Evaluation of the Histomorphological and Toxicological Changes in Rodents after treatment with Hydroethanolic Extract of the *Secamone Afzelii* Aerial Parts

G. O. Mbaka[1] T. E. Akala[2]

[1] Department of Anatomy, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria
[2] Department of Anatomy, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Ikenne, Nigeria

Address for correspondence G. O. Mbaka, PhD, Department of Anatomy, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria (e-mail: mbaaka2gm@gmail.com; mbaakagm@gmail.com).

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Abstract

Objective To evaluate the histomorphological and toxicological changes in rodents after treatment with hydroethanolic extract of the *Secamone afzelii* aerial parts.

Materials and Methods An acute toxicity study on Swiss albino mice of both sexes was performed. Administration of a single dose of 2,000 mg/kg $^{-1}$ of body weight (bwt) of *S. afzelii* extract by gavages to 5 mice showed no mortality; hence, a 1/10th dose was used as the highest therapeutic dose. The intra-peritoneal administration produced dose-dependent mortality, with median lethal dose (LD_{50}) being $\sim$ 281.8 mg/kg $^{-1}$. In a subacute toxicity study, Wistar rats received daily administration of the extract in the dose range of 50 to 200 mg/kg bwt for 35 days. Its effects on histological, biochemical and hematological parameters were evaluated.

Results There was an initial body weight decrease in all the treated animals in the first 7 days. Thereafter, increase in body weight was observed. The treated animals also exhibited organ weight increase when compared with the control. Statistically significant increase ($p < 0.05$) in aspartate aminotransferase (AST) occurred in the extract treated animals, whereas alanine aminotransferase (ALT) showed a statistically insignificant ($p \geq 0.05$) increase when compared with the control. Total plasma protein (TPP) and albumin (ALB) also exhibited insignificant ($p \geq 0.05$) increases, while total bilirubin (T. BIL) decreased compared with the control. Insignificant ($p \geq 0.05$) changes also occurred in the plasma creatinine and urea levels in the extract treated animals when compared with the control group. The liver tissue histology showed no hepatocellular damage. However, inflammatory changes occurred in the testicular tissue, in which a quantitative decrease in spermatogenic cells was observed due to extensive cellular necrosis, leaving only the basement layer of the seminiferous tubules.

Conclusion *S. afzelii* exhibited a high safety margin, an indication that it is safe for consumption. However, after chronic administration, it caused undesirable effect in the testes that could compromise its fertility function.

Keywords
- secamone afzelii
- tissue histology
- acute and subacute toxicities

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Introduction

The use of plants or compounds derived from plants as therapeutic agents against various human and animal diseases is an age-long practice. Phytomedicine is renowned as the most common form of alternative medicine and is used by ~ 80% of the world population mainly among the Asians and Africans, with growing awareness among the Europeans, Americans and other developed countries where modern medicine is predominantly used.[1,2] The popularity of phytomedicine has continued to increase in spite of the giant stride and wide application of modern medicine. Its rising popularity is accounted for mainly due to the advantage of being an efficacious and cheap source of medical care. The high reliance in herbal medicine may be due to misconception that herbal products are devoid of adverse and toxic effects associated with conventional and allopathic medicines.

Toxicity can be considered as the degree to which a chemical compound could harm humans, animals or plants. A toxic agent usually has a target organ; therefore, its toxicity profile can be measured by its effects on that organ.[3] More often, besides the primary target organ, the liver could also be affected because of its role in synthesis. A central concept to toxicity is that its effect is dose-dependent.[3] What this means is that, if consumed above the appropriate dose, medication, whether herbal or allopathic, will create toxic effect to the body system.

It is instructive to note that herbs used in the treatment of different ailments usually contain a wide range of chemical compounds, which apart from being of beneficial effect to the body may also contain ingredients that have adverse effects to its component parts.[4] Incidences of adverse effects and sometimes life-threatening conditions emanating from these herbal medicines have been reported.[5–8] A major factor to adverse health conditions caused by herbal medication is lack of standardization, resulting in indiscriminate use without appropriate dose. It is unfortunate that most of the cases of affected individuals are undocumented because the people predominantly affected are rural dwellers with high reliance on herbal medicine. The absence of reliable statistics of the affected cases has undermined the alarming nature of its negative impact, particularly to this vulnerable group. Although it is difficult to quantify the morbidity and mortality levels of the affected cases, their incidence calls for global attention to toxicity profiling of medicinal plants, even though they have been used for ages, to enable the documentation of their safety/risk potentials.

Secamone afzelii is an herbal therapy that has wide application. It belongs to the Asclepiadaceae family and is widespread in West and Central Africa.[9] It is mainly found in secondary forest and savanna thickets, being more common in abandoned fields and field boundaries growing in a wide range of climatic conditions, particularly in the sun or in the light shade.[9] Its medicinal value includes the use in the treatment of gonorrhea, cough and catarrh and as galactogogue.[10] Its leafy twig infusion is taken to treat sexually transmitted diseases, diabetes and schistosomiasis.[11] Its use in the treatment of benign prostatic hyperplasia (BPH) has also been reported.[12] The phytochemical study of *S. afzelii* revealed a high concentration of flavonoids, saponins, reducing sugars, coumarins and the triterpenoid friedelin.[13] Because of the wide application of *S. afzelii* by the traditional healers, it was found necessary to evaluate its toxicity profile.

Materials and Methods

Plant Materials

The aerial part of *S. afzelii* was collected from Ikenne-Remo, Ogun State, Nigeria. The plant sample was authenticated in the Forestry Research Institute of Nigeria (FRIN), Ibadan, where the voucher specimen was deposited in the herbarium (FHI/108940).

Preparation of Hydroethanol Extract of *Sacamone Afzelii*

The aerial part of the plant was dried in the sun within the temperature range of 30 to 42°C for 5 days before being subjected to size reduction with an electric grinder until it turned into a coarse powder. The coarse powder of the plant, weighing 780 g, was extracted with 90% aqueous ethanol in 3 cycles using a Soxhlet extractor. The crude extract was filtered with Whatman filter paper No. 4 (GE Healthcare UK ltd Buckghamshire HP7 9NA, UK) and the filtrate was concentrated *in vacuo* at 30°C to obtain 68 g of residue weight (8.7% w/w). The residue was stored in an air tight bottle kept in a refrigerator at 4°C until used.

Animals

Adult male Wister rats (200 ± 5 g) obtained from the Animal House of the University of Ibadan, Oyo State, Nigeria, were kept under the standard environmental condition of 12/12 hour light/dark cycle. They were housed in polypropylene cages (five animals per cage) and were maintained on mouse chow (Livestock Feeds Nigeria Ltd.) and provided with water *ad libitum*. They were allowed to acclimatize to the laboratory conditions for 12 days before the experiment. The use and care of the animals, and the experimental protocol were in strict compliance with the Institute of Laboratory Animals Research (ILAR) guidelines on the use and care of animals, in experimental studies.[14]

Acute Toxicity Study

Fifteen Swiss albino mice, fasted for 14 hours, were administered with *S. afzelii* extract dispersed in acacia solution (2% w/v) intraperitoneally in graded doses of 125, 250 and 500 mg/kg $^{-1}$, with 5 mice per group, until 100% mortality was recorded. The control group of 5 mice was given 0.3 ml/kg $^{-1}$ (bwt) of acacia solution. The median lethal dose (LD_{50}) was calculated using the method of Miller and Tanter.[15] Another group of 5 mice, fasted for 14 hours, were administered a single dose of 2,000 mg/kg $^{-1}$ (bwt) of *S. afzelii* extract by gavages and then observed for 7 days for mortality and physical/behavioral changes. The animals did not show any mortality at the dose administered; hence, the 1/10th dose, which was 200 mg/kg $^{-1}$ (bwt), was chosen as the highest dose, and graded dose decreases of 100 and 50 mg/kg $^{-1}$ (bwt) were also used as therapeutic doses.[15]

Subacute Toxicity Study

A total of 20 male and female ten-week-old rats weighing 140 to 150 g were randomly allotted, 5 per group, to the control and the extract-treated groups. After fasting the animals overnight, the control group received a dose of 0.6 ml mg/kg $^{-1}$ of acacia (2% w/v) solution and the treated received 50, 100 and 200 mg/kg $^{-1}$ of the extract dispersed in acacia (2% w/v) solution. The doses were administered by gavages daily for a period of 35 days.[16,17] The animals were observed closely for any behavioral changes, body weight changes and mortality and were later sacrificed for biochemical and hematological investigations and organs histological changes.

Biochemical Parameters

Following the sacrifice under mild diethyl ether, blood was collected via cardiac puncture in two tubes. The ethylenediaminetetraacetic acid (EDTA) tube was used to collect blood for the analysis of hematological parameters, while the second tube with heparin was used to separate plasma from blood for biochemical estimations. The collected blood was centrifuged within 20 minutes of collection at 4,000 rpm for 10 minutes to obtain the blood plasma, which was analyzed for total cholesterol, triglyceride and high-density lipoprotein cholesterol (HDL-cholesterol) levels by modified enzymatic procedures from Sigma Diagnostics by modified enzymatic method.[18] The plasma was analyzed for alanine aminotransferase (ALT) activity, aspartate aminotransferase (AST) activity, and creatinine by standard enzymatic assay methods.[19] The urea content was determined according to the Urease-Berthelot method.[20] The protein content was determined using enzymatic spectroscopic methods.[21] Total bilirubin was estimated using Jandrassik and Grof technique.[22] Albumin was determined based on its reaction with bromocresol green (Binding method).[23]

Hematological Parameters

Hematocrit (HCT) was estimated using the method of Ekaidem et al.[24] Hematocrit tubes were filled to mark with whole blood, and the bottom of the tubes sealed with plasticide and centrifuged for 4 to 5 minutes at 4,000 rpm using an HCT centrifuge. The percentage cell volume was read by sliding the tube along a "critocap" chart until the meniscus of the plasma intersected the 100% line. Hemoglobin contents were determined using the Cyanmethemoglobin (Drabkin) method.[24] Hematocrit was determined according to Ekaidem et al,[24] while white blood cells (WBCs) and its differentials (neutrophil, eosinophil, basophil, lymphocyte and monocyte) were determined as described by Dacie and Lewis.[25] The blood samples were analyzed for red blood cells (RBCs) using the hemocytometic method.[25]

Histology Slide Preparation

The organs were fixed in 10% formal saline for 10 days. They were later removed from the preservative and dehydrated in increasing concentrations of alcohol; 70%, 80%, 90% and absolute alcohol (100%). The organs were treated with acetone and then cleared in xylene for 30 minutes to enhance their tissue transparency, which was followed by impregnating and embedding them in paraffin wax. The embedded tissues were sectioned at 5 µm, mounted on slides and then rehydrated in descending grades of alcohol before staining with hematoxylin and eosin (H&E) stains.[15] The stained tissues were examined under a PoTop PD-PB sereies (OME-TOP systems co., ltd 6FL, No. 176-1 Jhongshan 2nd Rd., Lujhou Dist., New Taipei City 247, Taiwan) light microscope (fitted with camera) at high power magnification (X100) for changes in organ architecture and photomicrographs taken.

Statistical Analysis

All values were expressed as mean ± standard error of mean (SEM) and the statistical significance between treated and control groups was analyzed by means of the Student *t*-test, with $p < 0.05$ and $p < 0.01$ considered significant.

Results

Acute Toxicity Study

The acute toxicity study showed that the animals fed by gastric gavages tolerated up to 9 g/kg $^{-1}$ of the extract. The intraperitoneal (IP) administration produced dose dependent mortality with an LD_{50} of the extract at 281.8 mg/kg $^{-1}$ (►**Table1**).

Body and Organ Weights

The effect of the extract on the organ weight of the control and treated animals is shown in ►**Table 2**, and the percentage of increase in the body weight of the treated animals compared with the control is shown in ►**Fig. 1**.

A decrease in body weight was observed in all the treated animals in the first 7 days of the treatment. However, from day 21 on, particularly in the low and medium dose treatments, the body weight of the animals showed progressive weight increase until the end of the experiment. Generally, there was insignificant ($p > 0.01$) increase in the organ weight of the treated animals compared with the control group.

Tissue Histomorphology/Pathology

Figs. 2–5 show the histological studies of the effects of *S. afzelii* extract on target organs. Tissue morphological alterations were examined in the treated groups compared with the control.

Table 1 Acute toxicity determination of the aerial part of *Secamone afzelii* (intraperitoneal route) at a dose range of 125 to 500 mg/kg^{-1}

Dose	Motility	% Motility	Log Dose	Probit	Probit Approx.
125 mg/kg^{-1}	1/5	20	2.0969	4.1584	4.2
250 mg/kg^{-1}	2/5	40	2.3979	4.7467	4.7
500 mg/kg^{-1}	4/5	80	2.6989	45.8416	8.2

Table 2 Data on the organ weight (100 g^{-1}) in rats after sub-chronic treatment with *S. Afzelii* extract

Treatment	Mean organ weight per 100 g body $^{-1}$				
	Heart	Lung	Liver	Kidney	Testes
Control	0.3 ± 0.2	1.9 ± 0.5	5.8 ± 0.3	1.0 ± 0.3	2.7 ± 0.4
50 mg/kg^{-1}	0.5 ± 0.1	2.1 ± 0.2	4.0 ± 0.3	1.5 ± 0.0	3.7 ± 0.5
100 mg/kg^{-1}	0.5 ± 0.0	2.1 ± 0.2	4.0 ± 0.0	1.5 ± 0.2	3.7 ± 0.5
200 mg/kg^{-1}	0.5 ± 0.2	2.2 ± 0.1	4.2 ± 0.4	1.6 ± 0.3	3.8 ± 0.4

Mean ± SEM, ($n = 5$) $^{*}p < 0.05$ versus control group.

The hepatic tissue of the control (►**Fig 2A**) showed normal architecture. It exhibited typical parenchymal appearance, though with indistinct hepatic lobules. The polygonal shaped hepatocytes were arranged as irregular cord-like structures interspaced by sinusoids, which showed normal convergence toward the central vein. In the animals treated with 200 mg/kg^{-1} (►**Fig. 2B**), the hepatic parenchyma exhibited no apparent inflammatory changes. The hepatic portal was remarkable with no sinusoidal congestion.

►**Fig. 3A** showed normal testis in which a cross section of seminiferous tubules indicated a tubular basement membrane supporting multilayered germinal epithelium. In the germinal epithelium were few unremarkable Sertoli cells that provided support to the spermatogenic cells. The organization of the spermatogenic cells arrangement are in strata with the most primitive (large spermatogonia), closest to the basement, while spermatids smaller in size showed projection toward the lumen with their tails forming a wavy appearance. In the animals that received 200 mg/kg^{-1} (►**Fig. 3B**), marked inflammatory changes occurred. There was severe tissue and cellular necrosis in the seminiferous tubules resulting in marked depletion in spermatogenic cells mass at the multilayered germinal epithelium. The cross section of the tubules showed residue of very scanty sperm cells with basement membrane largely exposed.

The renal tissue of the control group (►**Fig. 4A**) showed the normal architecture of the renal cortical tissue, in which were renal corpuscles appeared as rounded structures surrounded by a narrow space, the Bowman's space. The cortical tubules seen in this section consisted mainly of proximal convoluted tubules with few of the distal convoluted tubules indicated. In the animals treated with 200 mg/kg^{-1} of the extract (►**Fig. 4B**), no noticeable distortion was observed in the glomerular complex, convoluted tubules and in the cellular interstices, respectively.

The cardiac tissue of the control group (►**Fig. 5A**) showed myocytes with deeply stained nuclei separated by unremarkable interstitium. In the treated animals (►**Fig. 5B**), the myocytes showed no variation compared with the control group.

Chronic Toxicity Study

The effects of the extract on the biochemical parameters were summarized in ►**Table 3**. There was significant increase ($p < 0.01$) in aspartate aminotransferase (AST) at the doses administered, whereas alanine aminotransferase (ALT) showed insignificant ($p \geq 0.01$) increase in the treated animals when compared with the control group. Total plasma protein (TPP) exhibited insignificant ($p \geq 0.05$) increase in all the doses administered. Albumin (ALB) equally showed insignificant increase in the two higher doses while significant ($p < 0.05$) increase was observed in the lowest extract dose. Total bilirubin (T. BIL) showed decrease compared with the control. There were insignificant ($p \geq 0.05$) changes in the plasma creatinine and urea levels in the treated groups when compared with the control group. Alkaline phosphate (ALP) increased at low and medium extract dose treatments but exhibited decrease at the highest dose when compared with the control group. In the lipid profile study, there was insignificant ($p \geq 0.05$) increase in total cholesterol (T. Chol.). Triglycerides (TG) exhibited marked

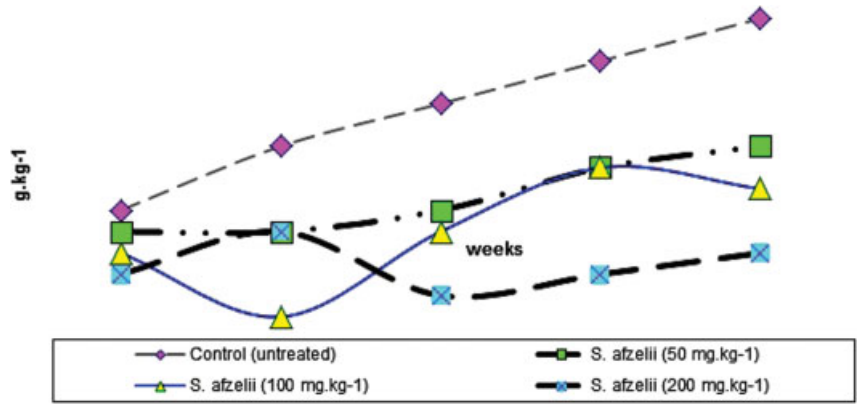

Fig. 1 Body weight differential in control and treated animals.

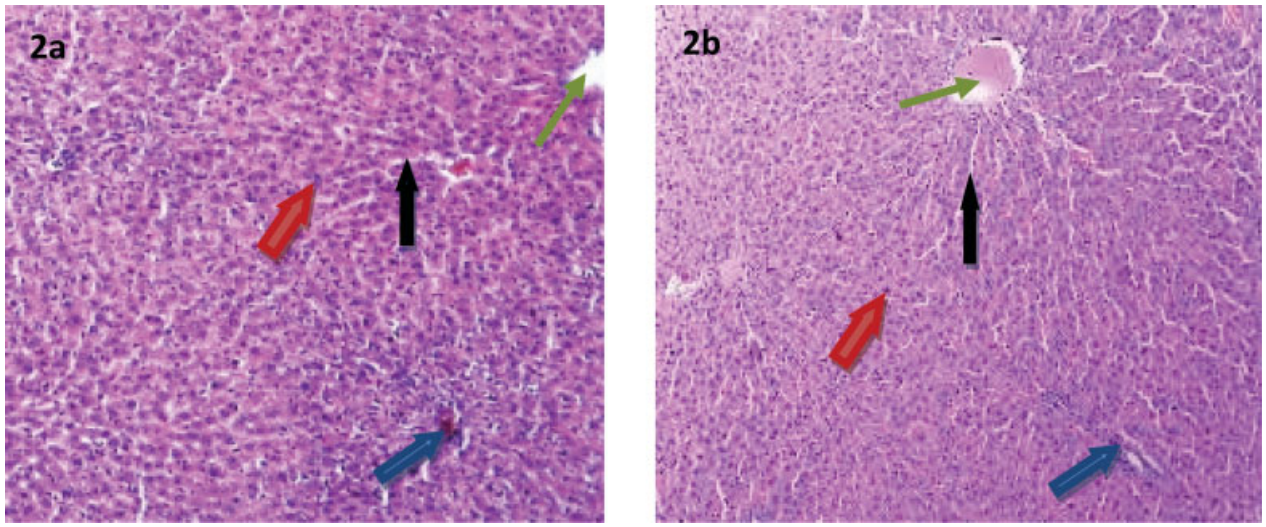

Fig. 2 (A) Photomicrograph of a cross section of normal hepatic tissue indicating portal tract (blue arrowed), hepatocytes (red arrowed), sinusoid (black arrowed) and central vein (green arrowed). Hematoxylin and eosin (H&E) stained. Mag. X100. (**B**) Photomicrograph of a cross section of hepatic tissue treated with 200 mg/kg^{-1} of the extract showed no abnormality. Hematoxylin and eosin stained). Mag. X100.

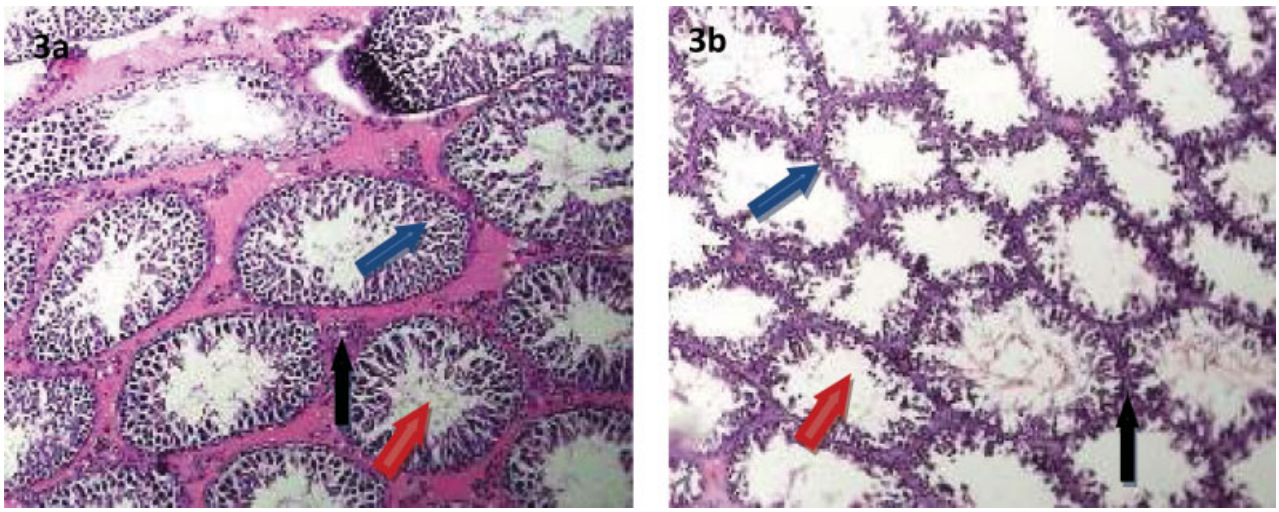

Fig. 3 (A) Photomicrograph of a cross section of normal testicular tissue indicating sperm cells (blue arrowed), interstitial cells of Leydig (black arrowed), and wavy tails of spermatozoa (red arrowed) (H&E stained). Mag. X100. (**B**) Photomicrograph of a cross section of tissue treated with 200 mg/kg^{-1} of the extract indicating quantitative sperm cells decrease, exposed basement membrane (blue arrowed) with wide lumen (red arrowed). Hematoxylin and eosin stained. Mag. X100.

increase at low and highest dose of administration while high density lipoprotein (HDL) showed decrease when compared with the control group.

The effects of *S. afzelii* extract on the RBC components and differentials were summarized in ►**Table 4**. Insignificant decreases ($p \geq 0.01$) were observed in the RBCS and haemoglobin content in all the groups compared with the control. The packed cell volume (PCV) decreased at low and medium doses but increased at the highest dose treatment, while WBC count showed insignificant increase in all the doses compared with the control. The RBC indices, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) exhibited insignificant increases ($p \geq 0.01$) when compared with the control group.

The effects of the extracts on WBC differentials are shown in ►**Table 5**. Lymphocytes exhibited significant increase ($p < 0.05$). Neutrophil showed increase that was marked at the lowest dose. There were insignificant increases ($p \geq 0.01$) in lymphocyte absolute (ABS) and in the mixed differential (MXD) when compared with the control group. Likewise, the platelets level increased markedly compared with the control.

Discussion

Plants used for medication are enriched with active principles. The type and the concentration of the active principle found in the diet and their metabolic clearance rate in the body are factors that may likely influence the toxicity of the plant.[15] Therefore, the safety of plant medicine for human use can be determined by means of a toxicological evaluation, which is usually performed in various experimental

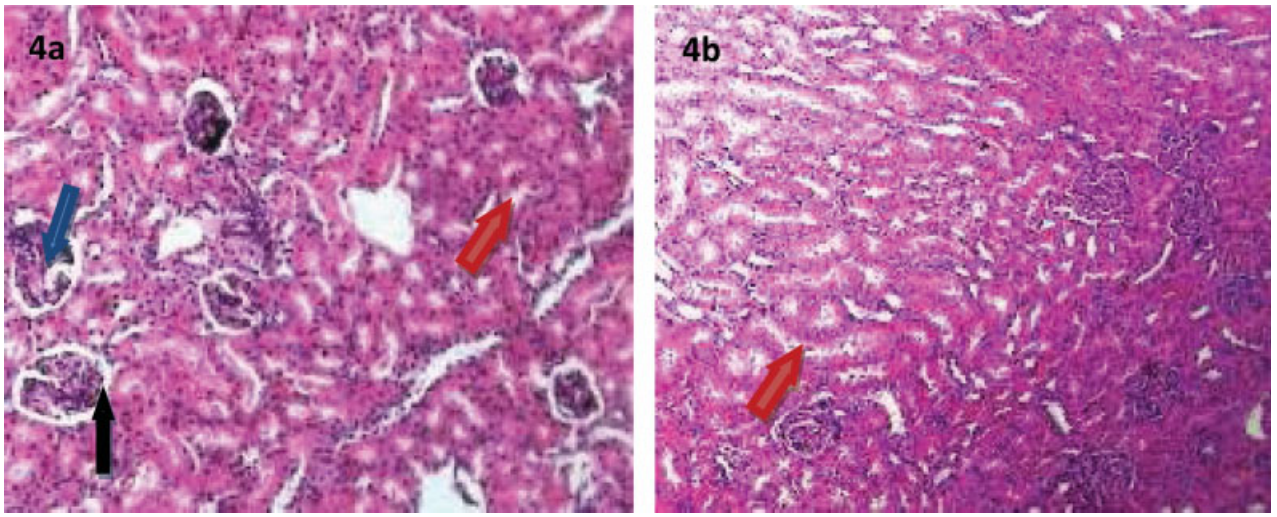

Fig. 4 (**A**) Photomicrograph of a cross section of normal renal tissue indicating glomerular complex (blue arrowed), Bowman capsule (black arrowed), and convoluted tubules (red arrowed). Hematoxylin and eosin stained. Mag. X100. (**B**) Photomicrograph of a cross section of renal tissue treated with 200 mg/kg^{-1} of the extract showed no abnormality. Hematoxylin and eosin stained. Mag. X100.

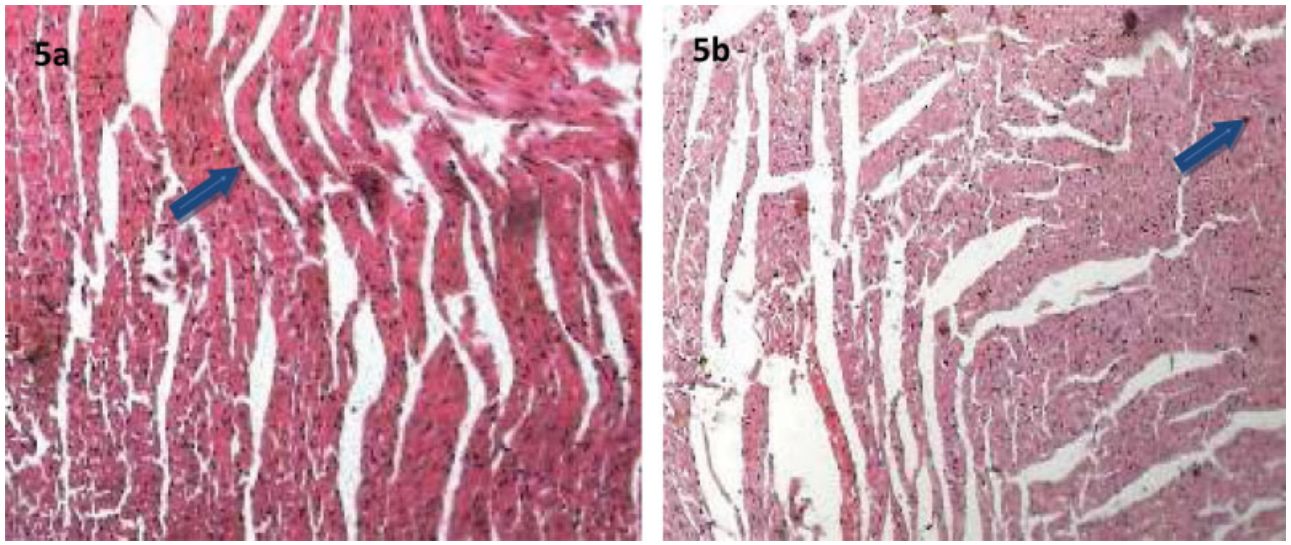

Fig. 5 (**A**) Photomicrograph of a cross section of normal myocardium indicating myocytes (arrowed) separated by interstitium. Hematoxylin and eosin stained. Mag. X100. (**B**) Photomicrograph of a cross section of cardiac tissue treated with 200 mg/kg^{-1} of the extract showed normal myocytes (arrowed) and normal interstitium. Hematoxylin and eosin stained. Mag. X100.

animal models to predict toxicity and to provide guidelines for selecting a safe dose in humans.

The acute toxicity study showed that *S. afzelii* extract may be generally regarded to be safe with remote risk of acute intoxication since the animals tolerated up to 9 g/kg $^{-1}$ bwt by gavages. More so, it showed much higher value than the World Health Organization's (WHO) toxicity index of 2 g/kg $^{-1}$ bwt.[7] The high degree of safety could be considered consistent with its popular use locally. Concerning the effect of the extract on the body weight of the animal, the result showed initial decrease in the treated group, which later exhibited gradual recovery after 7 days. Body weight loss is known to be caused by loss of appetite, malabsorption or the presence of anti-nutritional elements.[26] The improvement in weight, which was observed after 7 days, could therefore be due to adaptability. The gross anatomy of the organs revealed no detectable color changes, but organ weight increase was observed particularly in the testis. Increase in organ weight (organomegaly) has been noted as a sensitive indicator of adverse effect on organs, caused by standard toxicants.[27] The effect of the toxicant may have been responsible for the enlargement of the testis in this regard; more so, there was no correlation to the body size of the animal.

The liver is an important organ of the body because of its crucial role in various metabolic processes, and it is, therefore, particularly exposed to the toxic effects of exogenous compounds.[28] Alanine aminotransferase and AST are common liver enzymes because of their higher concentrations in hepatocytes, but only ALT is remarkably specific for liver function.[29] Aspartate aminotransferase, besides being found in the liver, is also found in equal amounts in heart, muscles, kidney, etc.[30] Hepatocellular injury leads to these enzymes release into the blood

Table 3 Blood chemistry values of rats in subchronic treatment with *S. Afzelii*

Parameter	Control	50	100	200
Total plasma protein (mg/dl^{-1})	65.9 ± 1.9	70.4 ± 2.7	70.8 ± 2.0	68.3 ± 3.2
Albumin (mg/dl^{-1})	36.6 ± 2.2	57.3 ± 1.5*	39.9 ± 3.0	39.1 ± 3.2
Total bilirubin (mg/dl^{-1})	1.4 ± 0.1	0.6 ± 0.0	0.6 ± 0.1	0.6 ± 0.0
AST ($iμ/L^{-1}$)	112.1 ± 4.4	123.2 ± 2.6**	124.5 ± 2.2**	127.9 ± 1.4*
ALT ($iμ/L^{-1}$)	59.5 ± 3.1	77.2 ± 1.4	60.7 ± 2.2	47.6 ± 2.4
Alkaline phosphatase ($iμ/L^{-1}$)	160.5 ± 2.4	170.2 ± 3.3	169.2 ± 3.0	141.3 ± 8.5*
Urea (mg/dl^{-1})	8.9 ± 1.6	7.9 ± 0.3	9.8 ± 0.6	9.4 ± 0.7
Creatinine (mg/dl^{-1})	39.5 ± 1.1	48.8 ± 0.1	46.7 ± 1.5	35.8 ± 3.7
Total cholesterol (mg/dl^{-1})	90.0 ± 0.2	87.4 ± 0.5	99.4 ± 0.4	95.3 ± 0.2
Triglycerides (mg/dl^{-1})	56.2 ± 0.4	77.5 ± 0.1*	58.6 ± 0.1	67.12 ± 0.4*
HDL-cholesterol (mg/dl^{-1})	41.1 ± 0.1	38.3 ± 0.1	38.2 ± 0.1	44.0 ± 0.1

Abbreviations: Alt, alanine aminotransferase; AST, aspartate aminotransferase; HDL, high-density lipoprotein.
Mean ± SEM, ($n = 5$) *$p < 0.05$; **$p < 0.01$ versus control group.

Table 4 Hematological values of rats in subchronic treatment with *S. Afzelii* extract

Treatment	RBC ($x10^6$)	Hb(g/dl)	PCV (%)	WBC ($x10^3$)	MCV (fl)	MCH (%)	MCHC (%)
Control	7.0 ± 0.1	10.5 ± 1.0	39.9 ± 0.9	4.6 ± 0.2	56.2 ± 1.2	16.1 ± 1.1	28.3 ± 01.1
50 mg/kg^{-1}	5.7 ± 0.5	10.3 ± 0.5	33.9 ± 2.0	9.2 ± 1.5	57.8 ± 1.1	17.6 ± 0.7	30.5 ± 1.6
100 mg/kg^{-1}	5.7 ± 0.2	10.1 ± 1.3	34.5 ± 0.2	7.6 ± 1.1	58.5 ± 4.11	18.0 ± 0.8	30.7 ± 1.1
200 mg/kg^{-1}	5.7 ± 0.8	10.4 ± 2.5	44.5 ± 2.5	5.2 ± 0.5	59.0 ± 7.3	18.4 ± 2.1	29.9 ± 0.9

Abbreviations: Hb, hemoglobin; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PCV, packed cell volume; RBC, red blood cell; WBC, white blood cell.
Mean ± SEM, ($n = 5$) *$p < 0.05$; **$p < 0.01$ versus control group.

Table 5 Quantitative data on white blood cell differentials in rats after sub-chronic treatment with *S. Afzelii* extract

Treatment	Neutrophil %	Lymphocyte %	Lymphocyte ABS	MXD %	Platelet %
Control	0.3 ± 0.1	62.6 ± 2.5	6.3 ± 1.2	23.3 ± 2.3	70.0 ± 1.6
50 mg/kg^{-1}	2.4 ± 0.8 **	73.9 ± 4.04*	6.8 ± 1.0	26.0 ± 4.0	84.4 ± 2.8*
100 mg/kg^{-1}	1.2 ± 0.2	82.5 ± 1.1*	6.3 ± 1.3	13.2 ± 1.2	62.2 ± 1.6*
200 mg/kg^{-1}	1.4 ± 0.3	71.9 ± 4.2 *	4.0 ± 0.4	25.4 ± 0.4	76.7 ± 4.2**

Abbreviations: Lymphocyte ABS, lymphocyte absolute count; MXD, mixed differential.
Mean ± standard error of the mean (SEM), ($n = 5$) *$p < 0.05$; ** $p < 0.01$ versus control group.

stream and their quantification in blood plasma is a useful biomarker to determine the extent and type of hepatocellular damage.[31] Hepatic tissue morphology of the treated groups showed normal cellular appearance with no hepatocellular damage. This finding also corroborated with the biochemical result in which the ALT plasma level was within the normal range. The appreciable increase observed in AST blood plasma could be attributable to the cytotoxic effect of the extract on other organs, since this enzyme is not liver-specific. In this case, the extract may have been implicated in testicular inflammatory changes. There was quantitative decrease in spermatogenic cells due to extensive cellular necrosis, leaving only the basement layer of the seminiferous tubules. Total plasma protein and ALB showed an increase in plasma levels, which was indicative that the extract helped to prevent oxidative damage to the liver. Increase in plasma level of these proteins is reported to have hepato-protective effect.[32] There was, however, a decrease in the plasma T. BIL level. Plasma bilirubin could be expressed as T.BIL, comprising of conjugated and non-conjugated, or as direct bilirubin, comprising only of the conjugated form. An increase in the plasma bilirubin level could be attributed to three major causes, such as hemolysis, biliary obstruction and liver cell necrosis.[33,34] The decrease observed in this study further confirmed that the extract had no deleterious effect on the liver. Alkaline phosphate showed insignificant increase at low and medium doses but decreased at the highest treatment dose. Alkaline phosphate is a marker enzyme present in high concentrations in the liver, and when hepatocytes are inflamed or damaged, these enzymes leak into the blood stream, leading to a rise in the plasma level of these enzymes.[35]

The kidney, much like the liver, plays a vital role in metabolizing toxic substances. Creatinine and urea, the two by-products of protein breakdown in the urinary system, are used as quantitative measure for kidney damage. An increase in the plasma creatinine level suggests kidney damage, more specifically damage to the renal filtration mechanism.[18,36] These end-products of protein metabolism, however, showed insignificant variation from the control group in this study, indicating that the risk of potential inflammatory challenge was minimal. Similarly, the tissue histology of the kidney of the extract-treated animals showed normal appearance, further confirming that the extract had no nephrotoxic effect. The extract also did not exhibit cardio-toxic effect, based on cardiac tissue morphology that showed normal appearance.

Hematological analysis of the plant extract is considered an important mode of assessing the toxicity of plants, having been noted as a major indicator of toxicity in phytomedicine.[8,37] There was a decrease in RBC, hemoglobin (Hb) and PCV values, except for the 200 g/kg^{-1} dose of the latter, suggesting decrease in erythropoiesis and the oxygen-caring capacity of blood and the amount of the oxygen delivered to the tissues.[38] The decrease in Hb level also suggested the likelihood of decrease in iron absorption. The RBC indices, MCHC, MCH and MCV exhibited insignificant increase, respectively. Increase in these parameters suggested that macrocytic anemia occurred, which is believed to be linked to iron deficiency.[39] The WBC count also showed increase, which might be due to challenge in body defense system. The WBC differentials, lymphocytes and neutrophils showed increase with more marked increase observed in lymphocyte which is the main effectors cell of the immune system.[40] Likewise, there was a stimulatory increase in lymphocyte that may have been triggered by the extract toxic challenge on the testis, which, in this case, appeared most vulnerable with extensive sperm cells damage. The increased neutrophil was indicative that the WBC differential was active as a phagocytic agent against foreign compounds. Neutrophil level is known to rise when there is an increased need for phagocytosis of damaged tissues.[7] The increase in the platelet count may have resulted from the stimulatory effect on thrombopoietin production.[41]

Conclusion

S. afzelii exhibited high safety margin, an indication that is safe for consumption. The prolonged administration of the extract did not provoke hepatotoxic, nephrotoxic and cardiotoxic effects. However, the quantitative decrease in spermatogenic cells in the extract treated group suggests that in chronic administration it might pose a threat to male fertility.

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