Scientific Research and Essay Vol.7 (15), pp. 1605-1613, 23 April, 2012
Available online at http://www.academicjournals.org/SRE
DOI: 10.5897/SRE09.334
ISSN 1992-2248 ©2012 Academic Journals

Full Length Research Paper

Toxicological studies on the leaf extract of *Aloe ferox* Mill. (Aloaceae)

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Accepted 5 October, 2009

***Aloe ferox* is renowned for, amongst others, its antihelminthic properties in livestock. However, there is dearth of information on toxicity of *A. ferox* in livestock. Therefore, toxicity effects of *A. ferox* were evaluated through acute, sub-acute and chronic toxicity tests. For each test, twenty-five rats were randomly grouped into five groups of five rats. Group 1 (control) was orally administered with 1 ml/day of distilled water and groups 2 - 5 received graded levels, 50, 100, 200 and 400 mg/kg of *A. ferox* aqueous leaf extract respectively, Mortality, behavioural and physiological changes were monitored for 72 h, 14 and 35 days in acute, sub-acute and chronic toxicity tests respectively, Relative organ weight was noted. Haematological, biochemical and macroscopic examinations, were conducted for sub-acute and chronic tests. No mortalities, behavioural or physiological changes were observed in all the toxicity tests ($p > 0.05$). Red blood cells, mean corpuscular haemoglobin, platelets and monocytes were elevated while neutrophils were lower than the reference range. Heart, lungs and kidneys were fairly enlarged; spermatogenic cells degenerated. Therefore, *A. ferox* is relatively non-toxic if used cautiously. Future work will focus on evaluating pharmacological properties of *A. ferox* aqueous leaf extract in order to authenticate its anti-helminthic use in livestock.**

Key words: *Aloe ferox*, aqueous extract, full blood count, histopathology, mortality, toxicology.

INTRODUCTION

Aloe ferox Mill., a spiky, succulent plant, is commonly known as bitter aloe or red aloe (English) (Reynolds, 1982). It is a tall, single stemmed (caulescent) plant that is indigenous to South Africa and has a wide distribution. It occurs in grassy fynbos in the south Western Cape and on the edges of the Karoo in the Eastern Cape Provinces of South Africa (Reynolds, 1982). It is one of the several plants that have been part of the traditional healing practices of South African people for centuries (van Wyk et al., 2002). Today the plant is renowned world-wide for its superior antiseptic, cleansing, moisturising and anti-inflammatory properties; thus, the healing properties stretch from the treatment of arthritis, skin cancer, burns, cuts and wounds, eczema, psoriasis, digestive and blood pressure problems (Van Wyk et al., 2002).

In spite of the importance of *A. ferox*, Aloes have been reported to cause hypertension (Morrow et al., 1980) renal tubule pigmentation and nephropathy (National Toxicology program, 2001) hyperplasia of the kidneys and testis (Zhou et al., 2003). Boudreau and Beland (2006) reported in their review that ingestion of *Aloes* is associated with diarrhoea, electrolyte imbalance, kidney dysfunction and conventional drug interactions. Most of the studies on *A. ferox* have, however, been centred on its healing

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Abbreviations: WBC, White blood cell; **RBC,** red blood cell; **RCDW,** red cell distribution width; **MCV,** mean cell volume; **MCH,** mean corpuscular haemoglobin; **MCHC,** mean corpuscular haemoglobin concentration; **AST,** aspartate transaminase; **ALT,** alanine transaminase; **ALP,** alkaline phosphatase; **GGT,** gamma-glutamyl transferase; **GLM,** general linear model; **ANOVA,** analysis of variance; **SAS,** statistical analysis system.

properties for humans (Kambizi et al., 2007; Steenkamp and Stewart, 2007; Viljoen, 2008; Kambizi and Afolayan, 2008). However, the healing properties of this plant are also crucial in animal production. In India, Aloe is used as an antihelminthic in livestock (Oronzo-Barocio et al., 1999, cited by Steenkamp and Stewart, 2007). Farmers in the Eastern Cape Province alleged that *A. ferox* is immensely useful in the control of gastro-intestinal parasites in village chickens (Dold and Cocks, 2001; Mwale and Masika, 2009), treatment of helmithiasis in livestock (Masika and Afolayan, 2003) and control of helminths in goats (Maphosa and Masika, 2010).

The leaf of *A. ferox* is crushed, mixed with cold water and this water is given to chickens of all age groups to drink, or leaves are crushed and left to stand in cold water to make an infusion for other livestock species. The safety of *Aloe* gel, latex and isolated compounds has been evaluated (Steenkamp and Stewart, 2007). Albert, the whole leaf contains compounds found in both the latex and gel, information on the safety of the fresh whole leaf of *A. ferox* to encompass all the compounds of the leaf of this plant is scanty. Therefore, an animal model experiment was conducted to determine the toxicity effects of *A. ferox* aqueous leaf extract on behavioural, physiological, haematological and histopathological changes of rats.

MATERIALS AND METHODS

Plant material collection

Fresh *A. ferox* leaves were collected from Centane district (32°38'63"S and 28°24'36"E; elevation 50 m) in the Eastern Cape Province of South Africa in October, 2007. The material was identified at Selmar Schonland Herbarium, Rhodes University Botany Department and a voucher specimen (MMAN, 2007/01) deposited at the University of Fort Hare Herbarium.

Aqueous extraction

The time period between plant collection and extract preparation was within 36 h. The collected leaves were washed in cold water to remove dirt. The spines around the leaves were removed using a knife after which the leaves were sliced. The sliced material, 200 g, was mixed with 100 ml of distilled water for easy crushing in an electric blender for 3 min (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. One percentage concentration was used to reconstitute the extract (re-suspended or re-dissolved in water to make stock solution; *A. ferox* aqueous extract) based on the proportions and methods used by resource-limited farmers and herbalists.

Animals and experimental design

Seventy-five Wistar rats, 6 - 8 weeks of age, of either sex 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 - 1800 h, that is, 12 h light-dark cycle). In each of the three toxicity tests conducted, acute, sub-acute and chronic, 25 rats were randomly grouped into five groups of five rats each. A completely randomised design was used in which group 1 (control) was orally administered with 1 ml of distilled water by means of a bulbed steel needle and groups 2 - 5 received graded dose levels; 50, 100, 200 and 400 mg/kg body weight of aqueous leaf extract of *A. ferox*, respectively.

The rats were allowed free access to standard commercial rat pellets (EPOL Feeds Ltd, South Africa), except for the acute toxicity where they were 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. (2005), where rats received a single dose of the graded dose levels of the test extract. Rats in group 1 (negative control) were administered 1 ml of distilled water *per os* and those in groups 2 - 5 were given 1 ml/kg body weight of 50, 100, 200 and 400 mg/kg doses, respectively. The initial body weights of the rats were recorded. The observations were made for any physiological and behavioural changes that include feeding behaviour, 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 extract of *A. ferox* once/day as explained in the acute test, for 14 consecutive days. The physiological and behavioural 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 on day 7 and 14 thereafter. The relative organ weight was calculated using the formula according to Chavalittumrong et al. (2004):

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

Haematological and biochemical assays

Rats were fasted overnight, anaesthetized using Halothane and sacrificed at the end of the experiment. Paired blood samples; one for heparinised tube for haematological evaluation and another for non-heparinised tube 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 Cell (WBC), Red Blood Cell (RBC) and differential leukocyte counts, Red Cell Distribution Width (RCDW), platelets, haematocrit,

haemoglobin estimation, Mean Cell Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC). The serum was assayed for glucose, creatinine, blood urea nitrogen, Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), Gamma-glutamyl Transferase (GGT), calcium, magnesium, inorganic phosphorus, protein and albumin (Bürger et al., 2005).

Histopathology

Immediately after collection of blood samples, rats were dissected and the liver, lungs, heart, spleen, kidneys and ovaries/testes were 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 labelled 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 haemorrhagic organ lesions, organ hypertrophy and/or hypotrophy.

Chronic toxicity test

The rats were orally administered with the test aqueous extract once daily for 35 consecutive days, according to Banu et al. (1997). The administration procedure is as described under the acute toxicity test. The initial 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 behavioural changes were observed daily as in the acute toxicity test. Haematological, biochemical and histopathological assays were performed as for the sub-acute toxicity test.

Data analyses

The obtained numeric data was tested for normality using the General Linear Model (GLM) Procedure of the Statistical Analysis System (SAS, 2004). Thereafter, the data was subjected to the Analysis of Variance (ANOVA) since it was normal and Dunnett's t test was computed to compare the treatment means against the control (SAS, 2004). Fisher's exact test was used to conduct chi-square test to determine if there was any relationship between the sub-acute and chronic toxicity tests in the histopathology examination (SAS, 2004).

RESULTS

Rat mortality and deviation from the norm

The aqueous extract of fresh leaves of *A. ferox* did not cause mortality of rats in the acute, sub-acute and chronic toxicity tests. For all the graded levels of the extract under the toxicity tests, there were neither behavioural nor physiological changes that were noticed in the experimental rats.

Body and organ weights

As indicated in Table 1, the final body weights of rats on 100 and 200 mg/kg body weight dose levels for the sub-acute test were not significantly different from the control. However, final body weights of rats on 50 and 400 mg/kg body weight dose levels were different from the control ($P > 0.05$), with those of rats under 50 mg/kg body weight having the highest body weights. For the chronic test the final body weights of rats were not different from the control ($P > 0.05$); except for the rats on 400 mg/kg body weight dose level which had lowest body weights (Table 1). The relative organ weights for rats under the sub-acute test were not different from the control, except for the liver for 50, 200 and 400 mg/kg doses, while for the chronic test the relative organ weights of all experimental rats were not different from the control ($P > 0.05$; Table 1).

Haematological and biochemical assays

For both the sub-acute and chronic toxicity tests, white blood cell count, Haemoglobin, lymphocytes, large unstained cells, eosinophils, basophils, Red Blood Cell count (RBC), neutrophils and monocytes of all the rats were not different from the control ($P > 0.05$). Platelets, MCV and MCH for sub-c-acute test were not significantly different from the control while for the chronic their values for the 400 mg/kg dose were different from the control. However, as shown in Tables 2 and 3; platelets and RBC values for both tests were higher than the reference range, MCV, MCH and neutrophils were lower than the reference range. For both toxicity tests, the red cell distribution width of rats was not different from the control ($P > 0.05$; Table 2) except for rats on 50 mg/kg dose under the sub-acute test. The MCHC values were low and marginally out of range for both tests. The 50 mg/kg dose was different from the control and had the highest value for sub-acute test.

Bilirubin total for rats under the 400 mg/kg dose was significantly different from the control for both tests. The parameter values were within range except for the 400 mg/kg dose of chronic test which was high. For the sub-acute test, bilirubin conjugated, albumin, AST, GGT, ALT parameters were not significantly different from the control. The values for AST and ALT were high and out of the reference range (Table 4). Albumin values were lower than the reference range. As indicated in Table 4; total protein for all experimental rats was not significantly different from the control and ALP was different from the control ($P < 0.05$) for the 200 and 400 mg/kg body weight dose Albumin values were almost into the range and ALP values were high and out of range.

Under the chronic test the majority of the serum biochemical parameters were within the range save for ALT, AST that were higher than the reference range; while calcium corrected and magnesium were slightly higher than the range (Table 5). The majority of the

Table 1. Relative organ and body weights of rats (±SE) orally administered with aqueous extract of *A. ferox* in sub-acute and chronic toxicity tests

Organ weight (g)	Dose (mg/kg body weight)					Standard error
	Control	50	100	200	400	
Sub-acute toxicity test						
Final body weight	116.60[a]	140.74[b]	115.02	125.66	138.10[b]	5.571
Liver	49.25[a]	41.13[b]	49.42	38.21[b]	37.32[b]	1.594
Heart	4.56	4.04	4.00	4.29	4.43	0.254
Kidney	10.12	9.34	9.58	10.99	9.77	0.715
Spleen	3.23	2.85	2.99	2.71	2.91	0.176
Lung	11.27	8.35	7.23	9.23	8.73	1.145
Chronic toxicity test						
Final body weight	230.78[a]	235.14	242.76	243.92	172.18[b]	9.371
Liver	33.79	32.30	34.12	30.36	32.88	1.426
Heart	4.26	4.00	4.00	3.86	4.29	0.214
Kidney	8.95	8.97	8.07	5.89	5.59	0.692
Spleen	2.60	2.23	2.33	2.31	2.79	0.206
Lung	6.39	6.14	6.35	6.11	6.87	0.629

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

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

Haematological Parameters	Control	*Aloe ferox* dose levels (mg/kg body weight)				Normal range
		50	100	200	400	
WBC ($x10^9$/l)	5.86±2.077	9.13±1.468	9.24±1.313	8.44±1.313	10.08±1.313	4-10
RBC ($x10^{12}$/l)	7.31±0.226	7.59±0.160	7.83±0.143	7.44±0.143	7.79±0.143	4.5-5.5
Haemoglobin (g/dl)	13.40±0.349	13.13±0.246	13.90±0.220	13.78±0.220	14.44±0.220	13-17
Haematocrit (l/l or %)	0.46±0.012[a]	0.47±0.008	0.48±0.007	0.48±0.007	0.49±0.007[b]	0.4-0.5
MCV (fl)	62.40±1.122	61.93±0.793	61.24±0.710	64.30±0.710	63.34±0.710	79.1-98.8
MCH (pg)	18.35±0.535	17.28±0.378	17.78±0.338	18.56±0.338	18.54±0.338	27-32
MCHC (g/dl)	29.40±0.420[a]	27.85±0.297[b]	28.90±0.266	28.86±0.266	29.26±0.266	32-36
RCDW (%)	11.00±0.942[a]	15.00±0.666[b]	12.42±0.596	13.22±0.596	12.48±0.596	11.6-14.0
Platelets ($x10^9$/l)	1002.50±109.454	994.25±77.396	993.80±69.225	838.00±69.225	1059.40±69.225	137-373
Neutrophils (($x10^9$/l)	0.14±0.152	0.23±0.108	0.38±0.096	0.23±0.096	0.40±0.096	2-7.5
Monocytes ($x10^9$/l)	1.37±1.326	2.86±0.938	3.58±0.839	2.54±0.839	4.27±0.839	0.18-0.8
Lymphocytes ($x10^9$/l)	3.78±1.187	4.51±0.839	3.67±0.751	4.35±0.751	3.56±0.751	1.00-4.00
LUC ($x10^9$/l)	0.50±0.447	1.38±0.316	1.48±0.283	1.20±0.283	1.74±0.283	
Eosinophils ($x10^9$/l)	0.05±0.017	0.09±0.012	0.09±0.011	0.09±0.011	0.07±0.011	0.00-0.45
Basophils ($x10^9$/l)	0.01±0.017	0.04±0.012	0.04±0.011	0.04±0.011	0.04±0.011	0.00-0.2

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

biochemical parameters were out of range for the sub-acute test. Bilirubin total, bilirubin conjugated, ALP, GGT, ALT and potassium were different from the control ($P < 0.05$).

The chemistry values (P-glucose, calcium, corrected calcium, inorganic phosphorus and magnesium) of the rats on all dose levels for both toxicity tests were not different ($P > 0.05$) from the control except for phosphorylated glucose (200 and 400 mg/kg doses) and inorganic phosphorus (50, 200 and 400 mg/kg doses) for the sub-acute test (Table 4).

For the UEC (Urea, Electrolytes and Creatinine) examination, sodium, potassium and urea of all the tested rats were not different from the control ($P > 0.05$) in the sub-acute test (Table 4) and in the chronic test only magnesium (100, 200 and 400 mg/kg doses) was differ-

Table 3. Haematological values (±SE) of rats treated with aqueous extract of *A. ferox* in a chronic toxicity experiment

Haematological Parameters	Control	*Aloe ferox* dose levels (mg/kg body weight)				Normal range
		50	100	200	400	
WBC (x10^9/l)	7.52±1.274	6.68±1.425	6.97±1.274	6.08±1.274	3.83±1.274	4-10
RBC (x10^{12}/l)	9.13±0.150	9.32±0.168	9.10±0.150	9.22±0.150	9.30±0.150	4.5-5.5
Haemoglobin (g/dl)	16.60±0.254	16.55±0.284	16.24±0.254	16.62±0.254	16.34±0.254	13-17
Haematocrit (l/l or %)	0.55±0.008	0.56±0.010	0.54±0.008	0.55±0.008	0.54±0.008	0.4-0.5
MCV (fl)	59.84±0.402[a]	59.45±0.450	58.92±0.402	59.40±0.402	57.94±0.402[b]	79.1-98.9
MCH (pg)	18.16±0.138[a]	17.75±0.154	17.86±0.138	18.04±0.138	17.58±0.138[b]	27-32
MCHC (g/dl)	30.36±0.155	29.88±0.173	30.30±0.155	30.36±0.155	30.34±0.155	32-36
RCDW (%)	10.98±0.193	11.60±0.216	11.10±0.193	10.78±0.193	10.60±0.193	11.6-14.0
Platelets (x10^9/l)	999.80±50.777[a]	1058.50±56.770	937.80±50.777	1149.60±50.777	1205.80±50.777[b]	137-373
Neutrophils ((x10^9/l)	0.47±0.103	0.43±0.115	0.60±0.103	0.27±0.103	0.33±0.103	2-7.5
Monocytes (x10^9/l)	2.65±0.576	2.27±0.644	2.13±0.576	2.26±0.576	1.65±0.576	0.18-0.8
Lymphocytes (x10^9/l)	2.86±0.860	3.01±0.961	3.40±0.860	3.01±0.860	1.39±0.860	1.00-4.00
LUC (x10^9/l)	1.46±0.274[a]	0.83±0.306	0.70±0.274	0.46±0.274	0.38±0.274[b]	
Eosinophils (x10^9/l)	0.08±0.019	0.09±0.021	0.12±0.019	0.06±0.019	0.07±0.019	0.00-0.45
Basophils (x10^9/l)	0.03±0.008	0.04±0.009	0.03±0.008	0.02±0.008	0.01±0.008	0.00-0.2

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

rent ($P < 0.05$) from the control (Table 5).

Histopathology

For the sub-acute test, the liver of rats on dose level 200 mg/kg body weight (Figure 1), and hearts of rats on the control, 50 and 200 mg/kg body weight dose levels were mildly affected. The hearts were showing signs of mild systemic hypertension. As illustrated in Figure 2, the lungs of rats for all the dose levels were showing signs of eccentric, peripheral and central hyperplasia. The kidneys showed evidence of arteriolar and medial hyperplasia. The testes of the rats on 200 mg/kg body weight dose level showed evidence of the formation of giant cells in the lumen of the seminiferous tubules. The effect of *A. ferox* on rat organs was not dose dependent except for the liver.

For the chronic test hypertrophy of large calibre arteries of lungs for rats under the control experiment was observed. The heart and kidney were affected for the entire dose levels save for the 50 mg/kg body weight and the control. The affected hearts revealed systemic hypertension with cardiac ventricular hypertrophy of the left ventricle and kidneys showed renal arterial and arteriolar medial hyperplasia. The liver of rats on 50 mg/kg body weight dose level showed evidence of acute inflammation of the bile duct and infiltration of portal tracts by polymorphonucleocytes, that is, the inflammation of the hepatic portal tract. The testis of the rats on 50 and 200 mg/kg body weight were affected. Giant cells were formed in the lumen of the seminiferous tubules and the spermatogenic cells degenerated. The effect of *A. ferox* on rat organs was not dose dependent except for the heart. There was no difference in the effect on rat organ between the sub-acute and chronic toxicity text ($P > 0.05$) except for the 200 mg/kg dose ($\chi^2 = 4.398$; $P < 0.05$).

DISCUSSION

The observation that oral administration of the aqueous extract of *A. ferox* did not cause any mortality or alter any behavioural and physiological state of rats in the acute, sub-acute and chronic toxicity tests indicates that the plant is not harmful at the level tested. This supports the reason why the plant is used widely as a therapeutic remedy both topically and orally and in consumer products (Viljoen, 2008).

Variation in the final body weight of the rats under the sub-acute toxicity could be attributed to the presence of anti-nutrients such as polyphynols and phytosterols (Du Toit et al., 2007; Venu, 2007) leading to reduced feed utilisation. However, gaining of weight by some rats could be ascribed to nutritive compounds of this plant as reported by Venu (2007) that *A. ferox* has higher nutrient concentrations than the popular *Aloe vera* plant. The decrease in the weight of the Liver could indicate that the plant has some deleterious effects on the rat organ.

In both the sub-acute and chronic toxicity tests most parameters for the full blood count examination did not differ from the control signifying that the plant does not negatively influence most haematological parameters but

Table 4. Sub-acute toxicity biochemical values (±SE) for rats treated with aqueous extract of *A. ferox*

Biochemical Parameters	Control	*Aloe ferox* dose levels (mg/kg body weight)				Normal range
		50	100	200	400	
LFT: Bilirubin total µm/l	9.75±0.806 [a]	10.50±0.806	9.40±0.721	12.00±0.806	14.60±0.721[b]	0-21
Bilirubin conjugated µm/l	3.50±0.517	3.00±0.517	3.40±0.463	4.00±0.517	5.00±0.463	0-6
Bilirubin unconjugated µm/l	6.25±0.285a	7.50±0.285a	6.00±0.258a	8.00±0.285a	9.60±0.258b	0-15
Total protein (g/l)	53.25±1.099	51.50±1.099	53.80±0.983	52.25±1.099	52.80±0.983	60-85
Albumin (g/l)	18.25±0.348	17.25±0.348	18.80±0.312	17.25±0.348	17.40±0.312	35-52
Globulin (g/l)	35.00±0.751	34.25±0.751	35.00±0.671	35.00±0.751	35.40±0.671	35-52
Alkaline Phosphatase (U/l)	565.50±36.559[a]	557.75±36.559	498.00±32.670	423.25±36.559[b]	302.40±32.670[b]	40-120
γ-Glutamyl Transferase (U/l)	5.00±1.188	7.50±1.188	5.00±1.063	5.50±1.188	7.00±1.063	0-60
Alanine Transaminase (U/l)	107.50±5.375	91.25±5.375	111.00±4.808	102.00±5.375	88.20±4.808	5-40
Aspartate Transaminase (U/l)	184.75±7.455	177.75±7.455	205.00±6.668	204.50±7.455	202.20±6.668	5-40
Chemistry test: P-Glucose (random) (mmol/l)	3.83±0.227[a]	3.20±0.227	4.24±0.203	1.83±0.227[b]	1.56±0.203[b]	4.1-11.1
Calcium (mmol/l)	2.39±0.032	2.40±0.032	2.37±0.028	2.40±0.032	2.35±0.028	2.05-2.56
Corrected calcium (mmol/l)	2.82±0.029	2.85±0.029	2.80±0.026	2.86±0.029	2.80±0.026	2.05-2.56
Magnesium (mmol/l)	1.55±0.081	1.73±0.081	1.56±0.072	1.68±0.081	1.58±0.072	0.65-1.10
P-inorganic (mmol/l)	3.50±0.162[a]	4.33±0.162[b]	3.30±0.145	4.45±0.162[b]	4.24±0.145[b]	0.8-1.4
UEC: Sodium (mmol/l)	137.50±0.735	139.25±0.735	138.20±0.658	137.50±0.735	137.40±0.658	135-147
Potassium (mmol/l)	5.78±0.157	5.48±0.157	5.62±0.141	5.73±0.157	5.96±0.141	3.3-5.3
Chloride (mmol/l)	101.00±0.765[a]	98.25±0.765	99.40±0.684	95.50±0.765[b]	95.20±0.684[b]	99-113
Urea (mmol/l)	6.55±0.389	6.35±0.389	6.90±0.348	6.85±0.389	7.66±0.348	2.6-7.0
Creatinine µmol/l	49.25±2.224[a]	52.25±2.224	44.80±1.989	59.00±2.224[b]	58.00±1.989[b]	60-120

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

has therapeutic properties (Reynolds, 1982). However, the aqueous extract of *A. ferox* affected other haematological parameters like the RBC, monocytes and platelets that were high confirming that the bone marrow of rats might have been induced by *A. ferox* to produce more RBC. This justifies the hypertensive state of the heart of rats that was noticed in histopathology examination, in order to cope with the copious amount of RBC produced. Nevertheless, increased synthesis of RBC leaves room for further validation as it could be that the plant possesses some potentially toxic substances.

Increase in monocytes could be attributed to the complex carbohydrate, acemannan, of *A. ferox*, that has immune stimulating properties (Magwa et al., 2006) and other substances like aloesin that are known to boost immunity (Yagi and Takeo, 2003; Du Toit et al., 2007). High blood platelet levels could also be attributed to *A. ferox* containing prostaglandins and fatty acids such as prostaglandin 1 series and gamma-linoleic acid that have beneficial effects on platelet aggregation (Venu, 2007). Nevertheless, *A. ferox* could have some detrimental effects that may lead to increased platelet levels and thus effect of *A. ferox* chemical constituent on blood platelets warrants investigation.

The MCV, MCH and neutrophils were lower than the recommended ranges confirming that the red blood cells of the rats were smaller than normal (microcytic) possibly because the plant was impairing the development of these blood parameters, through iron deficiency anaemia. Nonetheless, the lower MCV and MCH values particularly in the chronic toxicity test might not be

Table 5. Biochemical values (±SE) of rats treated with aqueous extract of *A. ferox* in a chronic toxicity experiment

Biochemical Parameters	Control	*Aloe ferox* dose levels (mg/kg body weight)				Normal range
		50	100	200	400	
LFT: Bilirubin total µm/l	9.50±2.952[a]	11.20±2.640	10.00±2.640	13.80±2.640	29.00±3.409[b]	0-21
Bilirubin conjugated µm/l	2.50±1.318[a]	2.80±1.179	2.80±1.179	3.80±1.179	16.33±1.522[b]	0-6
Bilirubin unconjugated µm/l	7.00±1.634a	8.40±1.461a	7.20±1.461a	10.00±1.461b	12.67±1.887b	0-15
Total protein (g/l)	61.00±1.367	61.80±1.223	58.80±1.223	59.20±1.223	62.33±1.578	60-85
Albumin (g/l)	19.00±0.396	18.80±0.354	18.60±0.354	18.00±0.354	20.67±0.457	35-52
Globulin (g/l)	42.00±0.971	43.00±0.869	40.20±0.869	41.20±0.869	41.66±1.121	35-52
Alkaline Phosphatase (U/l)	161.75±15.062[a]	139.80±13.472	155.60±13.472	152.60±13.472	97.00±17.392[b]	40-120
γ-Glutamyl Transferase (U/l)	8.25±2.644[a]	12.00±2.365	12.40±2.365	10.20±2.365	20.67±3.053[b]	0-60
Alanine Transaminase (U/l)	54.50±4.569[a]	48.40±4.087	63.20±4.087	58.20±4.087	87.00±5.276[b]	5-40
Aspartate Transaminase (U/l)	179.50±14.787	135.80±13.226	156.60±13.226	136.00±13.226	187.00±17.075	5-40
Chemistry test: P-Glucose (random) (mmol/l)	4.83±0.293	4.84±0.262	4.80±0.262	5.24±0.262	4.27±0.338	4.1-11.1
Calcium (mmol/l)	2.34±0.023	2.37±0.021	2.36±0.021	2.35±0.021	2.36±0.027	2.05-2.56
Corrected calcium (mmol/l)	2.76±0.020	2.79±0.018	2.79±0.018	2.79±0.018	2.75±0.023	2.05-2.56
Magnesium (mmol/l)	1.20±0.030	1.24±0.026	1.23±0.026	1.17±0.026	1.25±0.034	0.65-1.10
P-inorganic (mmol/l)	2.98±0.074	2.90±0.066	3.04±0.066	2.86±0.066	3.00±0.085	0.8-1.4
UEC: Sodium (mmol/l)	140.00±0.636	140.80±0.568	140.00±0.568	142.00±0.568	138.67±0.734	135-147
Potassium (mmol/l)	6.43±0.168[a]	5.88±0.151	5.60±0.151[b]	5.78±0.151[b]	5.33±0.195[b]	3.3-5.3
Chloride (mmol/l)	104.00±0.766	104.80±0.685	104.40±0.685	105.60±0.685	105.33±0.884	99-113
Urea (mmol/l)	6.75±0.311	6.24±0.278	6.54±0.278	6.08±0.278	7.23±0.359	2.6-7.0
Creatinine µmol/l	47.25±2.403	45.00±2.150	42.40±2.150	45.80±2.150	54.00±2.775	60-120

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

linked with the extract as this also happened to the control group. Possibility of deviation from the norm of blood parameters of the control group could be explained by the time of blood collection. Palmer (2004) explained that blood parameters are usually high when blood collection is done in the morning and afternoon than in the evening, of which in the current study blood collection was conducted in the morning.

Decrease in MCH (a calculation of the amount of oxygen-carrying haemoglobin inside the RBC) could be attributed to the smaller size of RBC (microcytic) that could have possibly been caused by the extract.

Both in the sub-acute and chronic toxicity tests there was a decrease in neutrophils (known as neutropenia) which could be accredited to damage to some parts of the bone marrow or an increase in the destruction of neutrophils in the body. Also, since neutropenia is related to stress this could indicate that the rats were stressed under the longer sub-acute and chronic toxicity tests, than the acute test, leading to the release of the corticosteroid hormone (The Merck Manuals, 2004).

In both toxicity tests, sub-acute and chronic, AST, ALT, GGT, bilirubin conjugated bilirubin and total protein findings show that the function of the liver was not affected by the extract, although more liver enzymes were produced and this concurs with the normality of the macroscopic analysis of most livers. The normality of the liver could be attributed to aloe-emodine, a derivative from the hydrolysis of aloin, which is a potent inhibitor of hepatic cell activation and proliferation (Arosio et al., 2000; Woo et al., 2002).

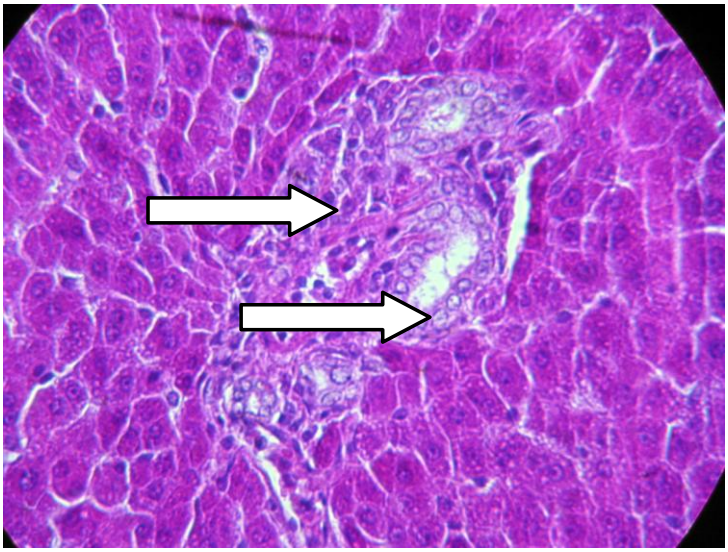
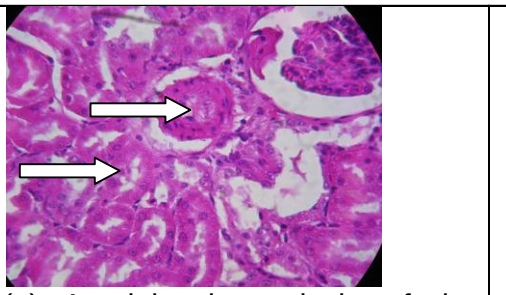
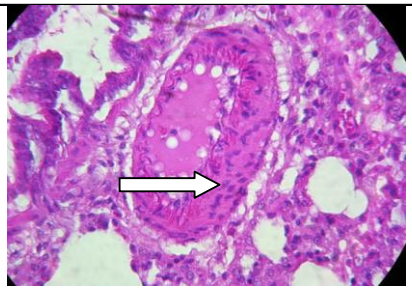


Figure 1. Photomicrograph of a liver of a rat orally administered with *A. ferox* aqueous extract at a dose of 200 mg/kg body weight for 14 days. The figure shows the proliferation of the bile duct (x400).



(a) Arteriolar hyperplasia of the media (x400) for 50 mg/kg dose at



(b) Central arteriole hyperplasia (x400) for 100 mg/kg dose

Figure 2. Photomicrograph of a lung of a rat orally administered with *A. ferox* aqueous extract at 50 and 100 mg/kg body weight doses for 14 consecutive days.

Inorganic phosphorus findings show that the kidneys might transiently not have been losing enough of the electrolytes thereby negatively affecting the acid-base balance of the blood. The macroscopic analysis of the kidney showed also some hyperplasia under the 100 mg/kg body weight dose indicating that the kidneys were forced to work more than normal in order to get rid of the electrolytes and wastes. This is supported by Boudreau and Beland (2006) who reported in their review that ingestion of Aloes is associated with electrolyte imbalance and kidney disfunction. Hyperplasia of lungs was noticed in almost all the dose levels including the control suggesting that the rats could have been chemically or physically injured before the commencement of the experiment leading to pneumonia. Therefore, it is imperative to have both the satellite group (Witthawaskul et al., 2003) at the end of the experiment and organs and blood testing of sample rats from the experimental groups before the toxicity experiment commences. A satellite group is a group of rats that serve as a recovery group by being left for some time after the experiment has been terminated and its organs and blood parameters will be tested later. The findings indicate that potential toxicity is pronounced when the plant is used for a longer period since more rat organs were affected in the chronic experiment compared with the sub-acute toxicity experiment. Also the effects were higher at higher dose levels. This is supported by Viljoen (2008) who purported that *A. ferox* should not be used in excess. Use of the aqueous extract of the plant is

detrimental to the reproductive organs and this concurs with Zhou et al. (2003).

Conclusion

Aloe ferox is relatively non-toxic if not used for few days such as 3 days of the acute toxicity test. The plant, at the highest dose level tested, did not cause any rat mortality, physiological or behavioural change in the acute, sub-acute and chronic toxicity tests. This could justify why *A. ferox* is increasingly used as an antihelminthic in livestock. However, the plant should not be excessively used as this could lead to the damage of the heart, lungs and kidneys, and degeneration of spermatogenic cells. Future work will, therefore, focus on evaluating the pharmacological properties of the *A. ferox* plant aqueous extract in order to authenticate its use in controlling helminths in animals.

ACKNOWLEDGEMENTS

Authors are indebted to the National Research Foundation, South Africa for funding the project. Authors are also grateful to the Agricultural and Rural Development Research Institute, Department of Livestock and Pasture Science and Department of Botany staff and students of the University of Fort Hare for technical support they rendered.

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