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Full Length Research Paper

Safety evaluation of aqueous extract of unripe berries of *Solanum aculeastrum* in male Wistar rats

O. M. Aboyade, M. T. Yakubu, D. S. Grierson and A. J. Afolayan*

Department of Botany, University of Fort Hare, Alice, South Africa.

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The acute and sub-acute toxicity of aqueous extract of the fresh, unripe berries of *Solanum aculeastrum* was investigated in male Wistar rats. In the acute toxicity study, toxicity symptoms such as hypoactivity, respiratory distress and epistaxis which disappeared 72 h post treatment, were observed in all the extract treated animals. Except for the 125 mg/kg body weight of the extract, all the other dose levels (250, 500 and 1000 mg/kg body weight) produced mortality in the animals whose latency was inversely proportional to the doses. The extract produced no histopathological alterations in all the organs except the lungs where there was evidence of follicle formation and interstitial diseases following the administration of 125 and 250 mg/kg body weight of the extract. Again, with 500 and 1000 mg/kg body weight of the extract, the lungs became characterized with massive expansion of the bronchial lymphoid tissue (BALT), extension of lymphocytes and plasma cells through the mascularies into the submucosa and mucosa. In the sub-acute toxicity study however, the 50 and 75 mg/kg body weight of the extract significantly ($P < 0.05$) increased the body weight of the animals by 9.23 and 20.02%, respectively. The extract decreased the weight of the liver whereas those of the lungs, spleen and the testes increased. All the dose levels also increased the concentrations of serum total protein, globulin, creatinine and MCV of the animals. Whereas the 50 and 75 mg/kg body weight of the extract increased the serum levels of albumin, urea, calcium, GGT, Hb and RBC, the 25 and 50 mg/kg body weight of the extract decreased the total and conjugated bilirubin. The 75 mg/kg body weight of the extract increased the levels of MCHC, WBC, Cl^-, total and conjugated bilirubin. Again, all the dose levels of the extract decreased the activities of serum ALP, ALT, inorganic phosphorus, MCH, platelets, lymphocytes, neutrophils, monocytes, eosinophils, LUC and basophils. The extract at 25 and 75 mg/kg body weight increased the RCDW and PCV levels respectively whereas the 75 mg/kg body weight of the extract reduced the RCDW. The extract at 25 mg/kg body weight decreased the serum AST activity, Hb, RBC, MCHC and WBC. The alterations in the haematological parameters, liver and kidney function indices as well as mortality observed in this study indicates that the aqueous extract of the fresh, unripe berries of *S. aculeastrum* is toxic and will adversely affect the normal functioning of the blood, liver and kidney of the animals. The follicular bronchitis observed in the lungs of the animals may be associated with immunodeficiency and hypersensitivity to the plant extract. Therefore, the extract is not completely safe as an oral remedy when repeatedly consumed on daily basis for 14 days at the doses investigated.

Key words: *Solanum aculeastrum*, safety, haematological parameters, functional indices, liver, kidney, histopathological alterations, oral remedy.

INTRODUCTION

Herbal remedies have been used in the management of various diseases from time immemorial. These remedies which are commonly self-medicated are more often with no proper dose regimen. Such indiscriminate use of medicinal plant simply because herbs are natural in origin, without recourse to safety and or adverse effects on biological system is worrisome (Aboyade et al., 2009).

*Corresponding author. E-mail: aafolayan@ufh.ac.za. Fax: +27 86 628 2295.

Therefore, it has become imperative to assess the safety or otherwise, the toxicity of plants used for medicinal purposes in folklore medicine. One plant widely used in traditional medicine of South Africa without information on its effect on biological system is *Solanum aculeastrum* Dunal. This plant, commonly known as goat bitter apple (English), is widely distributed in southern Africa (Watt and Breyer-Brandwijk, 1962) . It is a thorny, perennial plant that grows up to 2 - 3 m high with white flowers and lemon shaped berries that become greenish-yellow when ripe (Wanyonyi et al., 2002). The fruits of *S. aculeastrum* are used fresh, dried, boiled or ashed for the treatment of jigger wounds, gonorrhea, rheumatism as well as ringworm in cattle and horses (Agnew and Agnew, 1994; Watt and Breyer-Brandwijk, 1962). Ethnobotanical survey revealed that the berries are used in the treatment of breast cancer in the Eastern Cape Province of South Africa (Koduru et al., 2007a).

Preliminary pharmacological studies on the extracts of *S. aculeastrum* berries and leaves revealed that the plant parts possess antimicrobial, antitumor and antioxidant activities (Koduru et al., 2006a, b, c; 2007b). The steroidal alkaloids in the berries have also been reported to be active against schistomiasis and cancer (Drews and Van Staden, 1995; Koduru et al., 2007b; Wanyonyi et al., 2002). *S. aculeastrum* berries have been used widely for the management of several diseases by the traditional healers of South Africa without recourse to its safety or toxicity risk. For example, several studies have also validated the use of this plant as an antimicrobial and anticancer agents (Koduru et al., 2006 a, b, c; 2007b).

Again, study by Aboyade et al. (2009) have evaluated the toxicological effect of the repeated consumption of aqueous extract of the fresh berry, boiled fresh berry, oven-dried berry and boiled oven-dried berry of *S. aculeastrum* for 28 days at the doses of 1, 10 and 25 mg/kg body weight in male rats and concluded that the adverse effect on the normal functioning of the liver and kidney of the animals might be reduced by the drying and boiling treatments. Despite all these, information is still scanty on the toxic implications of the fresh, unripe berries of *S. aculeastrum* in animal model as used in the management of cancer by the traditional healers of South Africa. Therefore, this study was aimed at investigating the toxicological potentials of the aqueous extract of the fresh, unripe berries of this plant using male Wistar rats as model.

MATERIALS AND METHODS

Plant materials

Fresh, unripe berries of *S. aculeastrum* were collected from Kayalethu village in the Eastern Cape Province of South Africa. The fruit was authenticated by Prof. D.S Grierson of the Department of Botany, University of Fort Hare, South Africa and a voucher specimen (SA/Med 01) was deposited at the Giffen herbarium of the university.

Animals

Male rats (*Rattus norvegicus*) of Wistar strain weighing 187.29 ± 24.68 g were obtained from the Animal House of the Agricultural and Rural Development Research Institute (ARDRI), University of Fort Hare. The animals were housed in clean aluminum cages placed in well ventilated house with optimum conditions (temperature 23 ± 1^{O}C; photoperiod: 12 h natural light and 12 h dark; humidity: 45 - 50%). They were also allowed free access to food (Balanced Trusty Chunks (Pioneer Foods (Pty) Ltd, Huguenot, South Africa) and water. The cages were cleaned on daily basis. This study was carried out following approval from the ethical committee on the use and care of animals of the University of Fort Hare, Alice, South Africa.

Assay kits

The assay kits for creatinine, urea, calcium, phosphorus, chloride, magnesium, sodium, potassium, uric acid, albumin, bilirubin, alkaline phosphatase, gamma glutamyl transferase, alanine and aspartate aminotransferases were obtained from Roche Diagnostic GmbH, Mannheim, Germany. All other reagents used were of analytical grade and were supplied by Merck Chemicals (Pty) Ltd., Bellville, South Africa.

Preparation of extract

The fresh berries (500 g) were extracted in 1000 ml of cold distilled water for 48 h on a mechanical shaker (Stuart Scientific Orbital Shaker SO1, United Kingdom). The extract was filtered with a Buchner funnel and Whatman No 1 filter paper. This was later lyophilized using VirTis benchtop K freeze dryer (VirTis Company, Gardiner, NY) to yield 7.50 g which was reconstituted in distilled water to give the required doses used in this study.

Acute toxicity test

Thirty male rats were completely randomized into five groups (A - E) of six animals each and thereafter fasted (without food, but water) for 6 h before treatment. The animals from group A (the control) received orally 0.5 ml of distilled water (the vehicle) with the aid of oropharyngeal cannula while those in groups B, C, D and E were treated like the control except they received equal volume of the extract containing 125, 250, 500 and 1000 mg/kg body weight respectively. Clinical symptoms of toxicity, mortality and its latency displayed by the animals were observed for an initial 2 h following the administration of the extract and continued throughout the 7 days treatment period.

Sub-acute toxicity test

In this experiment, twenty animals were grouped into four (A-D) consisting of five each. The animals from the control group received orally, 0.5 ml of distilled water with the aid of oropharyngeal cannula, once daily for 14 days, while those in groups B, C and D were treated like the control except they received equal volume containing 25, 50 and 75 mg/kg body weight respectively. The animals were sacrificed 24 h after their 14 daily doses. The changes in the body weight of the animals as well as the absolute weight of the liver, lungs, kidney, spleen, heart and testes were also determined.

Preparation of serum

The procedure described by Yakubu et al. (2005) was used in the

Table 1. Acute toxicity of aqueous extract of unripe berries of *Solanum aculeastrum* in male Wistar rats (n = 6 ± SD).

Doses (mg/kg body weight)	D/T	Mortality (%)	Mortality latency (h)	Toxicity symptoms
Control (distilled water)	0/6	0	0	No clinical sign of f toxicity.
125	0/6	0	0	Hypoactivity, respiratory distress.
250	1/6	0.17	72.00 ± 0.0	Hypoactivity, respiratory distress.
500	2/6	33.30	48.00 ± 4.50	Hypoactivity, respiratory distress, epistaxis.
1000	3/6	50.00	24.00 ± 2.60	Hypoactivity, respiratory distress, epistaxis.

D/T = dead / treated rats. Mortality latency = time of death (in days) after the injection.

preparation of the serum. Briefly, under ether anaesthesia, the neck area of the rats was quickly shaved to expose the jugular veins. The veins after being slightly displaced (to prevent contamination of the blood with interstitial fluid) were sharply cut with sterile scalpel blade and an aliquot (2 ml) of the blood was collected into sample bottles containing EDTA (BD Diagnostics, Pre- analytical Systems, Midrand, USA) for the haematological analysis. Another 5 ml of the blood was allowed to clot for 10 min at room temperature and then centrifuged at 1282 g x 5 min using Hermle Bench Top Centrifuge (Model Hermle, Z300, Hamburg, Germany). The sera were later aspirated with Pasteur pipettes into sample bottles and used within 12 h of preparation for the assay (Malomo, 2000). The rats were thereafter quickly dissected in the cold and the organs (liver, kidneys, lungs, spleen, heart and testes) removed, freed of fat, blotted with clean tissue paper and weighed.

Determination of biochemical parameters

The serum was assayed for creatinine, urea, uric acid, aspartate (AST) and alanine amino transferases (ALT), alkaline phosphatase (ALP), potassium, sodium, calcium, phosphorus, chloride, magnesium, total bilirubin, total protein, albumin and gamma glutamyl transferase (GGT) using assay kits on Roche Modular Analyzer (model P800) Mannheim, Germany. The globulin concentration in the serum was computed using the expression of Tietz (1995). The haematological parameters such as the red blood count (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RCDW), large unstained cell (LUC), white blood cells (WBC), platelets, lymphocytes, neutrophils, monocytes, eosinophils and basophils were determined using the Horiba ABX Diagnostics - Pentra 80 (Montpellier, France).

Histopathological examination

The organs (liver, kidney, lungs, spleen, heart and testes) of the animals were fixed in 10% v/v formaldehyde, dehydrated through ascending grades of ethanol (70%, 90% and 95% v/v), cleaned in xylene and embedded in paraffin wax (melting point 56ºC) (Krause, 2001). Tissue sections were prepared as described by Drury and Wallignton (1973) and stained with hematoxylin/eosin. The photomicrographs were taken at x400 with a Canon Power Shot G2 digital camera (Melville, NY).

Statistical analysis

Data were expressed as mean of five replicates ± SD except in the acute toxicity studies and were subjected to one way analysis of variance (ANOVA). Means were separated by the Duncan's multiple range test using SAS and complemented with Student's t-test. Values were considered statistically significant at $p < 0.05$.

RESULTS

Acute oral toxicity study

Toxicity symptoms such as hypoactivity, respiratory distress and epistaxis were observed in all the extract treated animals. The surviving animals were however free of these signs of intoxication, 72 h post treatment (Table 1) . With the exception of the 125 mg/kg body weight of the extract, all the other dose levels (250, 500 and 1000 mg/kg body weight) produced mortality in the animals with latency that was inversely proportional to the doses (Table 1). The extract produced no histopathological alterations in all the organs except in the lungs. For example, the cardiac architecture was with no evidence of inflammation, fibrosis or ischemia. The lobular architecture of the liver, seminiferous tubules of the testes, medullary and cortical architecture of the kidney and the spleen cells was preserved (photomicrographs not shown).

In contrast however, there was follicle formation and interstitial diseases in the lungs of animals treated with 125 and 250 mg/kg body weight of the extract (Plates 1a and b, respectively). Again, at 500 and 1000 mg/kg body weight of the extract, the lungs became characterized with massive expansion of the bronchial lymphoid tissue (BALT) with extension of lymphocytes and plasma cells through the mascularies into the submucosa and mucosa (Plates 1c and d respectively). In addition, there was tracking of bronchitis into the terminal bronchioles. There was also marked narrowing of the bronchial lamina with acute pneumonic infiltrate in the alveoli (Plates 1a - d).

Subacute toxicity study

The repeated administration of 50 and 75 mg/kg body weight of the extract for seven days significantly ($P < 0.05$) increased the body weight of the animals by 9.23 and 20.02% respectively and this was sustained till the end of the 14 days experimental period. The absolute weight of the lungs, spleen and testes increased at all the dose levels of the extract whereas that of the liver decreased significantly. In contrast, the weight of the kidney and heart of the animals compared well with the control (Table 2).

The extract increased the concentration of serum total

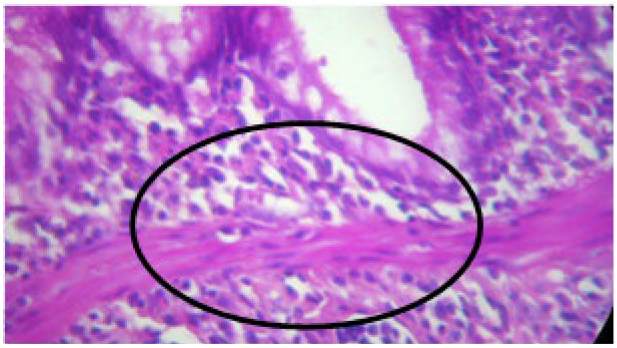

Plate 1a. Photomicrograph of male rat lung orally administered with 125 mg/kg body weight of aqueous extract of *S. aculeastrum* unripe berries for 7 days. The circled spot shows well preserved alveoli architecture with evidence of follicle formation (x400).

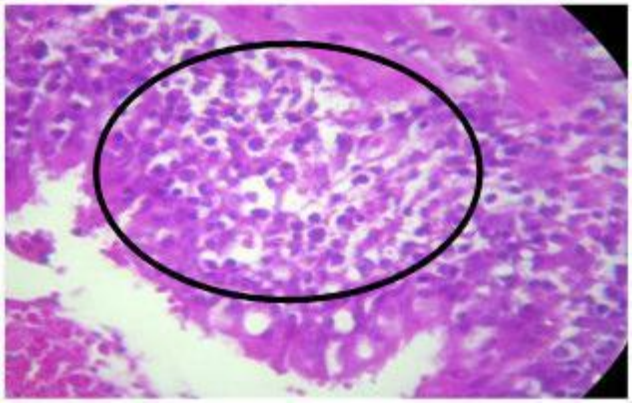

Plate 1b. Photomicrograph of male rat lung orally administered with 250 mg/kg body weight of aqueous extract of *S. aculeastrum* unripe berries for 7 days. The circled spot shows well preserved alveoli architecture with evidence of follicle formation (x400).

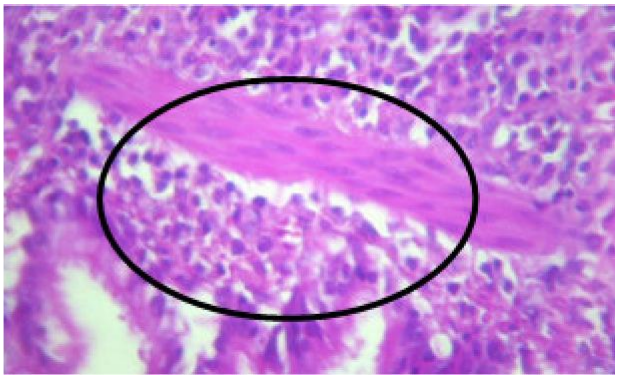

Plate 1c. Photomicrograph of male rat lung orally administered with 500 mg/kg body weight of aqueous extract of *S. aculeastrum* unripe berries for 7 days. The circled spot shows well preserved alveoli architecture with evidence of follicle formation, massive expansion of the bronchial associated lymphoid tissue (BALT) with extension of lymphocytes and plasma through the muscularis into the submucosa and mucosa. There is also acute pneumonic infiltrate in the alveoli as well as marked narrowing of the bronchial lamina (x400).

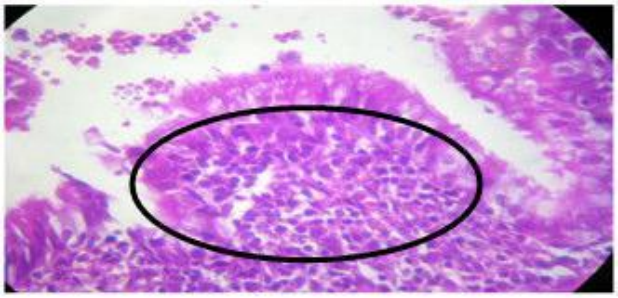

Plate 1d. Photomicrograph of male rat lung orally administered with 500 mg/kg body weight of aqueous extract of *S. aculeastrum* unripe berries for 7 days. The circled spot shows well preserved alveoli architecture with evidence of follicle formation, massive expansion of the bronchial associated lymphoid tissue (BALT) with extension of lymphocytes and plasma through the muscularis into the submucosa and mucosa. There is also acute pneumonic infiltrate in the alveoli as well as marked narrowing of the bronchial lamina (x400).

protein and globulin of the animals. Whereas the 50 and 75 mg/kg body weight of the extract decreased the serum albumin content, the 25 and 50 mg/kg body weight of the extract decreased the concentrations of total and conjugated bilirubin. In contrast, the 75 mg/kg body weight increased the levels of total and conjugated bilirubin (Table 3). Again, all the dose levels of the extract decreased the activities of serum ALP and ALT activity. The extract also produced dose specific responses in the activities of GGT and AST in the serum of the animals. For example, while there was decrease only in the activity of serum AST at 25 mg/kg body weight of the extract, the levels of GGT in the serum of the animals increased only at 50 and 75 mg/kg body weight of the extract (Table 3).

There was no significant alteration in the levels of sodium, potassium, magnesium and uric acid of the animals at all the doses investigated. While the concentration of creatinine in the serum of the animals was elevated at all the dose levels, that of inorganic phosphorus was reduced. The concentration of urea and calcium in the serum of the animals were also reduced at 50 and 75 mg/kg body weight of the extract, whereas, the level of chloride ions was elevated only at 75 mg/kg body weight of the extract (Table 4).

All the doses of the extract increased the MCV of the animals. The MCH, platelets, lymphocytes, neutrophils, monocytes, eosinophils, LUC and basophils were reduced significantly reduced (Table 5) . Whereas the levels of Hb and RBC decreased only at 25 mg/kg body weight of the extract, the 50 and 75 mg/kg body weight of

Table 2. Effect of aqueous extract of the unripe berries of *Solanum aculeastrum* on the weight of the body and absolute organs of male Wistar rats (n = 5, x ± SD).

	Body weight of the animals (g)			
	Control	**25**	**50**	**75**
*Day 0	178.00 ± 10.12	178.03 ± 7.94	189.01 ± 6.80	223.87 ± 6.98
Day 7	191.00 ± 10.11	191.03 ± 10.12	208.63 ± 8.39	238.80 ± 3.73
Day 14	194.30 ± 28.37	194.33 ± 28.39	202.03 ± 22.21	206.80 ± 6.41
	Organ weight (%)			
Liver	4.03 ± 0.09 ^{a}	3.52 ± 0.09 ^{b}	3.72 ± 0.07 ^{b}	3.97 ± 0.05 ^{b}
Lungs	0.83 ± 0.18 ^{a}	1.08 ± 0.21 ^{a}	1.14 ± 0.1 ^{b}	1.34 ± 0.07 ^{c}
Kidney	0.80 ± 0.05 ^{a}	0.76 ± 0.07 ^{a}	0.86 ± 0.18 ^{a}	0.81 ± 0.05 ^{a}
Spleen	0.27 ± 0.02 ^{a}	0.45 ± 0.06 ^{b}	0.44 ± 0.02 ^{b}	0.45 ± 0.09 ^{b}
Heart	0.41 ± 0.02 ^{a}	0.40 ± 0.03 ^{a}	0.42 ± 0.09 ^{a}	0.39 ± 0.01 ^{a}
Testes	1.03 ± 0.03 ^{a}	1.26 ± 0.07 ^{b}	1.46 ± 0.09 ^{c}	1.32 ± 0.02 ^{d}

Test values carrying superscripts different from their controls are significantly different ($p < 0.05$).
*Initial weight of the rats before treatment.

Table 3. Effect of aqueous extract of unripe berries of *Solanum aculeastrum* on some liver function parameters of male Wistar rats (n = 5, x ± SD).

	***Solanum aculeastrum* body weight (mg/kg)**			
	Control	**25**	**50**	**75**
Total bilirubin (µmol/L)	8.67 ± 0.08^{a}	7.33 ± 0.08^{b}	7.00 ± 1.00^{b}	8.00 ± 1.00^{a}
Conjugated bilirubin (µmol/L)	2.00 ± 0.00 ^{a}	1.33 ± 0.18^{b}	1.67 ± 0.18^{b}	3.00 ± 0.30^{c}
Total protein (g/L)	56.67 ± 1.15^{a}	69.33 ± 2.08^{b}	70.00 ± 2.00^{b}	70.33± 3.06^{b}
Albumin (g/L)	17.33 ± 0.58^{a}	17.00 ± 1.00^{a}	15.00 ± 0.65^{b}	11.33± 1.15^{c}
Globulin (g/L)	39.33 ± 1.53^{a}	52.33 ± 3.06^{b}	55.00 ± 1.00^{b}	59.00± 2.00^{c}
Alkaline phosphatase (U/L)	383.00 ± 1.14^{a}	261.00 ± 9.90^{b}	215.00 ± 8.49^{c}	244.50± 37.48^{b}
Gamma glutamyl transferase (U/L)	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	8.00 ± 0.61^{b}	5.50 ± 0.01^{c}
Alanine aminotransferase (U/L)	66.32 ± 2.08^{a}	55.33 ± 5.13^{b}	45.00 ± 3.61^{c}	37.67± 2.52^{d}
Aspartate aminotransferase (U/L)	158.50 ± 7.78^{a}	136.50 ± 9.19^{b}	166.50 ± 6.36^{a}	162.50 ± 3.54^{a}

Test values carrying superscripts different from the control for each parameter are significantly different ($p < 0.05$).

the extract increased these haematological parameters. In addition, the levels of MCHC and WBC decreased at 25 and 50 mg/kg body weight of the extract, whereas, the 75 mg/kg body weight of the extract increased the blood indices. Again, the 25 and 75 mg/kg body weight of the extract increased the RCDW and PCV levels respectively whereas the RCDW was reduced only at 75 mg/kg body weight of the extract (Table 5).

DISCUSSION

Herbal remedies are used by about 80% of the population in the developing countries (Saggu et al., 2007). Despite this widespread use, few scientific studies are available that ascertained the safety of these traditional remedies (Saggu et al., 2007). Therefore, the need to provide information on the toxic implications of these remedies cannot be over emphasized. In this study, the aqueous extract of the unripe berries of *S. aculeastrum* was investigated for its toxic implications in male rats using clinical and biochemical indices such as mortality, haematological and functional parameters of the liver and kidney. The various biochemical indices evaluated in this study are useful parameters that can be employed in assessing the toxic potentials of botanicals. The observed clinical signs of toxicity such as hypoactivity, respiratory distress and epistaxis as well as the incidence of mortality at 250, 500 and 1000 mg/kg body weight suggest that the extract is toxic to the animals at these doses. Since post-mortem examination was not carried out, it was not possible to ascertain the actual cause of the death of the animals. However, it is possible that the oral administration of the extract might have affected the respiration of the animals. The epistaxis observed in the animals may be associated with

Table 4. Effect of aqueous extract of unripe berries of *Solanum aculeastrum* on some renal function parameters of male Wistar rats (n = 5, x ± SD).

Parameters (mmol/l)	*Solanum aculeastrum* body weight (mg/kg)			
	Control	25	50	75
Sodium	137.67 ± 0.58^{a}	137.67 ± 0.58 ^{a}	137.67 ± 2.08 ^{a}	138.33 ± 0.58^{a}
Potassium	6.73 ± 0.32^{a}	6.27 ± 0.55 ^{a}	6.77 ± 0.76^{a}	8.07 ± 1.75^{a}
Calcium	2.51 ± 0.03^{a}	2.52 ± 0.06 ^{a}	2.41 ± 0.10^{a}	2.30 ± 0.10^{b}
Magnesium	1.15 ± 0.03^{a}	1.16 ± 0.08^{a}	1.16 ± 0.05^{a}	1.20 ± 0.22^{a}
Inorganic phosphorus	3.63 ± 0.15 ^{a}	3.07 ± 0.21^{b}	3.10 ± 0.40 ^{b}	3.10 ± 0.13^{b}
Chloride	102.00 ± 1.00^{a}	102.33 ± 0.58^{a}	103.33 ± 1.15^{a}	104.33 ± 1.15^{b}
Urea	7.07 ± 0.68^{a}	7.13 ± 0.81^{a}	6.90 ± 0.20^{a}	5.77 ± 0.15^{b}
Creatinine	42.00 ± 2.00^{a}	48.00 ± 3.46^{b}	49.33 ± 4.04^{b}	53.67 ± 7.50^{b}
Uric acid	0.09 ± 0.02 ^{a}	0.09 ± 0.01 ^{a}	0.10 ± 0.02 ^{a}	0.11 ± 0.01^{a}

Test values carrying superscripts different from the control for each parameter are significantly different ($p < 0.05$).

Table 5. Effect of aqueous extract of unripe berries of *Solanum aculeastrum* on haematological parameters of male Wistar rats (n = 5, x ± SD).

	Solanum aculeastrum body weight (mg/kg)			
	Control	25	50	75
Haemoglobin (g/L)	15.53 ± 0.42^{a}	14.63 ±0.71^{b}	16.63 ± 0.20^{c}	16.20 ± 0.20^{c}
Red blood cell (×10^{12}/L)	8.51 ± 0.11^{a}	8.26 ± 0.24^{a}	8.73 ± 0.03^{b}	8.88 ± 0.14^{b}
Mean corpuscular haemoglobin (pg)	18.80 ± 0.46^{a}	17.67 ±0.31^{b}	17.90 ± 0.17^{b}	18.27 ± 0.15^{c}
Mean corpuscular haemoglobin concentration (%)	31.57 ± 0.40^{a}	29.93 ±0.72^{b}	30.93 ± 0.27^{b}	36.67 ± 1.96^{c}
Mean corpuscular volume (fl)	55.53 ± 1.94^{a}	59.10 ±0.99^{b}	57.97 ± 0.63^{b}	57.23 ± 0.36^{b}
Cell distribution width (%)	12.40 ± 0.44^{a}	13.70 ±0.26^{b}	13.37 ± 0.99^{a}	11.20 ± 0.89^{c}
White blood cell (×10^{9} /L)	14.13 ± 1.46^{a}	7.76 ± 1.23^{b}	7.79 ± 2.22^{b}	20.97 ± 2.87^{c}
Lymphocytes (×10^{9}/L)	56.67 ± 2.63^{a}	4.01 ± 0.54^{b}	4.00 ± 1.21^{b}	8.16 ± 1.94^{c}
Neutrophils (×10^{9}/L)	5.43 ± 1.94^{a}	0.45 ± 0.16^{b}	0.53 ± 0.28^{b}	1.05 ± 0.27^{c}
Monocytes (×10^{9}/L)	28.17 ± 0.7^{a}	2.06 ± 0.24^{b}	2.82 ± 0.75^{b}	8.90 ± 3.46^{c}
Eosinophils (×10^{9}/L)	2.43 ± 0.25^{a}	0.12 ± 0.09^{b}	0.07 ± 0.04^{b}	0.05 ± 0.01^{b}
Basophils (×10^{9}/L)	0.43 ± 0.06^{a}	0.08 ± 0.04^{b}	0.13 ± 0.04^{b}	0.18 ±0.03bc
Large Unstained Cell (×10^{9}/L)	6.93 ± 1.19^{a}	1.00 ± 0.16^{b}	1.00 ± 0.14^{b}	1.33 ± 0.12^{b}
Platelets (×10^{9}/L)	942.50 ± 2.12^{a}	702.50 ±15.96^{b}	811.50 ± 4.95^{c}	838.50±11.92^{c}
Packed cell volume (L/L)	0.49 ± 0.02^{a}	0.49 ± 0.02^{a}	0.51 ± 0.02^{a}	0.53 ± 0.01^{b}

Test values carrying superscripts different from the control for each parameter are significantly different ($p < 0.05$).

platelet dysfunction (McGarry et al., 2005), as was revealed by the reduced platelet count in the present study. The increase in body weight of the animals treated with 50 and 75 mg/kg body weight of the extract for 14 days suggest that the normal growth in these animals was not impaired. This is in contrast to the least extract dosed group where no significant change in body weight was recorded. Therefore, it is unlikely that the extract will have adverse effect on the growth performance of the animals. This, however, contrast the loss of weight in the New Zealand rabbits, reported by Bello et al. (2005) following the oral administration of 750 mg/kg body weight of the aqueous extract of *S. melongena* fruit.

Organ weight is an index of swelling, atrophy or hypertrophy (Amresh et al., 2008). The increase in the weight of the spleen, lungs and testes following extract administration for 14 days may either imply hypertrophy or that growth of the organs is proportional to the growth of the animals. Again, the increase in the testes may be associated with anabolic effect of the extract arising from enhanced production of the secretory components of the testes (Yakubu et al., 2008), while that of the lungs may be due to hyperemia resulting from the ether anaesthesia. In addition, the reduction in the weight of the liver of the animals could possibly be due to cellular constriction. The absence of an effect on the weight of the heart and the kidney of the rats treated with the extract suggest that *S. aculaestrum* berry extract at the

doses investigated did not cause organ swelling, atrophy or hypertrophy of these organs. This is an indication that the extract has selective effect on the weight of the organs investigated in this study.

Again, since histopathological examination of these organs except the lungs showed lack of histoarchitectural alterations in the liver, kidney, spleen, heart and testes, therefore, the increase in the weight of the spleen and testes of the animals suggest that the growth of the organs was proportional to the growth of the animals. In contrast, the reduction in the weight of the liver notwithstanding the lack of histomorphorlogical changes indicates that the growth of the liver was not proportional to the growth of the animals. In addition, the follicular bronchitis observed in the lungs of the animals appeared to be a primary process with secondary acute pneumonia. This follicular bronchitis may be due to immunodeficiency and hypersensitivity of the animals to the plant extract (Corrin, 2000). This may account for the respiratory distress and consequently, death observed in the extract treated animals.

Evaluation of serum proteins such as albumin and globulin is a good criterion for assessing the secretory ability/functional capacity of the liver (Yakubu et al., 2003). While the elevated levels of serum total protein and globulin at all the doses of the extract could possibly imply increase in the functional activity of the liver caused by the component of the extract, the non-definite pattern on the conjugated and total bilirubin as well as albumin suggest adaptation attempt by the animals to the effect of the extract. These alterations will adversely affect the normal function of the hepatocytes.

There are many enzymes found in the serum that did not originate from the extracellular fluid. During tissue damage, some of these biomolecules find their way into the serum probably by leakage through altered membrane permeability (Akanji and Yakubu, 2000). Serum enzyme measurements are therefore a valuable tool in clinical diagnosis, providing information on the effect and nature of pathological damage to any tissue. The reduced levels of serum ALP and ALT at all the doses of the extract after 14 days suggest inactivation of the enzyme molecule *in situ* (Umezawa and Hooper, 1982) or inhibition of the enzyme activity at the cellular/molecular levels (Akanji et al., 1993; Karmen et al., 1965). The decrease in the activity of serum AST at 25 mg/kg body weight of the extract and the enhanced activity of GGT at 50 and 75 mg/kg body weight implies that the extract exhibited dose specific effect on these enzymes. The raised level of serum GGT at 50 and 75 mg/kg body weight might imply leakage of this enzyme from the tissues into the extracellular fluid.

Renal function indices such as serum electrolytes, urea, creatinine and uric acid can be used to evaluate the functional capacity of the nephrons of animals at the glomerular and tubular levels (Yakubu et al., 2003). The effect of the extract of *S. aculeastrum* berries was selective and dose specific on the kidney function indices investigated. Creatinine, urea and uric acids are major catabolic products of muscle, protein and purine metabolism respectively. Although, the levels of uric acid remained unaltered by the extract, the increase in the creatinine concentrations as well as decrease in urea further implies selective toxic effect of the extract. The elevated levels of creatinine at all the doses suggest glomerular dysfunction (Chawla, 1999). The extract might have interfered with creatinine metabolism leading to increased synthesis or the tissue might have compromised all or part of its functional capacity of tubular excretion (Zilva et al., 1991), as renal disease which diminish the glomerular filtration rate lead to creatinine retention. It may also be an indication of adverse effect on the muscle biochemistry or inability of the kidney to excrete creatinine. Reduction in the urea level at 50 and 75 mg/kg body weight might suggest abnormality in the physiological excretion of urea caused by a non-renal factor which is the extract in this study. The non-significant effect of the extract on sodium, potassium and magnesium as well as the reduction in calcium levels coupled with the increase in chloride ions at specific doses further supported the selective effect of the extract on the biochemical indices of toxicity investigated in this study. In addition, the reduction in the level of phosphorus by the extract could be due to tubular dysfunction (Chawla, 1999).

Let, in this study, the assessment of haematological parameters was used as important biomarkers for evaluating the haematotoxic potential of the extract of *S. aculaestrum* berry. The reduction in WBC, although at 25 and 50 mg/kg body weight of the extract suggest that the constituents of the extract might have destroyed or impaired the production of these blood cells. It has been reported that granulocyte- macrophage colony stimulating factor, interleukins IL-2, IL-4 and IL-5 regulate the proliferation, differentiation and maturation of committed stem cells responsible for the production of these blood cells (Guyton and Hall, 2000; Ganong, 2001).

Therefore, the extract might have reduced the production of these regulatory factors or interfered with the sensitivity of the committed stem cells responsible for the production of these white blood cells (Adebayo et al., 2005). The elevated WBC at the highest dose may suggest enhanced production of these blood cells. The reduced platelet level at all the doses investigated might be an indication of platelet dysfunction (McGarry et al., 2005), resulting probably from adverse effect on the bone marrow.

Furthermore, the reduction in RBC and Hb levels only at the least dose (25 mg/kg body weight) may imply an attempt by the animals to adapt to the effect of the extract as the blood indices were increased at the higher doses investigated in this study. MCHC, MCH, MCV relate to individual red blood cells while haemoglobin, RBC, RCDW, LUC and PCV relate to the total population of red

blood cells in the blood. The alterations in these parameters suggest localized, dose specific effect of the extract. Since lymphocytes, neutrophils, monocytes, eosinophils and basophils are related to the WBC, the decrease in WBC may as well account for the reduction in the levels of these factors and may adversely affect their normal functioning in the animals.

The biochemical data in the acute and sub acute studies present evidence of mortality as well as adverse effect on the absolute weight of some of the organs investigated, haematological parameters, liver and kidney function indices of male rats. Therefore, the aqueous extract of the unripe berries of *S. aculaestrum* is not safe as an oral remedy as it has the potentials of being nephrotoxic, hepatotoxic, haematotoxic and can also result in the death of animals.

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