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Full Length Research Paper

Assessment of the potential toxic effects of *Agave sisalana* Perrine ex Engelm in rats

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Agave sisalana **is commonly used by local communities to treat mucosal inflammation and control gastro-intestinal parasites in chickens. However, information on toxicity of** ***A. sisalana*** **is limited. This study conducted acute, sub-acute and chronic tests to assess the potential toxicity of the plant on rats. The** ***A. sisalana*** **aqueous leaf extract was prepared following the procedures of the communities. The test extract was orally administered to the rats using a bulbed steel needle. For each test, twenty-five rats were randomly divided into five groups each treated as follows: Group 1 (control) received 1 mL/day of distilled water and groups 2 to 5 received 50, 100, 200 and 400 mg/kg of** ***A. sisalana*** **test extract, respectively. Mortality, behavioral and physiological changes were monitored for 72 h, 14 and 35 days in acute, sub-acute and chronic tests, respectively. Haematological, biochemical and histopathological examinations were conducted in sub-acute and chronic tests. No mortality, behavioral or physiological changes were noticed in the acute test. Mortality rate for 50 and 100 mg/kg doses of sub-acute and chronic test respectively was 20%. Chronic test (200 mg/kg) had 40% mortality rate. For sub-acute and chronic tests, 100 and 200 mg/kg doses, mean corpuscular volume was low; potassium, alanine transaminase and aspartate transaminase were high. Cardiac vascular congestion was noted for all doses. Spleens of rats on 200 and 400 mg/kg had mild siderosis. The study concluded that** ***A. sisalana*** **is potentially toxic when used continually for more than 14 days especially in doses 50, 200 and 400 mg/kg body weight.**

Key words: *Agave sisalana* leaf extract, biochemistry, haematology, histopathology, livestock health

INTRODUCTION

Preliminary findings of the research on ethno-veterinary control of livestock diseases and parasites in rural communities by Mwale and Masika (2009) indicated that *A. sisalana* (Agavaceae) was the second most effective/ potent plant, after *Aloe ferox*, in controlling gastro-intestinal (GI) parasites in village chickens. Although, resource-limited farmers in the Eastern Cape Province of South Africa claimed that the medicinal plants are effective and safe to use, *A. ferox* was potentially toxic when used orally for more than 7 consecutive days (Maphosa and Masika, 2012), however, if the plant is used in less than 7 days, it is not toxic (Wintola et al., 2011; Celestino et al., 2013). Evaluation of the potential toxic effects of *A. sisalana* is therefore important. *A. sisalana* is exotic to

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Southern Africa (van Wyk and Gericke, 2003) and is commonly known as sisal, agave or sisal hemp. The plant is originally from South America and is widely cultivated in the warmer regions of the Americas. *A. sisalana* is an aloe-like plant that is short-stemmed and often rhizomatous. It is a multipurpose plant, normally cultivated for its fibre, food and ornamental value (Santacruz-Ruvalcaba et al., 1999; Gutiérrez et al., 2008; Sharma and Varshney, 2012). The plant contains various organic compounds of which saponins are outstanding (Makkar et al., 2007).

The saponins in *A. sisalana* are secondary metabolites which are mainly steroidal glycosides that have a bitter taste and act as mucosa irritants and have surfactant properties (Suyenaga et al., 2007). In addition the plant has toxic hepatopathy, nephropathy and hemoglobinuria properties (Silveira et al., 2012) and saponins in this plant have been known to have a lytic action on erythrocyte membranes (Francis et al., 2002). This haemolytic property is envisaged to be a result of the affinity of the glycone moety for membrane sterols especially cholesterol. Hence, *A. sisalana* is increasingly becoming important as a medicinal plant for livestock and human beings (Balick *et al.,* 2000; van Wyk and Wink, 2004). Balick *et al.* (2000) and van Wyk and Wink (2004) reported that the plant is used for the treatment of mucosal inflammation and digestive disorders.

According to a survey conducted by Giday *et al.* (2003), the Zay people in Ethiopia reported that the root of *A. sisalana* is used in the treatment of blackleg in cattle. The extract of *A. sisalana* demonstrated significant action on GI parasites for sheep and goats in vitro (Silveira et al., 2012). In Centane district in the Eastern Cape province of South Africa, the plant is widely used in controlling GI parasites in village chickens and the resource-limited farmers reported that it is safe to use (Mwale and Masika, 2009). Despite the invaluable uses of this plant, information on its potential toxicity properties is lacking. Acute, sub-acute and chronic toxicity tests were therefore conducted to evaluate the potential adverse effects of the aqueous leaf extracts of *A. sisalana* using a rat model. The hypothesis tested was that the toxicity of *A. sisalana* depends on the concentration of the extracts and the period of using the extract.

MATERIALS AND METHODS

Plant material collection

Fresh leaves of *A. sisalana* were collected from Centane district in the Eastern Cape Province of South Africa (32°38'63"S and 28°24'36"E; elevation 50 m) in October 2007. The plant materials were identified for appropriate nomenclature and characterization at Selmar Schonland Herbarium, at Rhodes University, Botany Department. The voucher specimen (MMAN 2007/02) was deposited at Giffen Herbarium of the University of Fort Hare.

Preparation of Aqueous extracts

The extract was prepared based on the proportions and methods used by resource-limited farmers and herbalists. The collected leaves were washed in cold water to remove dirt. The spines around the leaves of *A. sisalana* were removed using a knife after which the leaves were sliced into small pieces The sliced material, 200 g was mixed with 200 mL of distilled water to obtain 100% (w/v) extract and blended in an electric blender for five minutes (Githiori et al., 2003). The milled material was squeezed through a muslin cloth. The filtrate was freeze-dried at -50°C under vacuum using a lyophiliser (Savant Refrigerated Vapor Trap, RVT 4104, USA) and kept in a freezer at –20°C until use. In preparation for the experiments, the freeze-dried samples were re-dissolved in water. One percentage concentration of the freeze-dried samples of *A. sisalana* was used to make 50, 100, 200 and 400 mg/kg doses.

Experimental design

Seventy-five Wistar rats, 6 to 8 weeks of age of both sexes were used. The rats were bred in the animal house at the Agricultural and Rural Development Research Institute (ARDRI), University of Fort Hare under controlled environmental conditions (ambient room temperature 25 ± 2°C, and stand light from 0600 h to 1800 h i.e. 12 h light-dark cycle). In each of the three toxicity tests conducted, acute, sub-acute and chronic 25 rats were randomly divided into five groups each consisting of five rats. The treatments consisted of 50, 100, 200 and 400 mg/kg doses of *A. sisalana* aqueous leaf extract. A completely randomized design was used in which Group 1 rats (control) were administered orally (per os) with 1 mL of distilled water by means of a bulbed steel needle, and Groups 2 to 5 received graded dose levels of 50, 100, 200 and 400 mg/kg body weight of aqueous leaf extract of *A. sisalana* respectively.

The rats were allowed free access to standard commercial rat pellets (EPOL Feeds Ltd, South Africa), except for the acute toxicity group which was fasted for 16 h prior to the administration of the test aqueous extract. Clean water was provided *ad libitum* throughout the experimental period of 72 h, 14 days and 35 days for the acute, sub-acute and chronic toxicity tests, respectively. Ethical procedures for using Wistar rats were according to the University of Fort Hare ethics committee's and international standards (Austin et al., 2004; Marie, 2006).

Acute toxicity test

The test was conducted according to the method of Sawadogo et al. (2006), where rats received a single dose of the graded dose levels of the test extract. The initial body weights of the rats were recorded. Observations were made for any physiological and behavioral changes that included feeding behavior, increased or decreased activity due to drug reaction, stress and rat mortality. The rats were observed continuously for 3 h soon after administering the extract, then hourly for 72 h.

Sub-acute toxicity test

The method of Bürger et al. (2005) was followed in which rats orally received graded dose levels of the aqueous extracts of the plant once/day for 14 consecutive days. Physiological and behavioral changes were observed as in the acute toxicity test. The rats were observed daily for 14 days. The initial body weights of the rats were recorded on day one and seven, then on the 14th day. The relative organ weight was calculated using the formula of Chavalittumrong

et al. (2004):

Relative organ weight (kg) = [organ weight (g)/animal body weight (g)] x 1000

Haematological and biochemical assays were conducted for the rats as described below.

Haematological and biochemical assays

Rats were fasted overnight for easy sacrificing and collection of blood, anaesthetized using halothane, and sacrificed at the end of the experiment. Paired blood samples, one heparinized for haematological evaluation and the other non-heparinized for serum biochemical assay, were collected from experimental rats. The haematological and serum biochemical parameters were determined using Advia 2120 (Bayer, Germany) for haematology and Beckman DXC 00 (USA) for serum chemistry, respectively. Haematological parameters assayed for included white blood cells (WBC), red blood cells (RBC) and differential leukocyte counts, red cell distribution width (RCDW), platelets, haematocrit, haemoglobin estimation, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). The serum was assayed for glucose, creatinine, blood urea nitrogen (BUN), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), calcium, magnesium, inorganic phosphorus, protein and albumin (Bürger et. al., 2005). The reference ranges for haematology and serum biochemical values were according to Boehm et al. (2007).

Histopathology

Immediately after the collection of blood samples, rats were dissected and the liver, lungs, heart, spleen and kidneys removed. The organs were weighed individually on an electronic balance (August Sauter GmbH D-7470 Albstadt-Ebingen, Switzerland) and fixed in 10% buffered formalin in labeled bottles. The preserved samples were processed using the haematoxylin and eosin technique (tissue slides were stained with haematoxylin and eosin) and macroscopically examined (x400 magnification) for organ lesions, organ hypertrophy and/or hypotrophy (Attawish et al., 2004).

Chronic toxicity test

The rats were administered orally with the test aqueous extract once daily for 35 consecutive days (Banu et al., 1997). The body weights of the rats were recorded on day one and weekly thereafter. The relative organ weight was computed as in the sub-acute toxicity test. The physiological and behavioral changes were observed daily as in the acute toxicity test. Haematological, biochemical and histopathological assays were performed for the sub-acute toxicity test.

Statistical analyses

The numeric data obtained were tested for normality using PROC UNIVARIATE of SAS (2004), and the data was normally distributed. The effect of *A. sisalana* aqueous leaf extract on rat body and organ weight, haematology and serum biochemical parameters was analyzed using PROC GLM procedure of SAS (2004). Dunnett's t-test was computed to compare the treatment means against the control. Fisher's exact test was used to conduct chi-square test to determine if there was any association between the sub-acute and chronic toxicity tests in the histopathology examination (SAS, 2004).

RESULTS

Acute toxicity test

For all the graded levels of the plant aqueous extract, there was no rat mortality ($P>0.05$) of rats during the 72 h experimental period. There were neither behavioral nor physiological changes that were noticed in the experimental rats.

Sub-acute toxicity test

The aqueous leaf extract of *A. sisalana* caused 20% rat mortality for the 50 mg/kg body weight dose level. Final body weights of rats on 100, 200 and 400 mg/kg body weight dose levels were significantly higher ($P<0.05$) than weights for the control (Table 1).

The relative rat organ weights were not significantly different from the control except for the spleen for 100 and 400 mg/kg body weight dose levels that were higher than the control, and the kidney from rats on 400 mg/kg dose that had the highest weight ($P<0.05$) (Table 1).

Haematological and biochemical assays with respect to the sub-acute test

Red blood cell count, white blood cells, haemoglobin, haematocrit, MCH, MCHC, platelets, neutrophils, monocytes, lymphocytes, large unstained cells, eosinophils and basophils were not different from the control ($P>0.05$). The values of these blood parameters were within the reference range but monocytes of rats on 50 mg/kg dose were higher than the range (Table 2). Red cell distribution width and the MCV for rats on 100 and 200 mg/kg body weight dose levels were different from the control ($P<0.05$; Table 2) but the values were within the reference range.

Histopathology with respect to the sub-acute test

Cardiac vascular congestion was noted for the 200 and 400 mg/kg dose of the sub-acute test (Figure 1a and b). As shown in Figure 1c, the kidneys of rats for the 200 mg/kg dose had pigmented tubular casts and medial hypertrophy of the arcuate arteries.

Chronic toxicity test

Rat mortality for the 100 and 200 mg/kg body weight dose levels was 20 and 40%, respectively. Also, about

Table 1. Relative body and organ weights of rats (mean±SE) orally administered with aqueous leaf extract of *Agave sisalana* for 14 and 35 days.

Organ weight (g)	Dose (mg/kg body weight)				
	Control	50	100	200	400
Sub-acute toxicity (14 days)					
Final body weight (g)	261.22±11.660[a]	292.75±13.037[a]	172.38±11.660[b]	163.48±11.660[b]	160.14±11.660[b]
Liver	36.01±1.414	40.35±1.581	37.50±1.414	40.67±1.414	31.83±1.414
Heart	3.91±0.186	3.62±0.208	4.27±0.186	3.91±0.186	4.23±0.186
Kidney	8.40±0.363[a]	9.23±0.406[a]	9.28±0.363[a]	9.30±0.363[a]	9.98±0.363[b]
Spleen	2.35±0.228[a]	2.24±0.255[a]	3.82±0.228[b]	3.06±0.228[a]	3.47±0.228[b]
Lung	7.92±0.887	6.66±0.991	7.89±0.887	7.52±0.887	9.50±0.887
Chronic toxicity (35 days)					
Final body weight (g)	187.00±9.818[a]	177.20±9.818[a]	183.78±10.977[a]	248.33±12.675[b]	248.76±9.818[b]
Liver	37.43±2.123	38.80±2.123	38.43±2.374	36.10±2.741	32.69±2.123
Heart	4.71±0.209[a]	4.16±0.209[a]	4.09±0.234[a]	3.75±0.270[b]	3.74±0.209[b]
Kidney	10.90±0.504	10.74±0.504	10.90±0.563	10.11±0.650	9.00±0.504
Spleen	2.99±0.251	3.07±0.251	2.99±0.281	2.03±0.324	2.42±0.251
Lung	7.16±1.111	7.77±1.111	7.77±1.242	10.78±1.434	6.07±1.111

[ab] Values with different superscripts in the same row are different from the control ($P<0.05$).

20% of the rats of 200 mg/kg body weight dose level showed signs of laboured breathing. The final body weight of rats on 200 and 400 mg/kg body weight dose levels were significantly higher than those of the control. As indicated in Table 1, the relative rat organ weights were not significantly different from the control except the heart of rats on 200 and 400 mg/kg body weight dose groups.

Haematological and biochemical assays with respect to the chronic test

The haematological parameters in the chronic toxicity test were not different from the control ($P>0.05$). The values were all within the reference range except for monocytes at 100, 200 and 400 mg/kg doses and lymphocytes at 50, 100 and 200 mg/kg doses.

Chlorine, phosphorylated glucose, calcium, bilirubin total and corrected calcium findings were similar to those for the sub-acute toxicity test. As indicated in Table 3, bilirubin conjugated, albumin, GGT, magnesium, potassium, urea and creatinine were within the reference range. Total protein was within the reference range and the 400 mg/kg dose had a higher value than the control.

Sodium was within the range but the 100, 200 and 400 mg/kg doses had higher values than the control (Table 3). The inorganic phosphorus of rats in the 50 mg/kg dose group was higher than the reference range. The 100 and 200 mg/kg doses induced higher ALP values than the reference range. The extract caused high ALT values for doses 200 and 400 mg/kg, and AST for 100 and 400 mg/kg.

Histopathology with respect to the chronic test

The plant did not affect the heart, kidney, oesophagus, liver and the pancreas of the rats. Mild siderosis of the spleen was noticed in rats in the control group, 50 and 100 mg/kg doses. Splenic siderosis was observed for the 50 mg/kg dose of the chronic test (Figure 1d). There was no association ($P>0.05$) between the sub-acute and the chronic toxicity tests on the adverse effects caused on rat organs.

DISCUSSION

The observation that oral administration of the aqueous leaf extract of *A. sisalana* did not cause any mortality or alter any behavioral and physiological state of rats in the acute test is an indication that the plant is not harmful at the level tested. The fact that the plant extract did not cause mortality or change in behavior of rats signifies that the plant could be safe when used for a short period of time.

These findings are crucial since resource-limited farmers use these plants for 3 to 5 days when controlling gastro-intestinal parasites in village chickens. According to Francis et al. (2002) and Ribeiro et al. (2013), presence of saponins in the leaves of *A. sisalana* necessitates the plant to have the anti-helminthic properties.

Table 2. Sub-acute toxicity haematological values (mean±SE) for rats treated with aqueous extract of *Agave sisalana*.

Haematological parameter	Control	*Agave sisalana* dose levels (mg/kg body weight)				Normal range
		50	100	200	400	
WBC (x10^9/L)	9.78±1.355	9.50±1.750	7.55±1.355	4.53±1.355	6.00±1.355	4-10
RBC (x10^{12}/L)	4.99±0.145	5.1±0.187	4.70±0.145	4.75±0.145	5.02±0.145	4.5-5.5
Haemoglobin (g/dl)	15.06±0.236	15.63±0.305	15.12±0.236	15.16±0.236	15.64±0.236	13-17
Haematocrit (L/L or %)	0.49±0.007	0.51±0.009	0.49±0.007	0.50±0.007	0.50±0.007	0.4-0.5
MCV (fL)	82.16±0.729[a]	84.67±0.941[a]	85.14±0.729[b]	85.30±0.729[b]	84.00±0.729[a]	79.1-98.8
MCH (pg)	27.76±0.240	28.20±0.310	28.44±0.240	28.36±0.240	28.36±0.240	27-32
MCHC (g/dl)	34.06±0.233	33.47±0.300	33.64±0.233	33.44±0.233	34.12±0.233	32-36
RCDW (%)	12.76±0.290[a]	11.87±0.375[a]	11.20±0.290[b]	11.26±0.290[b]	11.78±0.290[a]	11.6-14.0
Platelets (×10^9/L)	368.00±47.403	239.67±61.198	225.80±47.403	273.40±47.403	232.80±47.403	137-373
Neutrophils ((x10^9/L)	4.34±0.126	4.51±0.162	4.44±0.126	4.63±0.126	4.37±0.126	2-7.5
Monocytes (x10^9/L)	0.64±0.357	1.74±0.461	0.36±0.357	0.66±0.357	0.22±0.357	0.18-0.8
Lymphocytes (x10^9/L)	4.00±1.050	3.39±1.356	3.40±1.050	1.21±1.050	2.22±1.050	1.00-4.00
LUC (x10^9/l)	0.67±0.126	1.10±0.163	0.62±0.126	0.52±0.126	0.54±0.126	-
Eosinophils (x10^9/L)	0.10±0.020	0.10±0.025	0.06±0.020	0.09±0.020	0.07±0.020	0.00-0.45
Basophils (x10^9/L)	0.03±0.006	0.03±0.008	0.02±0.006	0.02±0.006	0.01±0.006	0.00-0.2

[ab] Values with different superscripts in the same row are different from the control ($P<0.05$).

Saponins are in the form of triterpenic or steroidal glycosides, majority of which are in *A. sisalana,* like in most herbal plants, are steroidal saponins used also in the insecticides and synthesis of steroidal drugs in pharmaceutical industry as they have health promoting properties (Francis et al., 2002; Yokosuka and Mimaki, 2009; Ribeiro et al., 2013). As the plant was not harmful when used once, that is a single dose, this may justify why resource-limited farmers report that the plant is effective and non-toxic when used in the treatment of other human and veterinary conditions, because the farmers use the plant for a short period of time (Kaido et al., 1997; van Wyk and Gericke 2003). This is in agreement with Yokosuka and Mimaki (2009) who averred that some saponins such as the furostanol (11-15) and the saponins 2, 4, 5, 8 and 9 were not cytotoxic to HL-60 human promylelocytic leukemia cells, while others were moderately toxic. Given that the plant was not toxic under the acute toxicity test, it was important to determine its toxicity effects over a longer time period.

Mortality and behavioral changes were noticed both in the sub-acute and chronic tests suggesting the potential toxicity of the plant if used consecutively for a period of 14 days or more. Mortality was noticed in the 50 mg/kg dose and not higher dose, despite the fact that the weight was not altered and that more adverse effects were expected at higher dose levels. This could be that the concentration of the 50 mg/kg dose was not good enough to trigger weight changes and altering organs such as the liver and kidney in order to fight against the toxic substances.

In addition, since the crude aqueous extract of the plant were used, the stock solution that was used to reconstitute the 50 mg/kg body weight dose could have more toxic steroidal saponins since negative effects were noticed for the same dose both in the sub-acute and the chronic toxicity tests. According to Chen et al. (2011), the steroidal saponin 10 was the most cytotoxic when tested on cancer cells, thus it could be said that it was in high quantities in the 50 mg/kg dose compared to other steroidal saponins. Determination of the type of saponins in the aqueous leaf extract of *A. sisalana* is critical before toxicity studies are undertaken so as to determine their composition in the extract and hence their effects. Steroidal saponins could have

Table 3. Biochemical values (mean±SE) of rats treated with aqueous extract of *Agave sisalana* in a chronic toxicity experiment.

Haematological parameter	Control	*Agave sisalana* dose levels (mg/kg body weight)				Normal range
		50	100	200	400	
LFT						
Bilirubin total µM/L	5.80±1.029	6.00±1.029	4.75±1.151	7.00±1.627	6.60±1.029	0-21
Bilirubin conjugated µM/L	3.00±0.656	3.40±0.656	2.00±0.733	3.00±1.037	3.40±0.656	0-6
Bilirubin unconjugated µm/L	2.80±0.376[a]	2.60±0.376[a]	2.75±0.418[a]	7.00±0.590[b]	3.20±0.376[a]	0-15
Total protein (g/L)	61.00±0.774[a]	60.40±0.774[a]	61.50±0.865[a]	65.50±1.223[b]	63.40±0.774[b]	60-85
Albumin (g/L)	37.80±0.345	27.40±0.345	27.50±0.385	27.50±0.545	28.00±0.345	35-52
Globulin (g/L)	23.20±0.429[a]	33.00±0.429[b]	34.00±0.480[b]	38.00±0.678[b]	35.40±0.429[b]	35-52
ALP U/L	70.80±13.753[a]	68.80±13.753[a]	126.50±15.377[b]	152.50±21.746[b]	72.40±13.753[a]	40-120
GGT U/L	11.6±0.917	11.40±0.917	11.00±1.025	10.00±1.449	10.20±0.917	0-60
ALT U/L	33.80±3.307	41.00±3.307	38.25±3.697	47.50±5.229	40.20±3.307	5-40
AST U/L	22.40±26.817	6.80±26.817	0.09.75±29.982	49.00±42.402	0.06.00±26.817	5-40
Chemistry test						
P-Glucose (random) (mmol/L)	5.66±0.839	6.74±0.839	6.00±0.938	5.15±1.327	4.54±0.839	4.1-11.1
Calcium (mmol/L)	2.39±0.022	2.38±0.022	2.44±0.024	2.50±0.034	2.42±0.022	2.05-2.56
Corrected calcium (mmol/L)	2.34±0.023	2.33±0.023	2.39±0.026	2.45±0.037	2.36±0.023	2.05-2.56
Magnesium (mmol/L)	1.11±0.099	1.02±0.099	1.12±0.111	1.23±0.157	1.27±0.099	0.65-1.10
P-inorganic (mmol/L)	1.06±0.229	1.92±0.229	1.00±0.256	1.50±0.362	1.36±0.229	0.8-1.4
UEC						
Sodium (mmol/L)	139.20±0.415[a]	138.60±0.415[a]	140.50±0.464[b]	142.00±0.657[b]	140.20±0.415[b]	135-147
Potassium (mmol/L)	4.68±0.472	3.54±0.472	3.50±0.527	5.00±0.746	3.98±0.472	3.3-5.3

[ab] Values with different superscripts in the same row are different from the control ($P<0.05$).

seriously affected the membranes of the rats as they have an ability to irritate the mucosa and interact with the membrane sterols leading to toxicity, reduced feed conversion efficiency and hence growth retardation (Francis et al., 2002). This is supported by Flåøyen et al. (2002) and Silveira et al. (2012) who affirmed that toxicity depends on the quantity of the ingested saponins as well as the nature of the active compounds involved. The findings show how crucial it is to undertake studies that focus on determining the potential toxicity of *A. sisalana*. Studies have not been focused on medicinal use and potential toxic effects of *A. sisalana* mainly because the plant has been used more for fibre, beverages (mescal and tequila liquors) and ornamental values (van Wyk and Wink, 2004) than medicinal purpose. Few surveys have however, indicated the utilization of the plant for the treatment of uterine fibroids in women in New York (Balick et al., 2000) and blackleg in cattle in Ethiopia (Giday et al., 2003). Sharma and Varshney (2012) reported that the juice of the leaves lowers blood pressure in dogs and stimulates their intestinal movements. In addition, the authors reiterated that the plant may also be used as an abortifacient as it activates uterine motility. Silveira et al. (2012) asserted that

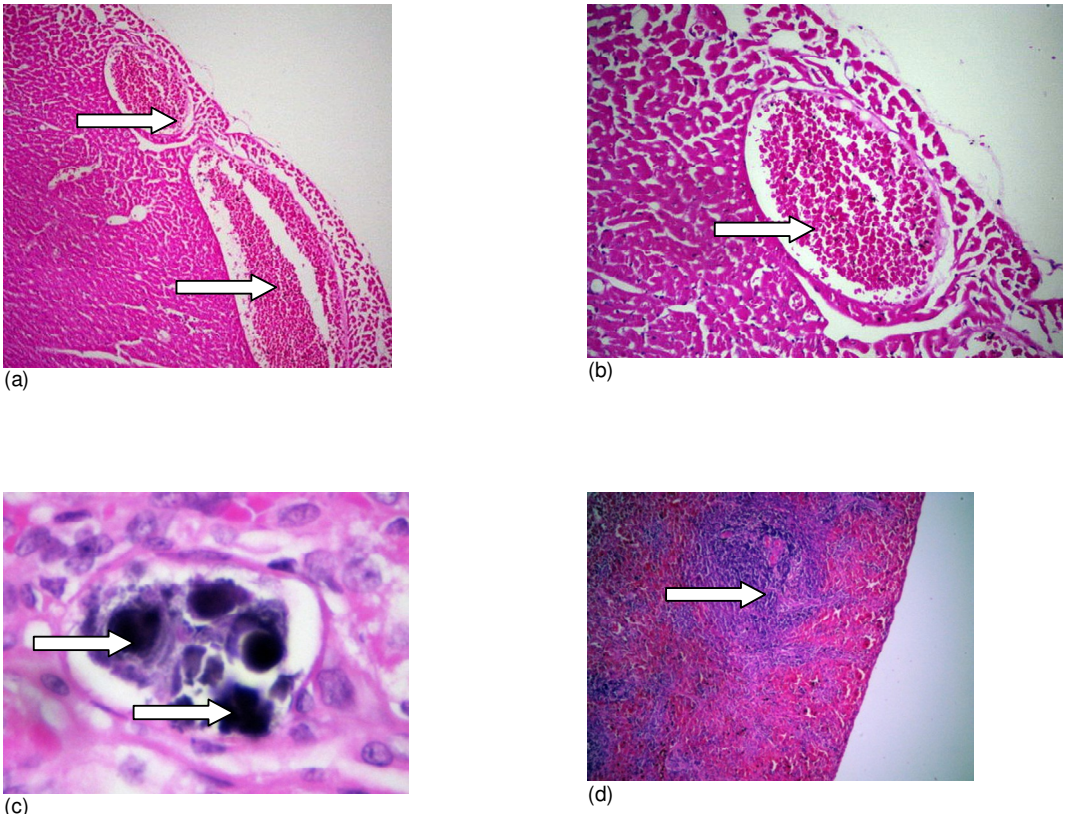

Figure 1. Effect of *Agave sisalana* on the heart, kidney and spleen of rats. (a) Cardiac vascular congestion (50x) - 200 mg/kg dose of the sub-acute test; (b) Cardiac vascular congestion (100x)-400 mg/kg dose of the sub-acute test; (c) Pigmented renal concretions (400x) -200 mg/kg dose of the sub-acute test; (d) Splenic siderosis (100x)-50 mg/kg dose of the chronic test.

A. sisalana extract inhibited egg hatching of the sheep gastro-intestinal nematodes demonstrating the anti-parasitic characteristics of the plant leaf extract.

Similar findings for haematology and serum biochemical parameters were obtained for both the sub-acute and the chronic toxicity tests signifying that the duration of exposure possibly did not have an adverse effect on blood and serum parameters. The findings of the current study show that *A. sisalana* caused an increase in organ weights and that there was a trend of affecting the body and organ weights with increase in the dose levels. Although, there is a strong correlation between the body weight and organ weight (Brown et al., 1926), the body weight differed from the control but not in the organs except the spleen. The obtained findings agree with the fact that some organs like the heart and the lung have an intermediate correlation with the body weight while the liver has low correlation (Yang et al., 1999). The relationship between organs and body weight entails that when the body weight varies as in the current study, the organs may not be influenced much leading to an insignificant difference. However, the observations that organ weight statistical differences were noted only for the spleen at 100 and 400 mg/kg, and the kidney at 400 mg/kg body weight warrants further investigation as more adverse effects on organs were expected at higher dose levels. Rat mortality was noted in the 100 and 200 mg/kg body weight and this could be due to the detergent effect of saponins as well as the damage to the membranes through peroxidation (Silveira et al., 2012). This is in agreement with Kayumba et al. (2008) who affirmed that long time exposure of workers in a sisal processing plant is associated with an increased risk of immunoglobulin E sensitization due to aero-allergens, as the tested workers had elevated serum immunoglobulin E levels.

Elevated monocytes and lymphocytes indicate an ongoing inflammation of particular organs of the rats (Haukeland et al., 2006). Monocytes are in the category of leukocytes and are responsible for regulating immunity against antigens in response to inflammation as well as replenishing damaged tissues. In the current study, these parameters were elevated probably to curb the inflammation of the heart and the kidney that was noticed in histopathology examination. Saponins in *A. sisalana* can lead to necrosis and red cell regeneration as they are

haemolytic and also can cause toxic hepatopathy, nephropathy and haemoglobinuria (Silveira et al., 2012). Hence these effects would elicit an increase in monocytes in the blood (monocytosis) in response to stress. Elevation of the transaminases, AST and ALT is attributed to the inflammation and/or injury to liver cells, a condition known as hepatocellular liver injury (Johnston, 1999; Palmer, 2004). This is because the steroidal saponins found in *A. sisalana* extract could have caused transient damage to liver cells resulting in the leakage of the transaminases into the bloodstream. High levels of ALP are a sign of possible blockage or injury to the bile ducts leading to inflammation of the bile ducts and cholestasis condition that is characterized by failure of bile flow (Palmer, 2004; Lin et al., 2008). This in turn causes the overflow of ALP out of the liver into the bloodstream.

Histopathology findings reveal the potential toxicity of *A. sisalana*, although the observed siderosis of the spleen was mild. Splenic siderosis which is also supported by the inflammation of the spleen of rats under the 100 and 400 mg/kg dose could be ascribed to excess iron absorption, usually dietary (Turner et al., 2006). Iron could also be provided by the extract leading to its accumulation in the spleen and hence inflammation of the spleen (Papakonstantinou et al., 2009). On the other hand, saponins in the plant are believed to damage the epithelial mucosa particularly the villi thereby obstructing the absorption of dietary micronutrients such as iron, and interfere with the absorption also of Vitamin A and E (Francis et al., 2002). The same authors asserted that saponins can increase the excretion of iron and magnesium thereby adversely affecting the function of the liver. This could explain why splenic siderosis was also observed for the 50 mg/kg dose of the chronic test. However, rats in the 200 mg/kg dose managed to withstand the proposed effect. It is, therefore imperative to conduct serological studies in order to determine the level of iron present in the test animal. In addition, chemical characterization of the aqueous extract *A. sisalana* will assist in verifying the actual types of saponins present as an array of saponins exit in the plant (Kanyumba et al., 2008; Yokosuka and Mimaki, 2009; Silveira et al., 2012) as well as any other potentially toxic substances.

Elevated urea and inorganic phosphorus indicate the malfunctioning of the kidney (Vorvick and Zieve, 2009). Tubular casts and medial hypertrophy of renal arteries were noticed in the 200 mg/kg dose of the sub-acute test indicating that the plant adversely affected the kidneys and hence filtration of minerals such as potassium and magnesium which were high in the blood. Turner et al. (2006) reported that high levels of potassium could be very dangerous as they can cause serious heart rhythm abnormalities, including cardiac arrest, and thus this could have precipitated the congestion of cardiac vessels of rats under the 200 and 400 mg/kg dose of the sub-acute test. Furthermore, renal tubular casts were lamellated and pigmented, showing features suggestive of mineral deposits especially calcium and iron probably due to increased calcium secretion or hypercalcaemia. On another note, high levels of potassium in the animal blood system may be due to feed, hence the source of excess potassium could be from the feed that the rats were provided with and/or from the plant extract.

Creatinine and potassium were however, within the reference ranges in the chronic test and were not affected in both the sub-acute and chronic toxicity tests. Nevertheless, the findings show that the plant is potentially toxic if used for 14 days (sub-acute toxicity test). This is supported by Love and Coverdale (1994) who reported that this likely appears when consumed in sufficient quantities, *A. americana* can cause poisoning in cattle and other animals. The authors reported that the toxicity manifested itself presumably as a myopathy and mainly affected the hind limbs; but most affected cattle seemed to recover from this effect. However, the real cause is unknown and this warrants further investigation.

The aqueous leaf extract of *A. sisalana* did not cause mortality, behavioral or physiological changes of rats under the acute test. However, mortality was observed in the sub-acute and chronic tests. Some haematological and serum biochemical parameters were altered in both the sub-acute and chronic toxicity tests. Few organ abnormalities were also observed in both the sub-acute and the chronic toxicity test. The aqueous leaf extract of *A. sisalana* is potentially toxic if used consecutively for more than 14 days. The studied plant should, therefore, not be used continuously for 14 consecutive days at the doses 50, 200 and 400 mg/kg body weight as it could damage the organs such as the heart, spleen and kidneys.

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