 400 and 800 mg/kg level following 90 days administration. Acute use of SCE is safe. Therefore, the resultant adverse effects observed on the lone liver enzyme (ALT) and the myocardial tissue at high doses following prolonged administration could be avoided if compliant use could be intermittent.

Keywords: LD$_{50}$, Haematotoxicity, hepatotoxicity, histopathology, rat, spathodea, toxicity

INTRODUCTION

The use of herbal medicines is a worldwide confluence of traditional and alternative medical practices, as it is in almost all, if not all non orthodox medical systems be it Traditional African Medicine (TAM); Ayurveda, Siddha etc. in the Asian region; homoeopathy and other forms of Complementary/ Alternative Medicines in the Western world. Good enough the World Health Organisation estimated that about 80% of the population of the developing world depends primarily on herbal medicines for basic healthcare (WHO, 2003). Furthermore, there is increasing reliance on herbal medicines in the industrialised world which is traceable to the extraction and development of several drugs, chemotherapeutics from these plants as well as from traditionally used rural herbal remedies (UNESCO, 1998). The high level of dependence on the herbal medicines both in the rural communities and industrialised world is often backed by the belief that the use of orthodox drugs is synonymous with encountering deleterious effects which are absent in these herbal medicines. Some scientific data has pointed out to the fact that herbal

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medicines are not without adverse effects and to add to it, revealed the realisation that there are possible interactions between contemporary drugs and herbal medicines that can produce deleterious effects. This erroneous impression is dangerously extended by parents and herbal treatment preferred for their children to conventional healthcare. There are too many instances where ignoring conventional treatment in favour of traditional medicine or CAM has ended up in regrettable consequences. It is noteworthy here that preference to CAM or traditional medicine is not usually only limited to mild conditions but also includes life-threatening conditions like cancer, diabetes, immunisation, HIV/AIDS etc. (Ernst, 2005). In the same write-up the author notes that some herbal preparations from India and China have been found to be contaminated with toxic heavy metals or strong prescription drugs and that puts the vulnerable children at particularly high risk of adverse effects and drug interactions. In the process of standardising herbal medicines it could be a laudable move if some effort is directed towards evaluating the safety of such before clinical use is authorised.

Spathodea campanulata Bignoniacea (*P.Beauv.*) is a "Large tree with a stout, tapering often somewhat buttressed trunk, branches thickish, marked with small white lenticels, subglabrous to thinly puberulent, reaches heights of 25 m; leaves usually opposite (rarely 3 at a node), very widely diverging, up to 50 cm long, dark green in colour. *Spathodea campanulata* invades both abandoned agricultural land and closed forest. It also invades natural ecosystems in the Cook Islands, Fiji, Guam, Hawaii, Samoa and Vanuatu. Although the African tulip tree favours moist and wet areas below 1000 m it grows up to 1,200 m in French Polynesia. *Spathodea campanulata* does not tolerate frost and demands full sun for fast growth and best flowering. The biggest trees grow in moist sheltered ravines. This species loves rich soil, but puts up with just about anything with a little fertility to it, including lime rock. It will survive a bit of salinity.

Spathodea campanulata invades agricultural areas, forest plantations and natural ecosystems, smothering other trees and crops as it grows becoming the prevailing tree in these areas. In Hawaii, there are major infestations tucked away in almost every rainforest valley along the northern and eastern slopes of Kaua'i, O'ahu and East Maui. In Uganda it is found in some rural homesteads as the boundary tree demarcating even grazing land. It is also found in the forests especially reserve areas in central and south western regions of the country.

The seeds are edible. In Singapore the timber is used for making paper. In West Africa the wood is used to make drums and blacksmith's bellows. The bark, flowers and leaves are also used in traditional medicine in its native home range, (http://www.floridata.com/

ref/s/spat_cam.cfm). The wood is difficult to burn and so the tree can be used in fire resistant landscaping. Buds contain liquid that will squirt out if they are squeezed or pierced, which children enjoy using as water pistols. The use of this tree as a medicinal plant in Uganda has been reported by Hamill *et al.*, 2003. The stem bark is extracted in banana beer (a local brew) and taken as treatment for diabetes. The water extract of the leaves is used as either an aphrodisiac for women or to improve on the production of vaginal fluid. Various medicinal uses have been described in different regions; in Rwanda it is used as an antidiabetic herbal remedy, (Niyonzima *et al.*, 1999). While in Nigeria, (Makinde *et al.*, 1988) and Cameroon, (Titanji *et al.*, 2008) it is used to treat malaria, it is reported to have analgesic and anticonvulsant applications among Nigerian traditional healers as well as possessing reversible organ toxicity, (Ilodigwe and Akah, 2009). With such wide spread use of this plant further broadened by the multiplicity of the diseases it is used to treat in various regions, it is but necessary that the toxicity or safety profile of such a plant be established. Several studies have established toxicity of herbal medicines even with specialised toxicity patterns and on specific organs, (Kalplowitz, 1997; Calixto, 2000; Jaouad *et al.*, 2004; Taziebou *et al.*, 2007). In the same line besides developing guidelines for quality control of the processing of traditional medical remedies, the WHO encourages that traditional medical practices be incorporated in contemporary medical practices apparently in order to put under control the potential of adverse effects from herbal preparations. In a similar bid most research on medicinal plants routinely includes toxicity evaluation especially at the preliminary stages, (Balogun *et al.*, 2011 and Ezeonwumelu *et al.*, 2011). Ilodigwe *et al.* (2010) studied the toxicity of the leaf extract as it is used in Nigerian folk medicine. No toxicity study has been done on the stem bark relating to the pattern of its use in Ugandan traditional medicine. Hence, the basis for the study of the toxicity of this medicinal plant. The present investigations were aimed at evaluating the acute and sub-chronic toxicity profiles following oral administration of SCE to rats.

MATERIALS AND METHODS

Environmental considerations: Prior to beginning the collection of plant material in collaboration with the Rukararwe Traditional Healers Partnership (RTHP), two seedlings of *Spathodea campanulata* were obtained from the RTHP medicinal plants nursery and planted near the KIU-WC staff residential area. This was in compensation for any eventual adverse environmental consequences of collecting the medicinal plant material. The trees are presently mature.

Plant material collection and extraction: The stem bark of *Spathodea campanulata* was collected locally from Rukararwe Traditional Healers Partnership premises in Bushenyi, Uganda. Collection was done between 9:00 and 11:00 am in the month of May 2012. Authentication was done in the Department of Science Laboratory Technology of Mbarara University of Science and Technology by Dr. Eunice Olet and a voucher (JIBI JAMES 001) deposited in the herbarium of the same department. The stem bark was shade-dried, powdered and taken for extraction. The plant material was extracted in 50% methanol using a soxhlet extractor (Quickfit) at the department of Pharmacology and Therapeutics of Mbarara University of Science and Technology. The crude extract was concentrated by distillation and further dried in an electrical oven (Mermmet®) at 40°C obtaining a yield of 40% w/w of plant material.

Laboratory animal acquisition and maintenance: Healthy young adult Wistar rats (*Rattus norvegicus*) of both sexes weighing 100-150 g bred and maintained at the Laboratory Animal Facility of the Department of Pharmacology and Toxicology, Kampala International University-Western Campus were used according to the NIH guide for the Care and Use of Laboratory Animals in Teaching and Research (NIH Publication No. 83-27, 1985). The animals were kept and maintained under ambient laboratory conditions of temperature, humidity, 12 h light/12 h dark cycle. The animals were allowed access to standard rodent feed (Nuvita®, Jinja Uganda) and tap water *ad libitum.* Prior to the experiments the animals were fasted overnight while maintaining their free access to tap water. All animal studies were conducted after obtaining prior approval from Mbarara University of Science and Technology Institutional Review Committee (MUST-IRC; Ref: MUIRC 1/7) according to the Uganda National Council for Science and Technology (UNCST) guidelines for laboratory animal care and use.

Acute toxicity evaluation: In order to carry out the acute toxicity evaluation of this SCE, the Organisation for Economic Cooperation and Development (OECD) guideline 425 of 2008 was adopted. The animals were deprived of food overnight prior to administration of the extract. In phase I, one male adult Wistar rat was administered 5,000 mg/kg SCE. The mouse was observed for signs of toxicity immediately and up to 24 h after administration and scored for mortality. The probable signs observed for, were ataxia, diarrhoea, sedation, anorexia, increased activity, piloerection etc. In Phase II, two male young adult Wistar rats were treated as above. They were observed for signs of toxicity as in phase I above. Twenty-four hours later they were scored for mortality. The same procedure was repeated using male adult Wistar rats weighing 100-150 g. The same animals were maintained under the usual laboratory conditions for two weeks and observed daily for any delayed toxic effects or mortality.

Sub-chronic toxicity evaluation: The test was carried out according to the Organisation for Economic Cooperation and Development (OECD) test guidelines which describes long-term repeated dose toxicity testing, TG425 version (OECD, 2008). Twenty-four adult male Wistar rats were divided into four groups of six rats each (n = 6). Group I served as the control receiving as treatment distilled water. Groups I to IV received orally 200, 400 and 800 mg/kg bodyweight of SCE respectively and daily for 90 days. They were observed daily for two hours after administration for any clinical signs of toxicity like diarrhoea, sedation, anorexia, hyperactivity, dieresis, piloerection etc. The bodyweights of the animals were taken every seventh day beginning from the day of initiation of administration of SCE for the experimental groups and the distilled water for the control group. The percentage bodyweight increments were calculated every two weeks according to the following formula; percentage bodyweight increment = (bodyweight after two weeks-initial bodyweight)/initial bodyweight x100. After the 90th day the animals were sacrificed by cervical dislocation. Blood was collected by cardiac puncture and preserved in both EDTA and plain vacutainer® tubes for haematological and biochemical analysis respectively. All the vital organs were harvested and carefully weighed. The relative mean organ weights were determined using the formula:

Relative mean organ weight = {Mean organ weight (g)/mean body weight on last day (g)} ×100%

Haematological assays: The haematological parameters determined from the complete blood count include Red Blood Cell (RBC), haemoglobin (HGB), haematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Red Cell Width (RDW), platelets (PLT) and Mean Platelet Volume (MPV) using a Beckman Coulter® Ac.T 5DIFF CP.

Biochemical assay: For the estimation of serum enzyme levels, the blood samples were allowed to coagulate for 30 min then centrifuged at 3,000 rpm for 10 min and the clear serum was separated and used for the estimation of biochemical hepatic and renal serum parameters like alkaline phosphatise (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyltranspeptidase (GGT), creatinine kinase and urea using a HUMASTAR® 180 automated chemistry analyzer.

Sample preparation for histopathology: The harvested organs were fixed in 10% saline formalin and preserved for histological studies. Histological examination was done on the preserved tissues (liver, kidney, lungs, heart, testes, stomach and intestines). The tissues were processed with microtome (Ernst Leitz Wetzler GMBH No. 537 Germany) and automated tissue processor, (USA). They were embedded in wax, sliced with the microtome and mounted on slides. The preparation was then stained with haematoxyline and eosin (Ganter and Jolles, 1970; Lucia *et al*., 2008). These tissues were then observed under a light microscope for possible histological changes and pictures taken with an eyepiece-attached digital camera (Sony Cybershot12.1pixels).

Statistical analysis: The results are presented as means±SEM. Statistical significance between control and extract treated groups were determined using a one way Analysis of Variance (ANOVA) with the Dunnets post hoc test. A p-value <0.05 was considered statistically significant.

RESULTS

Acute toxicity study: Administration of SCE to both mice and rats by oral intubation at a dose of 5,000 mg/kg did not cause any lethality. So the LD_{50} of the crude extract is high above the limit dose used in this study (5,000 mg/kg). During the two weeks post administration observation for probable delayed toxic effects, the animals (rats and mice) were seen to be normal as usual. No delayed toxic effects were observed.

Sub-chronic toxicity:
The effect of SCE on body weight: The general trend in the weight profile of the animals was that there was an increase in the bodyweights as seen in Table 1 showing the mean bodyweights and the standard error of the mean. Compared to the control, there was no significant difference in the mean bodyweights (p<0.05) at the doses of SCE used. The calculated percentage increments on the mean bodyweight indicate that there was an overall increase in the percentage increment after every two weeks, that is, the percentage by which the bodyweight increased was higher after every two weeks, (Table 2). However, the percentage increment on the mean bodyweight of the animals started dropping at the sixth week. The end-result was an increase in the bodyweight increments or acceleration of bodyweight increase within the first four weeks.

The effect of SCE on organ weight: The effect of SCE on organ weights was viewed in terms of its relativity to the final bodyweight, that is, the bodyweight on the sacrifice day (91st day). The percentages of the relative organ weights for the SCE-treated groups were similar and close in value for most of the considered organs; liver, kidneys, testes, lungs and heart relative to those of the control group. As for the stomach, the relative organ weight value was higher (2.67 and 2.97% respectively) for the 400 and 800 mg/kg of the SCE-treated groups compared to that of the control group (1.73%), (Table 3).

The effect of SCE on biochemical parameters: With reference to Table 4, the following observations were made on the biochemical parameters considered; serum ALP, GGT and urea values for the SCE-treated groups were lower compared to the control. Serum AST, ALT and creatinine values were higher in the SCE-treated groups than in the control. However, the only statistically significant (p<0.05) increase was observed in serum ALT at the SCE 800 mg/kg level.

The effect of SCE on haematological parameters: Significant changes (p<0.05) were observed in the haematological parameters. As can be seen on Table 5, while the levels of WBC, RBC, RDW and HCT dropped significantly (p<0.05) at the 800 mg/kg, the

Table 1: Effect on Bodyweight Mean ± SEM n = 6 p≥0.05

Treatment	Dose (mg/kg)	Bodyweight (g) on							
		Day 1	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84	Day 91
Control	10 mL/kg	104.0±4.1	127.0±4.8	162.6±4.7	174.8±7.9	190.9±5.5	191.6±4.3	210.6±4.8	210.7±5.5
SCE	200	103.1±2.6	120.6±2.3	125.8±2.3	137.1±3.2	135.7±3.3	124.3±3.6	123.5±4.4	127.6±5.4
SCE	400	101.5±6.9	122.5±6.9	149.5±6.6	158.8±7.2	175.5±7.5	185.6±8.4	196.4±9.3	204.1±8.4
SCE	800	103.6±5.7	117.8±6.2	156.7±5.7	174.6±6.8	187.7±8.1	192.7±8.6	194.6±12.5	202.2±14.1

Table 2: The effect of SCE on bodyweight increments in Rats

Treatment group	Percentage mean weight increments calculated every two weeks						
	14	28	42	56	70	84	91*
Control	21.1	28.0	7.5	9.2	0.4	9.9	0.05
SCE 200 mg/kg	11.4	3.0	6.4	-1.4	-6.1	-0.5	2.4
SCE 400 mg/kg	20.7	22.7	6.2	10.5	5.8	5.8	3.9
SCE 800 mg/kg	10.9	33.0	10.1	7.5	2.7	1	3.9

*The day of sacrifice is not up to two weeks from the previous date

Table 3: Relative mean organ weight

Treatment group	Relative mean organ weight as a Percentage					
	Liver	Kidneys	Testes	Stomach	Lungs	Heart
Control	3.83	0.73	1.31	1.73	1.00	0.37
SCE 200mg/kg	3.20	0.73	1.30	1.35	1.07	0.37
SCE 400mg/kg	3.90	0.77	1.20	2.67	0.82	0.36
SCE 800mg/kg	4.01	0.78	1.30	2.97	0.99	0.36

Table 4: The effect of SCE on biochemical parameters

Treatment group	Biochemical parameters					
	ALP U/I	AST U/I	ALT U/I	GGT U/I	CREATININE mg/dL	UREA mg/dL
Control	944±210.5	28.3±8.4	62.8±18.1	109±43.3	0.71±0.15	119.8±16.5
SCE 200 mg/kg	506±187	385.3±137	192.4±32.6	66±22.9	0.66±0.8	58±8
SCE 400 mg/kg	602±124.2	472.7±212.7	245.5±73.8	124.2±56.7	0.91±0.21	99.3±24.9
SCE 800 mg/kg	763.3±153.1	586±279	365.7±111.8*	56.7±15.5	0.87±0.08	94.7±6.1

Values are Mean±SEM (n = 6); *value statistically significant at $p < 0.05$ compared to the control

Table 5: The effect of SCE on haematological parameters; (a) The Effect of SCE on Blood count

Treatment	WBC (x10³/µL)	RBC (x10⁶/µL)	RDW %	PLT (x10³/µL)
Control	5.4±0.34	8.3±0.23	11.3±0.29	321.7±74.3
SCE 200 mg/kg	5.64±0.67	8.29±0.28	11.54±0.25	275±70.8
SCE 400 mg/kg	3.2±0.6*	5.45±1.36	10±0.73	310±110.4
SCE 800 mg/kg	2.3±0.3*	3.34±1.14*	8.7±1.12*	53.83±20.32

WBC-White blood cell counts; RBC-Red blood cell counts; RDW-Red blood cell distribution; PLT-Platelet counts; Values are Mean±SEM (n = 6); *values statistically significant at $p < 0.05$ compared to the control

Table 5: (b) The Effect of SCE on Hemoglobin Concentration and Mean Corpuscular value

Treatment	HGB g/dL	HCT %	MCV fl	MCH pg	MCHC g/dL	MPV fl
Control	15.30±.50	43.60±1.48	52.00±0.52	18.32±0.23	35.10±0.24	8.20±1.10
SCE 200 mg/kg	15.16±0.58	43.20±1.76	51.80±0.58	18.26±0.27	35.08±0.30	8.62±1.18
SCE 400 mg/kg	14.50±0.77	30.37±5.9	66.60±9.16	47.40±17.9	62.22±16.49	7.40±0.36
SCE 800 mg/kg	13.3±1.36	20.37±4.44*	74.67±8.29*	65.33±15.11*	79.85±13.88*	11.13±1.89

HGB-Hemoglobin Concentration; HCT-Hematocrit values; MCV-Mean Corpuscular volume; MCH-Mean Corpuscular haemoglobin; MCHC-Mean Corpuscular Haemoglobin Concentration; MPV-Mean Platelet Volume; Values are Mean±SEM (n = 6); *values statistically significant at $p < 0.05$ compared to the control
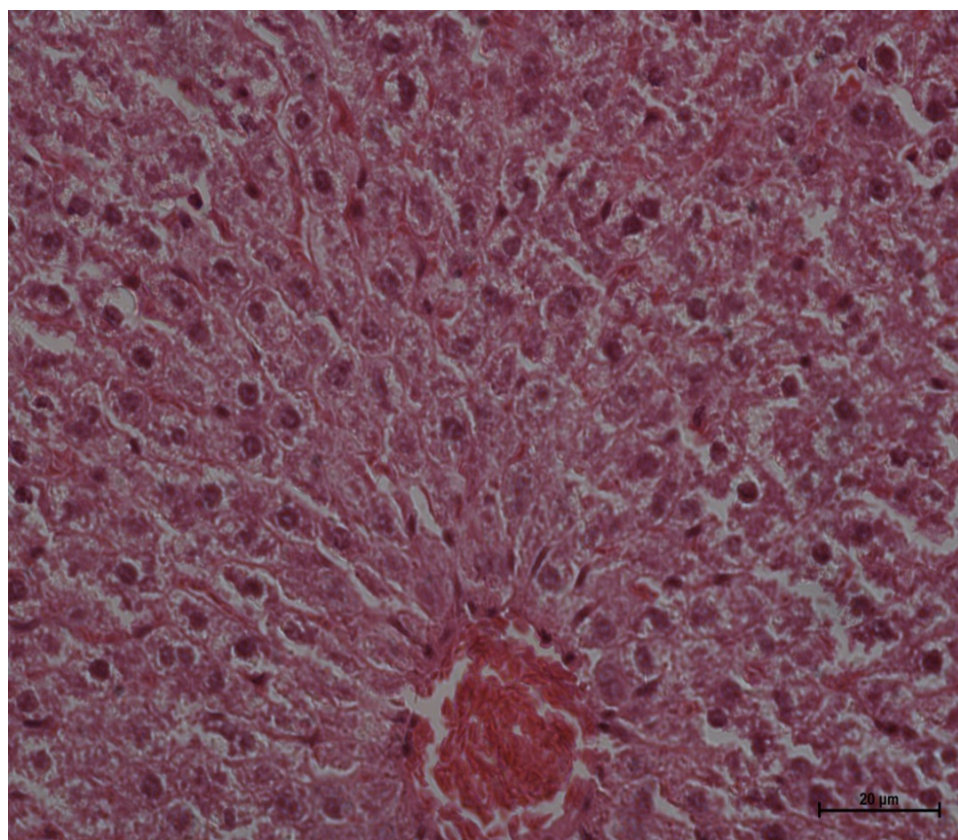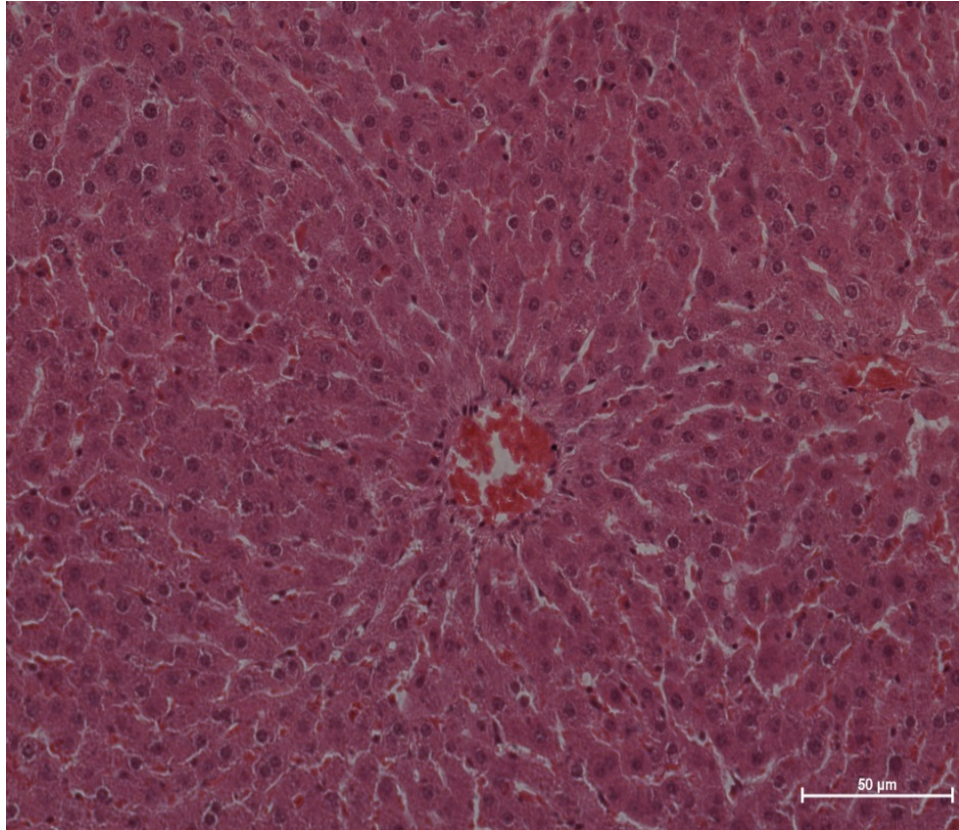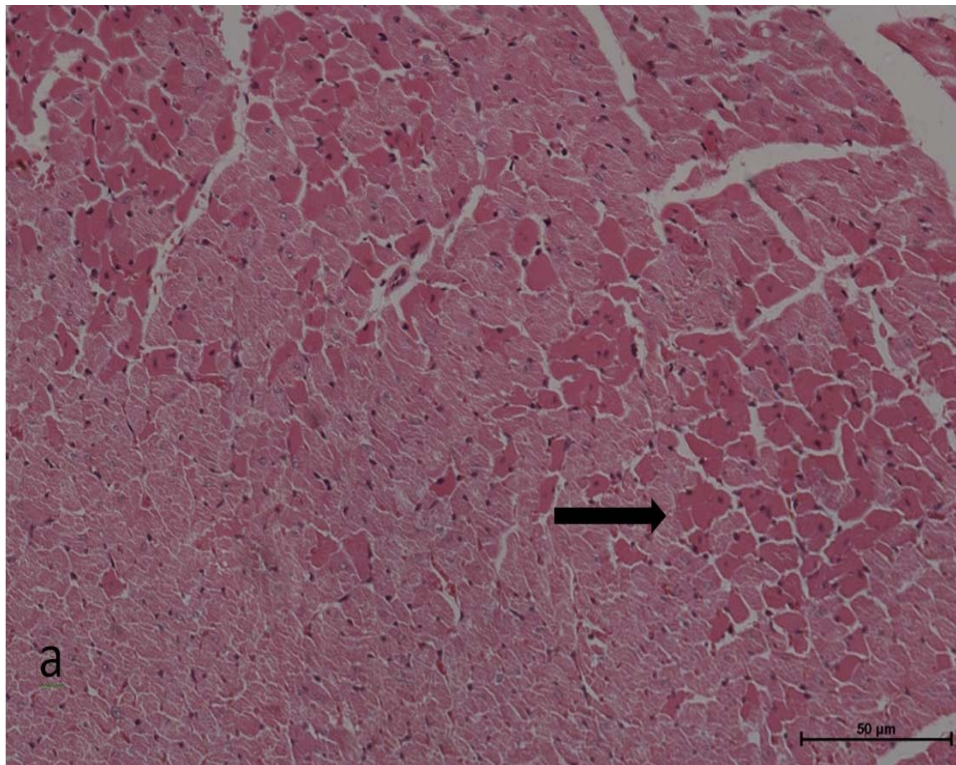
Fig. 1a and b: a: Liver from experimental animal (SCE 800 mg/kg) showing vacoular degeneration of the hepatocytes; b: Liver normal (control animal) showing normal architectural arrangement of hepatocytes with normal looking hepatocytes
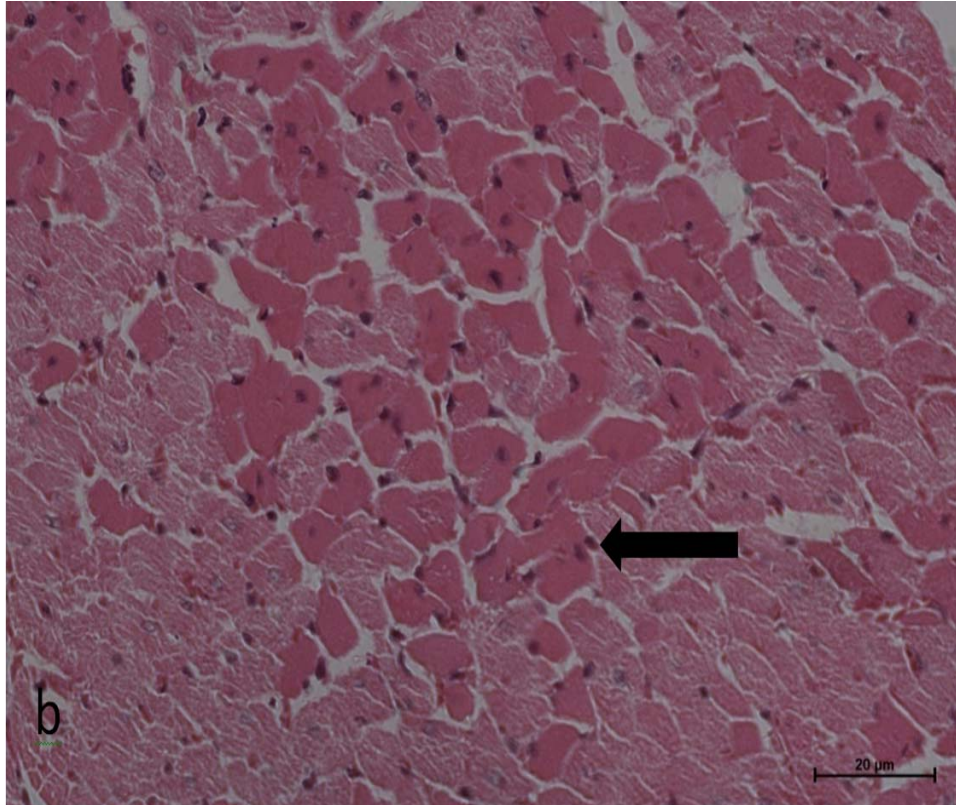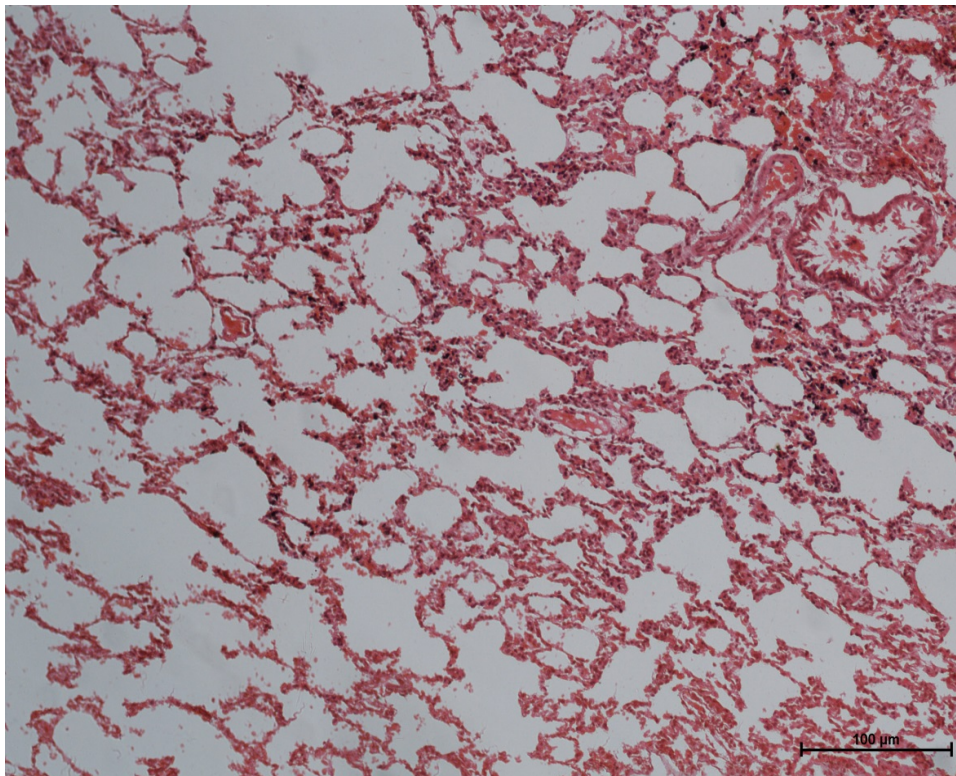
Fig. 2a and b: a: Heart from experimental animal, showing degeneration of myocardial cells which appeared more eosinophilic (arrow) than the normal cells; b: Heart, higher magnification of Fig. 2a, from experimental animal (SCE 800 mg/kg) showing degeneration of myocardial cells
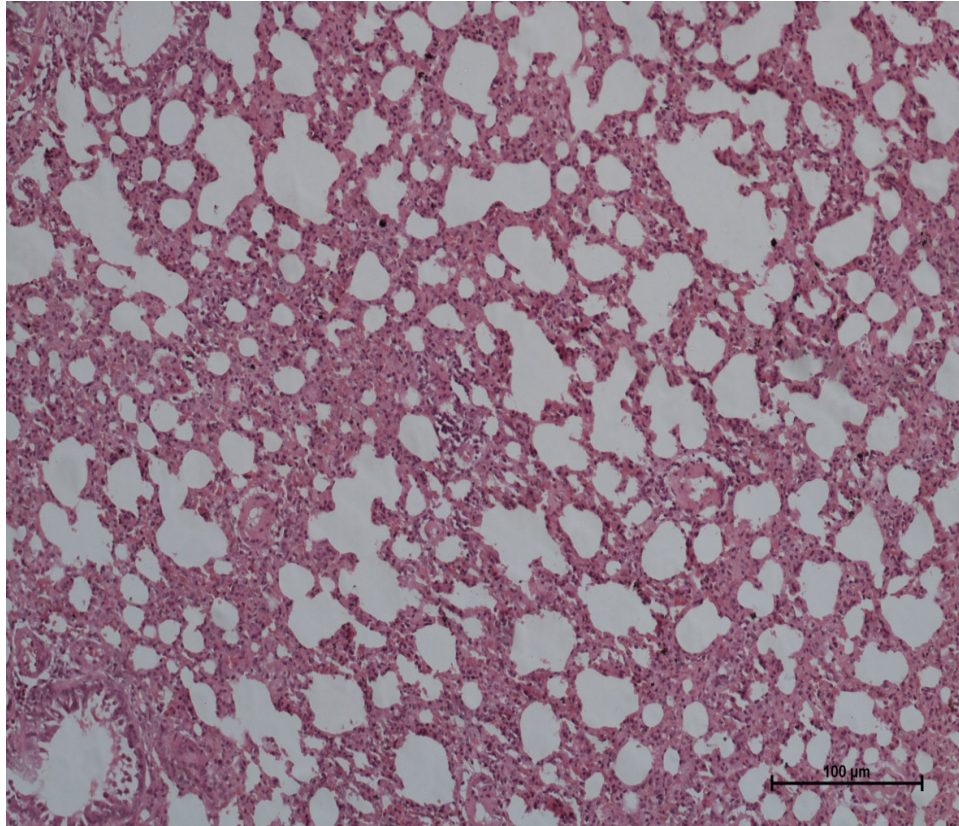
Fig. 3a and b: a: Lung from control animal. Not the normal thin interalveolar septae; b: Lung from experimental animal (SCE 800 mg/kg) with thickening of the interalveolar septae
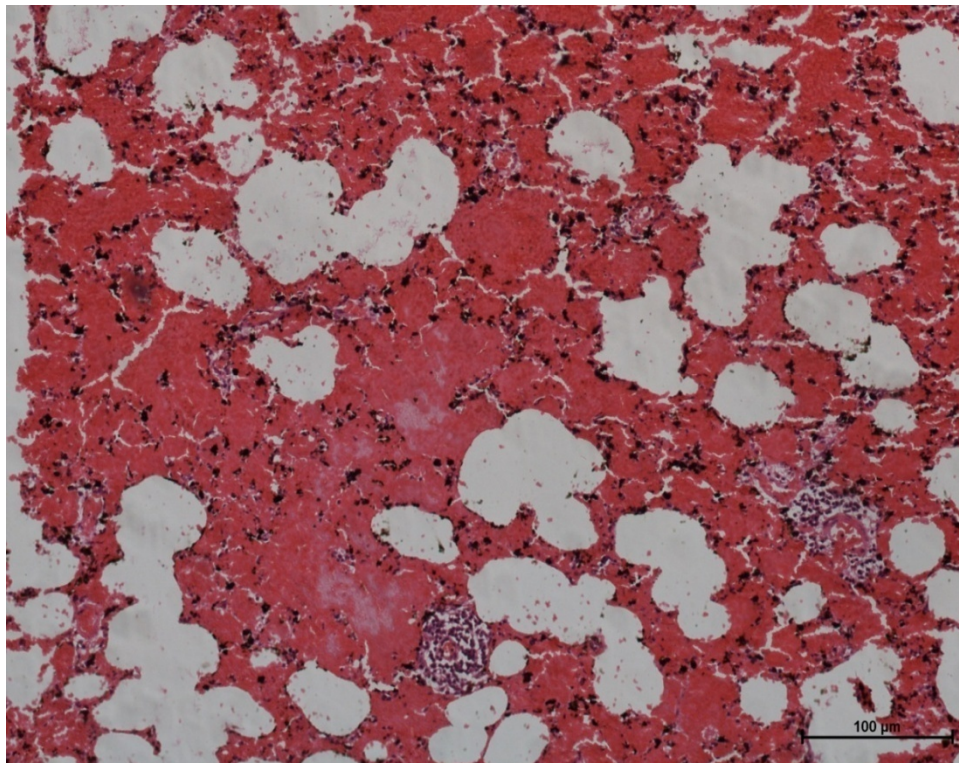

Fig. 4: Lung from experimental animal (SCE 800 mg/kg) showing pulmonary parenchymal haemorrhage

WBC value alone behaved in the same manner at 400 mg/kg in the SCE-treated groups. There was however a significant elevation (p<0.05) of MCV, MCH, MCHC levels at 800 mg/kg in the treated animals compared to the control group.

The effect of SCE on tissues: Light microscopic examination of different organs showed some tissue lesions due to the effects of the extract (SCE). Microscopic examination of the liver revealed vacuolar degeneration (Fig. 1b). Lesions of the heart of the animals treated with 400 and 800 mg/kg bodyweight of SCE indicated wide spread degeneration of individual myocardial cells with focal areas of myocardial necrosis (Fig. 2a and b). Focal areas of myocardial, epicardial and subepicardial hemorrhages were observed. Mild mononuclear cell infiltration was mostly common with the 800 mg/kg SCE-treated group. The lung of animals treated with 400 mg/kg SCE commonly indicated thickening of the inter-alveolar septae as well as marked parenchymal hemorrhage (Fig. 3b and 4). Hyperplasia of peribronchial lymphoid tissue, perivascular infiltration with mononuclear cells and pulmonary edema were occasionally observed. Lung from the 800 mg/kg SCE-treated animals showed similar effects but with more frequent occurrences of alveolar emphysema and perivascular infiltration with mononuclear cells. The sections of the testes of the rats treated with both 400 and 800 mg/kg SCE were observed to show mild interstitial edema. The sections of the stomach examined included the glandular portion and the cornified squamous portion. The glandular mucosae of the SCE-treated rats were vacuolated. Sections of the kidney showed mild to moderate degeneration of the tubular epithelial cells at the doses 400 and 800 mg/kg of SCE.

DISCUSSION

Acute toxicity studies of SCE in both mice and rats indicated that the LD50 value of the extract is high above 5,000 mg/kg bodyweight. This implies that the said extract is sufficiently safe for single-dose use as it is applied in traditional medicine in Uganda for the treatment of diabetes mellitus and other ailments, (Hamill *et al*., 2003). The use of some medicinal plants in traditional medicines in various parts of the world has been established to be safe as well, (Ogwal-Okeng *et al*., 2003; Balogun *et al*., 2011 and Ezeonwumelu *et al*., 2012). This safety profile following single dose administration should however not be mistaken for that following chronic use. There is a need to investigate the likely toxic effect of long term use as is the case with compliant treatment of diabetes mellitus, which treatment is a lifetime process.

The SCE consumption by rats for a period of 90 days did not influence the increase in body weight.

Comparing the treated groups to the control the gain in body weight did not have any significant difference (p<0.05) in the increase. The increase in body weight experienced by the animals was a normal growth process owing to the fact that the experiment was started with growing young adult rats. Unlike other traditional herbal portions which have been found to cause bodyweight loss (Ezeonwumelu *et al*., 2011), SCE does not cause it. Traditional use may not be associated with weight reduction as side effects that can complicate its use. There is similarly, no significance in the drop of the increments of the percentage weight increments that was observed singly in the SCE 200 mg/kg treated group given that all the increments decline after the sixth week of the experiment. This parameter does not signify any deterrent in bodyweight increase since it is neither severe nor dose-dependent. Bodyweight loss could be caused through such mechanisms as loss of appetite, malabsorption or the presence of anti-nutritional elements, (Ilodigwe *et al*., 2010) and advanced age. Increased (organomegaly) or decreased (atrophy) organ weight (relative or absolute) has been noted as a sensitive indicator of adverse effect on organs caused by standard toxicants, (Dioka *et al*., 2002). The relative organ weights expressed as a percentage mostly appeared similar to the control values though the stomach values appeared higher at the 400 and 800 mg/kg dose that leaves a lot to be desired in the tendency for causing organomegaly at the level of the stomach. Histological analysis may have to be considered to conclude the existence of such pathology.

The considered biochemical parameters ALP, GGT, AST, creatinine and urea that did not show any statistically significant difference (p<0.05) indicate that the effect of SCE treatment may not appreciably negatively influence hepatic function as well as the renal elimination process. This fact varies with the observation of Ilodigwe *et al*., 2010 on the ethanolic extract of the leaves of *Spathodea campanulata*. Creatinine and urea are the chosen parameters that give an estimate of renal capacity to eliminate some metabolites. Non significant rise in ALP and GGT levels precisely indicates the non existence of cholestatic liver disease at the doses studied since high ALP levels are characteristic of cholestasis, (Aniagu *et al*., 2005). The ALT level that is significantly higher (p<0.05) than the control at 800 mg/kg level is one of the key liver aminotransferases and a reliable indicator of specific liver congestion or injury. Some plant extracts are observed to cause liver damage in a similar manner but the liver is such a readily regenerating organ that more often the hepatotoxicity may be reversible after some time following discontinuation of the use of the causative agent, (Ilodigwe *et al*., 2010). It should also be borne in mind that whether hepatotoxicity is temporal or long lasting will depend

on the type of phytochemicals present in the plant material or if it were contaminated by other hapatotoxic organic agents like afflatoxins.

Changes in haematological parameters have been noted as an important indicator of toxicity of medicinal plants just like other haematotoxic agents, (Dioka *et al*., 2002). The significant drop noticed in the values of haematological components such as WBC, RBC, RDW and HCT at 800 mg/kg and WBC alone at 400 mg/kg gives an indication that prolonged use of SCE may cause appreciable anaemia especially at high doses though curiously enough there was no statistically significant difference in the value of haemoglobin (HGB). A severe drop in the values of MCV, MCH and MCHC levels occurs in the condition of thallasaemia. The significant rise in MCV, MCH, MCHC levels at 800 mg/kg body weight in the treated animals compared to the control group are indicative of the fact that this extract may instead cause a reversal of the signs of thallasaemia which is an added advantage that may be exploited for therapeutic use.

Organ tissue damage is considered as an important target for xenobiotic toxicity, (Klaassen and Watkins, 2010). Organ effects resulting from exposure to xenobiotics occurs as lesions of varying category depending on the duration, concentration, route of exposure, potency among other factors. Most of the organs examined after the 90 days administration of SCE did not show signs of severe or permanent injury. The effects on the kidney, stomach, testes, lungs and liver were mild to moderate lesions which could be repaired following discontinuation of the SCE administration. The tissues could recover from such injury. However, the heart seemed to be more vulnerable to the administration of SCE considering the wide spread distribution and degree of the lesions observed on the myocardium. The observed myocardial necrosis with haemorrhage at the doses 400 and 800 mg/kg is an irreversible lesion that may have long lasting and devastating cardiac defects.

CONCLUSION

SCE is practically safe when used as a single dose treatment. Prolonged use of this extract may not have any influence on bodyweight but may cause an increase in stomach weight and injurious lesion on the myocardium. Prolonged use of this extract may also cause haematological and hepatological injury. However, these observed injuries may be avoided by intermittent use and could eventually be reversed by discontinuation.

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CONFLICT OF INTEREST

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