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Full Length Research Paper

Toxicity evaluation of the aqueous leaf extract of *Gunnera perpensa* L. (Gunneraceae)

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The objective of the study was to determine the potential toxicity of *Gunnera perpensa* through acute, sub-acute and chronic toxicity tests. For each test, 25 rats distributed in 5 groups of 5 rats per group were used. Group 1, the negative control, were orally administered with distilled water and groups 2 to 5 *G. perpensa* extract at 50, 100, 200 and 400 mg/kg doses, respectively. Change in behaviour and mortality was recorded. In addition, haematology, serum biochemical assays and histopathology examinations were conducted for sub-acute and chronic tests. Neither rat mortality nor changes in behaviour were noted for acute test. Rat mortality for 400 mg/kg dose of sub-acute and 200 mg/kg of chronic test was 20%. For sub-acute and chronic tests, platelets and monocytes were higher than the reference range, while mean cell volume was low. Creatinine, aspartate transaminase, magnesium, inorganic phosphate and potassium were low in the sub-acute while alkaline phosphatase and inorganic phosphate were low for the chronic test. Mild splenic siderosis and renal inflammation was observed in the sub-acute test. The plant is potentially toxic when used consecutively for a long period.

Key words: Aqueous extract, *Gunnera perpensa*, haematology, mortality, serum biochemical parameters, toxicity.

INTRODUCTION

Gunnera perpensa (Haloragaceae or Gunneraceae) is commonly known as river pumpkin (English) or Imphuzi lomlambo/Igangashane (Xhosa) (Dold and Cocks, 1999). In an era, where more and more people are turning towards natural remedies, *G. perpensa* is becoming one of the top natural remedies for a variety of skin conditions, medical ailments and in the control of gastro-intestinal parasites in village chickens (Van Wyk and Gericke, 2003; Mwale and Masika, 2009). Village chickens are one of the mainstays for the livelihoods of resource-limited farmers (Sonaiya, 2007). The productivity of these chickens is, nonetheless, hampered by gastro-intestinal parasite infestation. The parasites are ideally controlled through the use of commercial drugs. These commercial drugs are, however, expensive and out of reach of most resource-limited farmers, inaccessible in some instances and may be in bulky packages that are unsuitable for the resource-limited farmers or misused leading to toxicity or parasite resistance (Waller 2006; Molento, 2009). Consequently, the resource-limited farmers use medicinal remedies such as *G. perpensa* as an alternative control of gastro-intestinal parasites in village chickens (Dold and Cocks, 2001; Mwale and Masika, 2009).

Albeit, medicinally used plants like *G. perpensa* are assumed to be non-toxic, many are potentially toxic and there is little attention on the safety of *G. perpensa* to both humans and chickens. A previous toxicity assessment of the plant using the brine shrimp assay, found it to be toxic at 10 mg/ml (McGaw et al., 2005). The authors recommended further toxicity evaluation of *G. perpensa* on mammalians. Therefore, this study was conducted to

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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.

evaluate the potential toxicity of *G. perpensa* through the acute, sub-acute and chronic toxicity tests using rats.

MATERIALS AND METHODS

Plant collection

Fresh leaves of *G. perpensa* were collected from Centane district (32°38'63''S and 28°24'36''E; elevation 50 m) of South Africa in October 2007. The plant was identified at Selmar Schonland Herbarium of Rhodes University, Botany Department. Voucher specimen (MMAN 2007/03) was deposited in the Giffen Herbarium at the University of Fort Hare.

Plant material extraction

The extract was prepared based on the proportions and methods used by resource-limited farmers and herbalists. The plant material was extracted through crushing 200 g of *G. perpensa* fresh leaves mixed with 400 ml of water in an electric blender for 5 min, after which the extract was squeezed through a muslin cloth to obtain 50% (w/v). The filtrate was freeze dried at -50°C under vacuum using a lyophiliser (Savant refrigerated vapour trap, RVT 4104, USA) and kept in a freezer at -20°C until use. The filtrate was reconstituted in water to make aqueous stock solution of 50, 100, 200 and 400 mg/kg body weight graded dose levels.

Animals used and experimental design

Seventy-five Wistar rats, weighing 150±10 g, of either sex were used. The rats were bred in the Animal House of the Agricultural and Rural Development Research Institute (ARDRI), University of Fort Hare under standardised environmental conditions (ambient room temperature 25±2°C and standard light from 0600 to 1800 h that is, 12 h light-dark cycle). The rats were allowed free access to standard commercial rodent pellets (EPOL Feeds Ltd, South Africa). Clean water was provided *ad libitum* throughout the experimental period. In each of the three toxicity tests conducted, acute, sub-acute and chronic, 25 rats were randomly distributed into 5 groups of 5 rats per group. A completely randomised design was used in which group 1 (Control) orally received 1 ml of distilled water by means of a bulbed steel needle and groups 2 to 5 received graded dose levels; 50, 100, 200 and 400 mg/kg body weight of the aqueous extract of *G. perpensa,* respectively. Ethical procedures for using Wistar rats were according to the guidelines of the University of Fort Hare ethics committee's and the international standards (Austin et al., 2004; Marie, 2006).

Acute toxicity

The acute toxicity test was conducted according to the method of Sawadogo et al. (2006), where rats received orally a single dose of the graded dose levels of the test extract. Observations were made for physiological and behavioural changes that include feeding behaviour, increased or decreased activity due to drug reaction and stress, and rat mortality. Rats were observed continuously for 3 h soon after administering the test extract, then hourly for 72 h.

Sub-acute toxicity

The method of Bürger et al. (2005) was followed in which rats in group 1 (Control) received 1 ml of distilled water and groups 2 to 5 received 1 ml of each of the graded dose levels of the aqueous leaf extract of *G. perpensa* per os for 14 consecutive days. Observations for physiological and behavioural changes were as discussed above. Initial body weights of the rats were recorded on day one and on weekly basis thereafter. The relative organ weight was calculated following the formula of Chavalittumrong et al. (2004).

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

Haematology and biochemical assays

Rats were fasted overnight, anaesthetized using halothane and sacrificed at the 14th day. Paired blood samples, heparinised and non-heparinised, were collected for haematological and serum biochemical assays, respectively.

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 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). 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 (Cole et al., 1986; Bürger et. al., 2005).

Histopathology

Immediately after collection of blood samples, rats were dissected and liver, lungs, heart, spleen, oesophagus and kidneys were removed. The organs were weighed individually using 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 and stained using the haematoxylin and eosin technique and macroscopically examined (x400 magnification) for organ lesions like haemorrhages, organ hypertrophy and/or hypotrophy (Attawish et al*.,* 2004).

Chronic toxicity test

Rats were orally administered with the test aqueous extract once daily for 35 days according to Banu et al. (1997). Observations for physiological and behavioural changes were as for the acute toxicity test. Body weight measurement and relative organ weight calculations, haematology and biochemical assays and histopathology examination were as stated earlier.

Data analyses

The obtained numeric data was tested for normality using the general linear model (GLM) procedure of the statistical analysis system (SAS, 2004). Realising that the data was normal, it was subjected to the analysis of variance (ANOVA) and Dunnett's t-test was computed to compare treatment means against the control mean using GLM procedure of 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

Acute toxicity test

The aqueous extract of fresh leaves of *G. perpensa* did not cause rat mortality during the 72 h observation period ($P > 0.05$). The rats did not show any signs of toxicity, behavioural or physiological changes for all the dose levels tested ($P > 0.05$).

Sub-acute toxicity test

Rat mortality was 20% for the 400 mg/kg body weight dose level. There was a significant difference in the final body weights of rats on 100, 200 and 400 mg/kg body weight doses ($P < 0.05$; Table 1). As also shown in Table 1, the relative rat organ weights were not significantly different from the control except for the spleen of rats on 100 and 400 mg/kg body weight doses.

Haematology and biochemical assays

White blood cells, red blood cell count, MCH, MCV, platelets, neutrophils, lymphocytes and basophils of rats for all the dose levels were not significantly different from the control ($P > 0.05$; Table 2). However, platelets for 100 and 400 mg/kg dose were higher than the reference range, while MCV for 50 and 100 mg/kg dose were lower than the reference range. As indicated in Table 2, haemoglobin, haematocrit, MCHC red blood cell distribution width, large unstained cells and eosinophils of rats were not significantly different from the control ($P > 0.05$) and were within the reference range. Monocytes for rats under all dose levels were significantly different from the control ($P < 0.05$) and were higher than the reference range.

Calcium, albumin, corrected calcium, chlorine, magnesium, bilirubin total, bilirubin conjugated, GGT and ALP of all the rats were not significantly different from the control ($P > 0.05$; Table 3) but magnesium of rats on 50, 100 and 400 mg/kg were lower than the reference range. As indicated in Table 3, urea, creatinine, sodium, total protein, ALT and phosphorylated glucose of rats were not significantly different from the control but creatinine values were lower than the reference range. Potassium and AST of rats on doses 100 and 400 mg/kg body weight were significantly different from the control ($P < 0.05$) and lower than the reference range. Inorganic phosphorus for rats on 50, 100 and 200 mg/kg was significantly different from the control and lower than the reference range.

Histopathology

Relatively few abnormalities were detected for *G. perpensa*. The plant did not affect the heart, liver, pancreas and oesophagus for the tested doses ($P > 0.05$). No organ damage was noticed for the 200 and 400 mg/kg body weight ($P > 0.05$). Mild splenic siderosis was predominant in rats that were on the 50 and 100 mg/kg body weight doses (Figure 1).

Chronic toxicity test

For the 200 mg/kg body weight dose, rat mortality was 20% and rat paralysis (20%) was noticed a week after the commencement of the experiment. *G. perpensa* caused diarrhoea in 20% of the rats on the 200 mg/kg dose, from the 1st to the 3rd week of the experimental period. As indicated in Table 1, the final body weight of rats on doses 200 and 400 mg/kg were different from the control ($P > 0.05$). The relative organ weights were not significantly different from the control except for the spleen of rats under 100 and 400 mg/kg (Table 1).

Haematology and biochemical assays

Values of the white blood cells, red blood cells, MCH, neutrophils, lymphocytes and basophils, LUC and eosinophils were similar to those of the sub-acute test. Haemoglobin, haematocrit, MCHC and red cell distribution of rats were significantly different from the control (Table 4), but were within the reference range. Platelets and MCV were not significantly different from the control ($P > 0.05$) but were within the reference range. Monocytes for rats on 50, 200 and 400 mg/kg were significantly higher than those of the control.

Urea, calcium, albumin, corrected calcium, bilirubin total, bilirubin conjugated, total protein, phosphorylated glucose, GGT and sodium and chlorine of all the rats were similar to values of the sub-acute test. Inorganic phosphorus and ALP values for the 200 mg/kg dose were lower than the reference range. Potassium for 200 and 400 mg/kg doses was significantly different from the control and higher than the reference range ($P < 0.05$; Table 5). Creatinine and magnesium were not significantly different from the control and were within the range, while AST for the 100, 200 and 400 mg/kg dose was significantly higher than the control value.

Histopathology

G. perpensa was fairly non-toxic; it did not damage the heart, liver, oesophagus and spleen of the rats. Peribronchial, sub-pleural and pulmonary interstitial inflammation (infiltrate) was noticed in rats that were on the 50, 200 and 400 mg/kg doses (Figure 2). The plant induced renal eosinophilic luminal casts in convoluted tubules, focal interstitial nephritis and acute tubular

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

Organ weight (g)	Dose (mg/kg body weight)				
	Control	50	100	200	400
Sub-acute toxicity					
Final body weight	234.98±6.202[a]	162.62±6.202[b]	188.78±6.202[b]	167.66±6.202[b]	238.73±6.934[a]
Liver	36.86±1.629	42.01±1.629	37.18±1.629	41.79±1.629	35.64±1.822
Heart	7.25±1.418	4.30±1.418	4.37±1.418	4.53±1.418	4.08±1.586
Kidney	8.44±0.542[a]	9.49±0.542[a]	9.39±0.542[a]	10.59±0.542[b]	9.65±0.606[a]
Spleen	3.06±0.243	2.83±0.243	2.79±0.243	2.83±0.243	2.54±0.271
Lung	11.23±2.523	14.30±2.523	7.95±2.523	7.94±2.523	6.25±2.821
Chronic toxicity					
Final body weight	296.90±10.583[a]	274.80±10.583[a]	242.68±10.583[b]	196.68±11.832[b]	177.06±10.583[b]
Liver	33.04±1.133[a]	33.44±1.133[a]	31.01±1.133[a]	38.56±1.267[b]	35.63±1.133[a]
Heart	3.90±0.212[a]	4.00±0.212[a]	4.25±0.212[a]	4.46±0.237[a]	4.77±0.212[b]
Kidney	9.96±0.218	9.98±0.218	10.33±0.218	10.19±0.244	10.52±0.218
Spleen	2.83±0.315	2.49±0.315	2.86±0.315	3.98±0.353	3.77±0.315
Lung	7.90±0.749	7.22±0.749	6.40±0.749	6.76±0.838	6.81±0.749

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

Table 2. Haematological values (±SE) for rats treated with aqueous extract of *G. perpensa for* 14 days.

Haematological parameters	Control	*G. perpensa* dose levels (mg/kg body weight)				Normal range
		50	100	200	400	
White blood cell ($x10^9$/l)	5.59±0.916	6.89±0.916	7.08±0.916	5.51±0.916	6.66±1.024	4-10
Red blood cell ($x10^{12}$/l)	5.37±0.180	5.64±0.180	5.58±0.180	5.45±0.180	5.12±0.202	4.5-5.5
Haemoglobin (g/dl)	15.70±0.275	15.80±0.275	15.30±0.275	15.68±0.275	15.45±0.308	13-17
Haematocrit (l/l or %)	0.53±0.013	0.51±0.013	0.50±0.013	0.51±0.013	0.52±0.014	0.4-0.5
Mean cell volume (fl)	81.00±0.742	78.98±0.742	78.32±0.742	80.28±0.742	83.18±0.830	79.1-98.8
Mean corpuscular haemoglobin (pg)	21.12±1.128	18.30±1.128	17.80±1.128	18.54±1.128	19.05±1.261	27-32
Mean corpuscular haemoglobin concentration (g/dl)	33.74±0.353	34.02±0.353	33.58±0.353	33.78±0.353	33.13±0.395	32-36
Red cell distribution width (%)	12.14±0.355	11.80±0.355	11.28±0.355	11.52±0.355	12.53±0.397	11.6-14.0
Platelets ($x10^9$/l)	292.20±60.86	314.60±60.86	422.20±60.86	276.60±60.86	435.25±60.043	137-373
Neutrophils (($x10^9$/l)	4.30±0.076	4.38±0.076	4.50±0.076	4.42±0.076	4.37±0.085	2-7.5
Monocytes ($x10^9$/l)	0.33±0.471[a]	0.0±0.471[a]	0.86±0.471[b]	0.0±0.471[a]	0.44±0.527[b]	0.18-0.8

Table 2. cont.

Lymphocytes (x10^9/l)	2.37±0.684	4.03±0.684	2.97±0.684	3.29±0.684	3.15±0.764	1.00-4.00
Large unstained cells (x10^9/l)	0.86±0.082^a	0.46±0.082^b	0.60±0.082	0.44±0.082^b	0.63±0.092	
Eosinophils (x10^9/l)	0.04±0.014^a	0.08±0.014	0.12±0.014^b	0.10±0.014^b	0.07±0.016	0.00-0.45
Basophils (x10^9/l)	0.03±0.006	0.03±0.006	0.02±0.006	0.02±0.006	0.02±0.007	0.00-0.2

^{ab}, Values with superscripts in the same row are significantly different from the control (P < 0.05).

Table 3. Biochemical values (±SE) for rats treated with aqueous extract of *G. perpensa for* 14 days.

Biochemical parameter	Control	*G. perpensa* dose levels (mg/kg body weight)				Normal range
		50	100	200	400	
LFT: Bilirubin total µm/l	8.00±1.306	7.40±1.306	4.00±1.306	8.80±1.306	5.00±1.460	0-21
Bilirubin conjugated µm/l	3.80±0.687	2.30±0.687	1.80±0.687	4.40±0.687	1.13±0.768	0-6
Total protein (g/l)	68.80±1.095	67.00±1.095	68.40±1.095	68.60±1.095	68.75±1.224	60-85
Albumin (g/l)	47.40±0.444	47.20±0.444	47.60±0.444	48.20±0.444	47.25±0.497	35-52
Alkaline phosphatase (U/l)	88.20±11.750	47.20±11.750	47.60±11.750	50.20±11.750	87.75±13.137	40-120
γ-Glutamyl transferase (U/l)	9.80±0.942	10.00±0.942	11.20±0.942	9.00±0.942	12.25±1.054	0-60
Alanine transaminase (U/l)	28.40±3.010	22.00±3.010	17.80±3.010	19.20±3.010	19.00±3.365	5-40
Aspartate transaminase (U/l)	14.20±23.062^a	20.40±23.062^a	00.00±23.062^b	36.40±23.062^b	2.25±25.785^b	5-40
Chemistry test: P-Glucose (random) (mmol/l)	4.30±0.545	6.22±0.545	7.92±0.545	5.44±0.545	5.35±0.610	4.1-11.1
Calcium (mmol/l)	2.35±0.015	2.39±0.015	2.34±0.015	2.36±0.015	2.34±0.017	2.05-2.56
Corrected calcium (mmol/l)	2.80±0.014	2.85±0.014	2.79±0.014	2.80±0.014	2.80±0.016	2.05-2.56
Magnesium (mmol/l)	0.67±0.086	0.58±0.086	0.37±0.086	0.66±0.086	0.40±0.096	0.65-1.10
P-inorganic (mmol/l)	0.96±0.073^a	0.18±0.073^b	0.03±0.073^b	0.24±0.073^b	0.95±0.082^a	0.8-1.4
UEC: Sodium (mmol/l)	143.00±0.556	143.20±0.556	142.80±0.556	141.80±0.556	143.50±0.622	135-147
Potassium (mmol/l)	4.10±1.202^a	3.92±1.202^a	2.66±1.202^b	5.38±1.^{202a}	2.60±1.344^b	3.3-5.3
Chloride (mmol/l)	100.00±0.856^a	103.80±0.856^b	105.60±0.856^b	103.80±0.856^b	103.75±0.957^b	99-113
Urea (mmol/l)	6.16±0.410	5.96±0.410	5.50±0.410	5.24±0.410	5.20±0.458	2.6-7.0
Creatinine µmol/l	67.40±5.248	59.00±5.248	57.20±5.248	57.40±5.248	49.25±5.867	60-120

^{ab}, Values with superscripts in the same row are significantly different from the control (P < 0.05)

necrosis in the doses 400 mg/kg (Figure 2). As shown in Figure 1, mild splenic siderosis was noticed for the rats on the 400 mg/kg dose. There was no significant difference in the effect of *G. perpensa* on rat organs between the sub-acute and chronic toxicity tests.

DISCUSSION

Gunnera perpensa did not cause rat mortality and change in behaviour of rats under the acute toxicity test. This indicates that, utilisation of the plant for a short period of time is not associated with toxicity. These findings are crucial since resource-limited farmers use the plant for 3 to 5 days when controlling gastro-intestinal parasites in village chickens (Mwale and Masika, 2009). This may justify why resource-limited farmers purport that the plant is effective and non-toxic

Table 4. Haematological values (±SE) of rats treated with aqueous extract of *G.perpensa* for 35 days.

Haematological parameters	Control	*G. perpensa* dose levels (mg/kg body weight)				Normal range
		50	100	200	400	
White blood cell (x10^9/l)	9.44±1.619	8.34±1.619	6.69±1.810	9.75±1.810	8.17±1.810	4-10
Red blood cell (x10^{12}/l)	4.04±0.154	4.10±0.154	4.05±0.172	3.56±0.172	4.25±0.172	4.5-5.5
Haemoglobin (g/dl)	15.96±0.255^a	16.22±0.255^b	16.55±0.285^b	14.55±0.285^b	16.20±0.285^b	13-17
Haematocrit (l/l or %)	0.50±0.007^a	0.50±0.007^a	0.51±0.007^a	0.46±0.007^b	0.50±0.007^a	0.4-0.5
Mean cell volume (fl)	85.68±0.627	85.16±0.627	86.13±0.701	83.75±0.701	83.83±0.701	79.1-98.9
Mean corpuscular haemoglobin (pg)	27.68±0.213	27.82±0.213	28.30±0.238	27.05±0.238	27.50±0.238	27-32
Mean corpuscular haemoglobin concentration (g/dl)	32.76±0.194^a	33.36±0.194^a	33.65±0.217^b	32.73±0.217^a	33.53±0.217^a	32-36
Red cell distribution width (%)	11.90±0.376^a	11.86±0.376^a	11.43±0.420^a	13.55±0.420^b	11.65±0.420^a	11.6-14.0
Platelets (x10^9/l)	264.80±62.249	194.00±62.249	216.75±69.596	156.50±69.596	140.50±69.596	137-373
Neutrophils ((x10^9/l)	4.47±0.130	4.64±0.130	4.48±0.145	4.69±0.145	4.97±0.145	2-7.5
Monocytes (x10^9/l)	0.37±1.016^a	0.88±1.016^b	0.31±1.135^a	2.46±1.135^b	1.67±1.135^b	0.18-0.8
Lymphocytes (x10^9/l)	5.84±1.052	4.05±1.052	3.92±1.176	3.84±1.176	2.67±1.176	1.00-4.00
Large unstained cells (x10^9/l)	1.14±0.280	1.16±0.280	0.88±0.313	1.08±0.313	1.23±0.313	
Eosinophils (x10^9/l)	0.09±0.023	0.11±0.023	0.06±0.025	0.15±0.025	0.11±0.025	0.00-0.45
Basophils (x10^9/l)	0.04±0.007	0.03±0.007	0.04±0.008	0.04±0.008	0.03±0.008	0.00-0.2

^{ab}, Values with superscripts in the same row are significantly different from the control ($P < 0.05$).

Table 5. Biochemical values (±SE) of rats treated with aqueous extract of *G.perpensa* for 35 days.

Biochemical parameter	Control	*G. perpensa* dose levels (mg/kg body weight)				Normal range
		50	100	200	400	
LFT: Bilirubin total µm/l	6.40±0.848	3.60±0.848	4.00±0.848	3.75±0.948	7.40±0.848	0-21
Bilirubin conjugated µm/l	2.80±0.337	2.00±0.337	2.00±0.337	2.00±0.377	4.00±0.337	0-6
Total protein (g/l)	69.20±0.562^a	67.20±0.562	67.40±0.562	66.00±0.628^b	66.60±0.562^b	60-85
Albumin (g/l)	47.80±0.364	47.40±0.364	46.80±0.364	47.50±0.407	47.80±0.364	35-52
Alkaline phosphatase (U/l)	61.80±15.023	59.80±15.023	74.00±15.023	26.25±16.796	41.40±15.023	40-120
γ-Glutamyl transferase (U/l)	11.80±0.898	13.80±0.898	13.00±0.898	11.50±1.004	12.00±0.898	0-60
Alanine transaminase (U/l)	22.00±2.964^a	21.80±2.964	32.20±2.964	23.50±3.314	34.00±2.964^b	5-40
Aspartate transaminase (U/l)	25.00±11.968^a	5.20±11.968^b	40.20±11.968^a	42.25±13.381^a	108.20±11.968^b	5-40
Chemistry test: P-glucose (random) (mmol/l)	5.02±0.338	5.76±0.338	5.10±0.338	5.70±0.378	4.16±0.338	4.1-11.1
Calcium (mmol/l)	2.38±0.021	2.43±0.021	2.37±0.021	2.42±0.024	2.38±0.021	2.05-2.56
Corrected calcium (mmol/l)	2.82±0.019	2.89±0.019	2.83±0.019	2.87±0.021	2.82±0.019	2.05-2.56
Magnesium (mmol/l)	1.06±0.030	1.02±0.030	1.04±0.030	1.05±0.033	1.17±0.030	0.65-1.10
Inorganic phosphorus (mmol/l)	1.12±0.094^a	1.00±0.094^a	1.12±0.094^a	0.60±0.106^b	1.34±0.094^a	0.8-1.4

UEC: Sodium (mmol/l)	141.00±0.539[a]	140.80±0.539	142.40±0.539	141.75±0.602	138.80±0.539[b]	135-147
Potassium (mmol/l)	4.92±0.328[a]	5.00±0.328[a]	4.88±0.328[a]	5.93±0.367[b]	7.48±0.328[b]	3.3-5.3
Chloride (mmol/l)	103.20±0.617	105.20±0.617	104.20±0.617	105.50±0.690	104.20±0.617	99-113
Urea (mmol/l)	6.26±0.253	6.14±0.253	6.74±0.253	6.93±0.283	6.92±0.253	2.6-7.0
Creatinine µmol/l	67.00±2.121	66.80±2.121	67.20±2.121	75.50±2.371	62.80±2.121	60-120

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

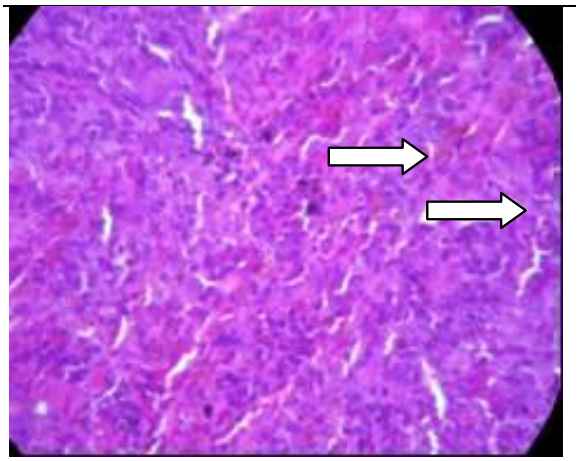
when used in the treatment of other human and veterinary conditions (Kaido et al., 1997; Van Wyk and Gericke, 2003). Besides, it is reported that, the Zulus consume the fresh leaves of *G. perpensa* as a vegetable (Phillips, 1917, cited by Drewes et al., 2005). Given that *G. perpensa* was not toxic under the acute toxicity test, it is important to determine the toxicity of *G. perpensa* over a longer time period.

The findings of 20% mortality in sub-acute (400 mg/kg dose) and the chronic toxicity (200 mg/kg) tests indicate that, the plant is potentially toxic if used for several consecutive days. In addition, *G. perpensa* caused diarrhoea in rats under the 200 mg/kg dose for the chronic test during the 1st and 3rd week of the experimental period showing that, the plant might consists of chemical compounds that tend to upset the gastrointestinal tract (GIT). Although, *G. perpensa* mainly has uterotonic activity (McGaw et al., 2005), its extract is used as a colic remedy and was shown to inhibit the maximal response of ileum to the standard agonists, acetylcholine (Kaido et al., 1997). Both the uterus and the GIT are made of smooth muscles that are mediated similarly by biologically active substances such as flavonoids, tannins and anthraquinones whose activity is mediated through calcium channels for enhancing contractility in the uterus and the GIT (Odenthal and Ziegler, 1988; Amos et al., 1998). This might explain why in the current study the aqueous extract of *G. perpensa* induced diarrhoea in rats. Furthermore, the plant caused hind leg paralysis in 20% of the rats under the 200 mg/kg dose of the chronic test, signifying that the plant could potentially have toxins that bind to sodium channels in nerve cells thereby preventing sodium ions from being conducted properly (Rush et al., 2006; Kukreja et al., 2009). The other possibility is that, the plant might have toxic compounds that tend to reduce free calcium levels inside the nerve terminals or reduce sensitivity of the nerve terminal to calcium (Tricarico et al., 2006). It could also be that the toxic compounds induced blood clots that blocked the femoral arteries leading to the paralysis of the hind legs. More work will have to be done to determine the pathogenesis of the paralysis.

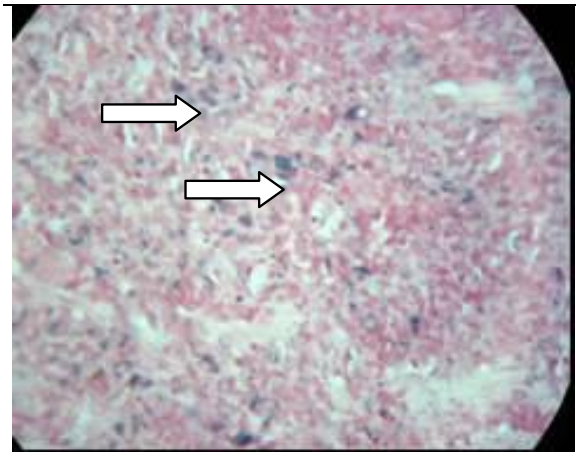
Fluctuations in body weight of rats could be attributed to variations in feed utilisation and probably the diarrhoea that was noticed in the rats. Diarrhoea is associated with loss of nutrients and dehydration thereby leading to loss in weight (Checkley et al., 2008). Adverse effects of *G. perpensa* on the spleen are supported by histopathology results that showed a mild splenic siderosis in the rats that were under both the sub-acute and the chronic toxicity tests. Splenic siderosis is the accumulation of haemosiderin which is an iron-storage protein, in the spleen leading to the inflammation of the organ. This could be attributed to high blood iron levels probably initiated by the plant or inhibition of iron metabolism (Murray et al., 1984; Papakonstantinou et al., 2009). Testing for levels of iron in the blood of test animals and iron levels in the plant is vital; or any heavy metal that might be present in the plant.

Elevation of monocytes could however, be an indication of alteration of the body functions due to diseases or foreign body matter since monocytes are critical for the maintenance of host defence together with other leukocytes (Traves et al., 2004). Elevation of monocytes is also related to inflammation of organs such as the lungs (Haukeland et al., 2006) as confirmed by the histopathology findings where lung inflammation was observed for rats under 50, 200 and 400 mg/kg dose in the chronic test. Lungs are reported to be important sites of host defence (Zeidler et al., 2000) containing increased concentration of neutrophils, monocytes and other leukocytes. This could also explain why inflammation of the lungs was observed in the chronic toxicity test. Chemical elucidation of *G. perpensa* is scanty (McGaw et al., 2005) but Watt and Breyer-Brandwijk (1962) however, reported of the presence of a bitter substance, celastrin that could be attributed to the potential toxicity of *G. perpensa.* The potential toxicity effects of *G. perpensa* were also reported by McGaw et al. (2005) at 10 mg/ml concentration of *G. perpensa* extracts of hexane, dichloromethane, acetone, methanol and ethanol/water.

High AST in the chronic test is associated with liver disease or hepatocelluar liver injury leading to the leakage of the transaminase enzyme into the blood stream (Palmer, 2004). In the current study, there was however, no detectable liver damage. Since AST is found also in other organs



a



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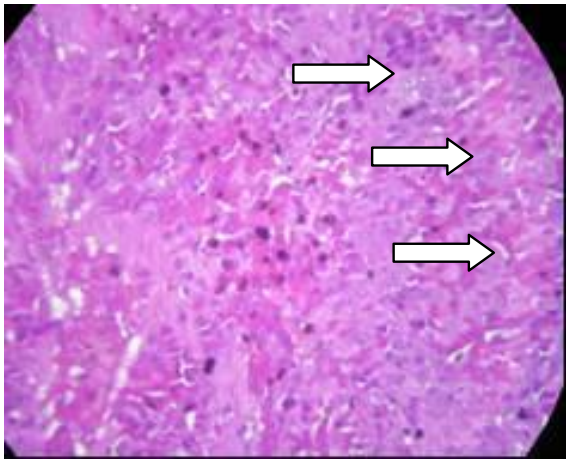

c

Figure 1. Mild splenic siderosis for sub-acute test at 100 mg/kg dose a 400x magnification. (a) the affected spleen shows positive signs for iron staining (400x); (b) mild splenic siderosis for the chronic test at 400 mg/kg dose (400x); (c) Mild splenic siderosis for the chronic test at 400 mg/kg dose (400x).

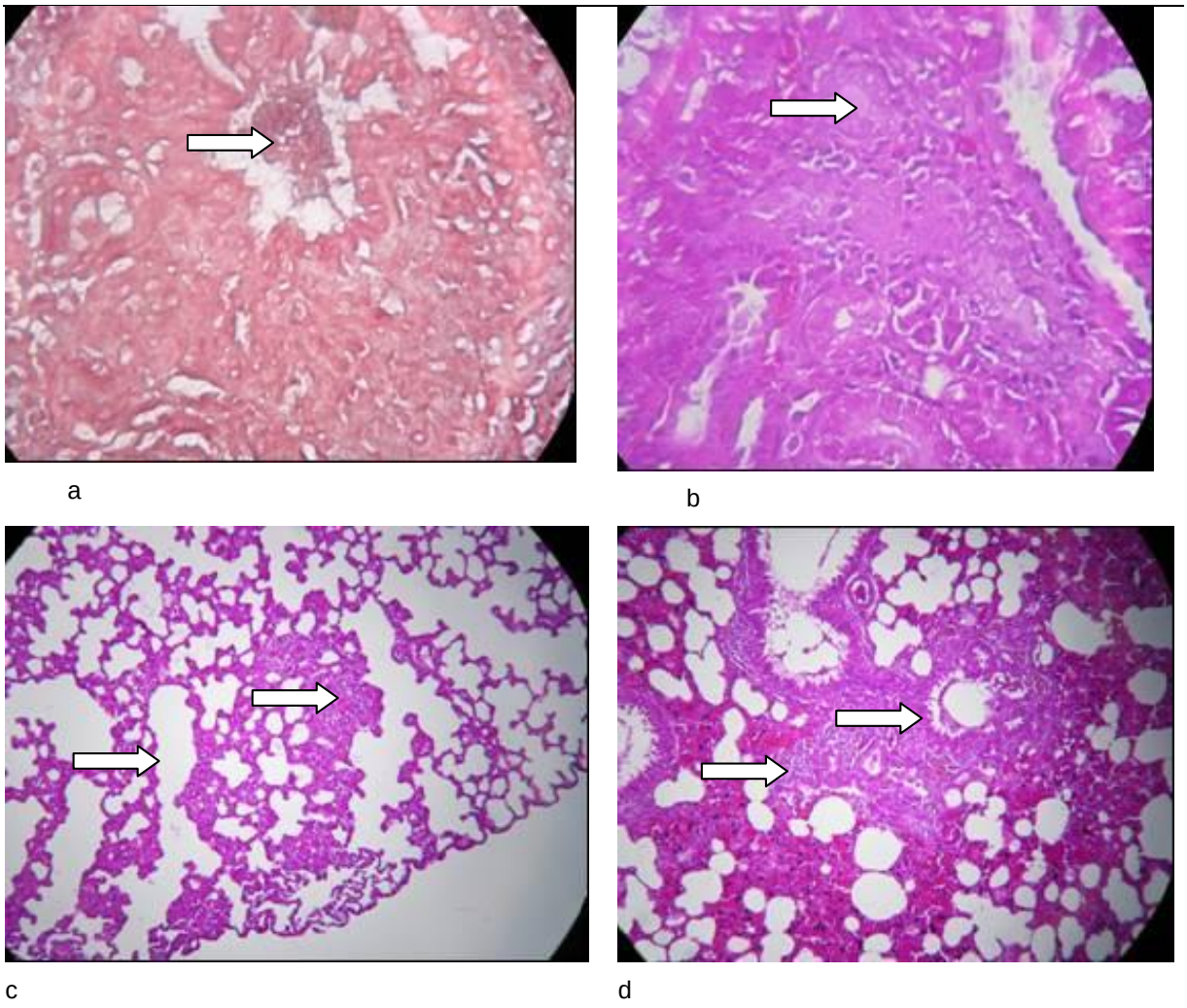

Figure 2. The effect of *G. perpensa* on the lung and kidneys. (a) Nodular pulmonary infiltration (50 mg/kg dose) (100x); (b) renal interstitial nephritis (400 mg/kg dose) (400x); (c) pulmonary interstitial infiltration (200 mg/kg dose) (100x); (d) nodular pulmonary infiltration (400 mg/kg dose) (100x).

such as the kidneys, heart and muscles, the high levels of AST detected could have been from the muscles or kidney as supported by the damage to the kidneys observed in the current study. Palmer (2004) reported that, injury to the liver is confirmed if GGT and ALT are also elevated since they are predominantly found in the liver. In this case, GGT, a cholestatic liver enzyme was within the reference range and ALP was not elevated.

High ALP was observed which relates to a liver disease characterized by possible blockage, injury or inflammation of the bile ducts leading to the impairment or failure of bile flow (cholestasis) (Palmer, 2004). When blockage or inflammation of the bile ducts occurs, ALP can overflow into the bloodstream, and the enzyme typically becomes markedly elevated; approximately ten times the upper limit of the normal range. In the present study, elevated levels of ALP could however, not be attributed to liver damage as the blood parameter is also found in other organs that include the bones intestines, kidneys and placenta (American Proficiency Institute, 2003). Hence, in the current findings, the possible reason why ALP and AST were elevated could be attributed to the time of blood collection. The blood was collected in the morning and Palmer (2004) explains that, these liver function enzyme are usually high when blood collection is done in the morning and afternoon than in the evening.

Creatinine is the best routine blood test for measuring how well kidneys are working. It is a waste product produced by muscles and released through the kidneys (Turner et al., 2006). Low creatinine levels in the blood under the sub-acute test may indicate that, less creatinine is released by the muscles for excretion or most creatinine is lost by the kidneys; due to malfunctioning, the kidneys might be incapable of retaining appropriate quantities for the body system. Low potassium in the sub-acute test could be that the rats were not getting enough from the

feed, or that the rats were incapable of utilising the feed efficiently. High potassium levels observed under the chronic test could be explained by the damage of the kidneys impeding loss of extra potassium through urine (Turner et al., 2006). High levels can be very dangerous as they can cause serious heart rhythm abnormalities, including cardiac arrest hence, caution need to be exercised.

Although, McGaw et al. (2005) postulated that, *G. perpensa* extracts cannot be regarded as being highly toxic in relation to results obtained for other plant extracts where the extracts are toxic at 0.03 mg/ml concentrations, care is necessary when utilising the plant since the current findings revealed that, *G. perpensa* is potentially toxic when used consecutively for long period of time. In addition, further research on the plant is crucial to ensure that the benefits of the resource are realised and that, novel corrective measures have been put in place to guard against the potential toxicity effects of *G. perpensa*.

Conclusions

G. perpensa did not cause rat mortality or change in behaviour in the acute toxicity test. However, the plant is potentially toxic as it caused mild splenic siderosis and mild renal inflammation in the sub-acute and chronic toxicity tests. Although, most haematological and biochemical parameters were not affected, albumin was lower than the reference range and monocytes, ALP, ALT and AST values were elevated. Therefore, there is need for exercising caution when using the plant consecutively for a longer time period. Future work will focus on the pharmacological properties of *G. perpensa*.

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