Evaluation of the acute and subchronic toxicity of the aqueous root extract of *Talinum triangulare* (Jacq.) Willd. [Portulacaceae]

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ABSTRACT

Background: *Talinum triangulare* (Jacq.) Willd [Portulacaceae] is commonly consumed as vegetable and used for medicinal purposes. Its root is used as tonic and for managing hypertension, asthma, schistosomiasis, scabies and fresh cut wounds. The aim of this study is to evaluate the toxicity of the aqueous root extract of *T. triangulare* (ARTT).

Methods: Acute toxicity tests of ARTT administered orally at 1,250−10,000 mg/kg and intraperitoneally at 125−1,250 mg/kg were carried out using albino mice. Subchronic (90 days) toxicity test of ARTT (10, 50 and 250 mg/kg; p.o.) was also performed using albino rats.

Results: The LD_{50} obtained for oral and intraperitoneal acute toxicity tests were 5,623 mg/kg and 398 mg/kg respectively. In the subchronic toxicity test, ARTT significantly ($p < 0.05$) reduced body weight and food intake of male and female rats. It significantly ($p < 0.05$) altered heart, pancreas, testes and ovaries weights of male and female rats. Histological examination revealed pathological changes in the structure of rats' lungs and kidney tissues. Red blood cells, hemoglobin, hematocrit and platelet count reduced significantly ($p < 0.05$) in female rats while white blood cells count reduced in both male and female rats. Significant ($p < 0.05$) increase in triglycerides and cholesterol were noted for male and female rats respectively. Alteration of biochemical indices of renal and hepatic functions among other effects were also observed.

Conclusion: These results show that ARTT induces weight loss, anorexia, pancytopenia, nephrotoxicity and other toxic effects. This finding indicates that the ingestion of ARTT poses significant health risk.

Key words: — Water leaf, weight loss, toxicity, sperm count, dyslipidaemia, kidney

1. INTRODUCTION

Talinum triangulare (Jacq.) Willd [Portulacaceae], a perennial herb with fleshy green leaves, succulent stem and pink flowers is commonly known as water leaf because of its high moisture content of almost 91% per 100 g of edible leaf [1]. Its leaf and soft stem are usually cooked and consumed as vegetable with its composition of nutritional components well reported in literature [2], [3]. Various parts of *T. triangulare* are used for various disorders in traditional medicine practice. Ameh and Eze [2] reported that the leaf and root are either used separately or in combination with other herbs for gastrointestinal disturbances or as diuretic. They also reported the use of the leaf and root for dressing fresh cuts and for managing schistosomiasis and scabies. The use of the leaf and root extract for the management of asthma has also been reported [4]. The whole plant's polysaccharides are protective against carbon tetrachloride-induced hepatotoxicity [5]. The root on its own is used in the form of a decoction to manage hypertension [6]; as a tonic for general fatigue and as replacement for ginseng in the management of inflammation [7]. It is known to share similar properties with ginseng [8] and is also used for managing prostate enlargement as well as measles. The anti-diarrhoeal effect of the aqueous extract [9] and molluscidal activity of the ethanolic extract [10] of the root have been demonstrated. An ethno-botanical survey by Fred-Jaiyesimi and Ajibesin [11] revealed that certain regions in the South-western part of Nigeria have observed a potential for *T. triangulare* root ingestion to result in toxicity. Ameh and Eze [2] also reported its use for rat poison preparation in the Eastern part of Nigeria. In spite of this, the root of *T. triangulare* is still ingested for medicinal purposes in various parts of the world including the regions of Nigeria where its potential toxicity was noted. According to Werjia [8], it is still considered healthy and added to stews and soups. Therefore, in the absence of experimental evidence for its toxic potential and mode of toxicity, its harmful potential may easily be waived off as

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mere anecdotal evidence. The question of the dose at which toxicity is observed also need to be addressed. In addition, some medicinal plants in today's alternative medicine practise have also been known to be poisonous to animals and even humans. It is best to rely on evidence from experimental findings rather than empirical or anecdotal reports to help users make informed decisions regarding their use. The aim of this study is to evaluate the toxic potential of the aqueous root extract of *T. triangulare* (ARTT) using sub chronic toxicity test.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Chemicals

Formalin (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and other reagents used were all of analytical grade.

2.1.2 Plant collection, identification and authentication

Fresh root of *Talinum triangulare* was harvested from a farm in Abule-Ado town in Ikorodu, Lagos State, Nigeria. The plant identification was done by Mr. T.K. Odewo, a Taxonomist and former superintendent of the Forestry Research Institute of Nigeria (FRIN) Ibadan, where a voucher specimen was preserved (voucher No FHI 107620). It was authenticated by Prof. J.D. Olowokudejo of Botany Department, Faculty of Science, University of Lagos, Nigeria.

2.1.3 Plant extract preparation

This was done following a slight modification of the method described by Adeyemi *et al*. [9]. The fresh root of *T. triangulare* was washed with distilled water, air-dried and then chopped into smaller pieces. The chopped root (100 g) was boiled in 1 L of distilled water for 1 hour after which it was left to cool at room temperature. The solution was filtered using cotton wool and filter paper. The filtrate was then evaporated to dryness in an oven at 40 °C. The dried extract gave a yield of 6.09%.

2.1.4 Experimental animals

Albino mice (average weight 23 g) and albino rats (average weight 135 g) of both sexes were used for the study. They were obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos, Nigeria. They were maintained under standard environmental conditions (23-25 °C temperature and 12 h/12 h light/dark cycle) and allowed access to feed and water *ad libitum*. Experimental protocols were carried out in accordance with the United States National Institute of Health's Guide for the Care and Use of Laboratory Animals [12].

2.2 Methods

2.2.1 Oral and intraperitoneal acute toxicity tests in mice

This test was performed following a slight modification of the method described by Omotoso *et al*. [13]. Briefly, after acclimatization, mice were divided into 13 groups of 5 mice each and fasted for 12 hours prior to the experiment. Six groups of mice were intraperitoneally administered ARTT (125, 250, 500, 750 and 1250 mg/kg) and distilled water (10 ml/kg) respectively. Seven other groups were orally administered ARTT (1250, 2500, 3750, 5000, 7500 and 10,000 mg/kg) and distilled water (10 ml/kg) respectively. The mice were then monitored for any form of abnormalities or behavioural changes for 2 hours following administration. At 24 hours post treatment, they were examined for mortality or any other sign of toxicity. The LD_{50} values of the extract for both routes of administration were determined using probit-log dose analysis.

2.2.2 Subchronic toxicity study in rats

2.2.2.1 Study design and assessment of the effect of subchronic ARTT treatment on body weight

This was carried out following guidelines provided by the Organization for Economic Cooperation and Development in 2007 [14]. Eighty (80) albino rats of both sexes were randomly allotted to 4 groups of 10 male rats and 4 groups of 10 female rats; each group was housed separately in polypropylene cages. The groups of animals were daily administered distilled water (10 ml/kg), ARTT at 10, 50 and 250 mg/kg orally respectively for 90 days. These 3 doses of ARTT represent one-fifth of the most pharmacologically active dose, the most pharmacologically active dose and five times the most pharmacologically active dose respectively [13]. The most pharmacologically active dose was determined from studies (data not published) on ARTT conducted in our laboratory. After 90 days of ARTT exposure, blood samples were collected via ocular puncture before humanely sacrificing the rats and carefully excising vital organs for further analyses. In the course of exposure and by the end of the exposure period, the rats were observed for alterations in body weight, food intake general morphology and behaviour. Biochemical, and haematological analyses of bio-specimens collected from the rats following cessation of exposure was also performed.

2.2.2.2 Assessment of the effect of subchronic ARTT treatment on vital organ weight and histology
By the end of the exposure period, the weight of vital organs including the brain, heart, lungs, spleen, kidneys, lungs, pancreas, testes (for male rats) and ovaries (for female rats) were determined following careful excision of each organ from humanely sacrificed animals. The weight of each organ was standardized to 100 g body weight of each animal. After weighing, the organs were quickly grossly examined and then stored in 10% formosaline for histological examination. In processing their tissues for histology, sections of the tissues were obtained using digital rotary microtome before staining of the sections following Mayer's Hematoxylin and Eosin staining technique for examination by light microscopy [15].

2.2.2.3 Assessment of the effect of subchronic ARTT treatment on haematological indices
Blood samples collected into ethylenediaminetetraacetic acid (EDTA) treated bottles were used for haematological analyses. Red blood cells, haemoglobin, haematocrit, white blood cells, platelet and lymphocyte counts were determined using an automated haematological analyser (Mindray, BC-2800, Shenzhen, China).

2.2.2.4 Assessment of the effect of subchronic ARTT treatment on serum biochemical indices
Serum biochemical parameters assayed for include aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total proteins, albumin, total bilirubin, creatinine, urea, uric acid, total cholesterol, triglycerides, low density lipoprotein and high-density lipoprotein following standard procedures. Serum electrolytes were analysed using standard procedures of flame photometry for sodium and potassium; cresol phthalein complexone method for calcium and titrimetric method for bicarbonate and chloride Serum samples were also analysed for indicators of oxidative stress including super oxide dismutase (SOD) using the method of Sun and Zigma [16]; reduced glutathione (GSH) using the method of Sedlak and Lindsay [17]; catalase (CAT) using the method of Sinha [18] and lipid peroxidation marker, malondialdehyde (MDA) using the method described by Soon and Tan [19].

2.2.2.5 Semen analysis of ARTT-treated rats
Semen samples were collected from the *Cauda epididymis* of male rats and analyzed for sperm count, morphology and motility using standard procedures earlier described [20].

2.3 Statistical Analysis
The results of the studies are expressed as mean ± standard error of the mean (SEM). The difference between the mean of control and treated groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using Graph pad prism software (La Jolla, CA, USA). The statistical value, $p < 0.05$ was set as the statistically significant difference level.

3. RESULTS

3.1 Effect of ARTT in oral and intraperitoneal acute toxicity tests in mice
Following oral exposure to relatively high doses of ARTT, an LD_{50} value of 5,623 mg/kg was obtained. For the intraperitoneal acute toxicity test, ARTT gave an LD_{50} value of 398 mg/kg (Figure 1).

3.2 Effect of ARTT in subchronic toxicity test in rats
3.2.1 Effect of ARTT on weekly changes in body weight and food intake
The extract at all treated doses significantly ($p<0.05$) decreased body weight of female rats. Food intake was also significantly ($p<0.05$) reduced at 250 mg/kg for both male and female rats (Table 1).

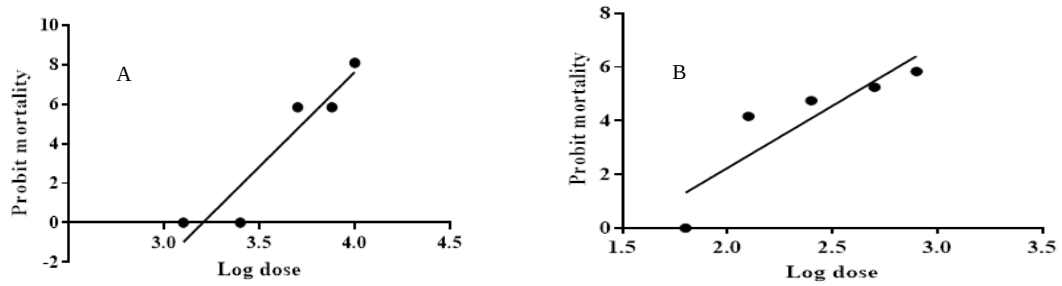

Figure 1: Graph of log dose vs probit mortality in mice following A) oral administration of ARTT (n= 5) LD_{50} = 5623.41 mg/kg. B) intraperitoneal administration of ARTT (n= 5) LD50 = 398 mg/kg.ARTT – aqueous root extract of *T. triangulare*

Table 1: Effect of subchronic ARTT treatment on body weight and food intake of exposed rats

Parameter Assessed	Sex	Control	ARTT (10 mg/kg)	ARTT (50 mg/kg)	ARTT (250 mg/kg)
Body weight (g)	M	179.50 ± 1.90	164.80 ± 7.10	172.10 ± 4.89	140.50 ± 8.34*
	F	180.40 ± 1.49	157.10 ± 2.23*	134.20 ± 5.15*	152.70 ± 3.99*
Food intake (g)	M	248.80 ± 0.61	248.50 ± 0.64	248.80 ± 0.59	238.30 ± 0.72*
	F	248.90 ± 0.71	245.50 ± 0.74	247.70 ± 0.99	235.30 ± 0.25*

Values are mean ± S.E.M (n = 5). *p < 0.05 vs control (One way ANOVA followed by Tukey's multiple comparison test). Control group received 10 ml/kg body weight of distilled water. M – Male, F – Female, ARTT –aqueous root extract of *T. triangulare*

3.2.2 Effect of subchronic ARTT treatment on vital organ weight and histology

The extract significantly ($p < 0.05$) increased the weight of male rats' hearts at 10 and 250 mg/kg; female rats' hearts at 10 and 50 mg/kg; male rats' liver at 10 and 250 mg/kg; female rats' pancreas at 10 and 50 mg/kg; testes at 10 and 250 mg/kg and ovaries at 10 – 250 mg/kg. It however decreased the weight of male rats' brain at 10 – 250 mg/kg; male rats' pancreas at 10 mg/kg as well as female rats' spleen at 10 mg/kg (Table 2). Interstitial inflammation with thick air-blood barriers were observed in lung tissues of male and female rats exposed to 50 and 250 mg/kg of ARTT. Renal vascular congestion was observed in kidney tissues of male rats exposed to 250 mg/kg of ARTT and interstitial inflammation in kidney tissues of female rats exposed to 50 and 250 mg/kg ARTT (Table 3).

3.2.3 Effect of subchronic ARTT treatment on haematological indices

The extract significantly ($p < 0.05$) reduced red blood cells, haemoglobin, haematocrit and platelets at 50 and 250 mg/kg, and reduced total white blood cells and lymphocytes at 10, 50 and 250 mg/kg among female rats. Among male rats, it significantly ($p < 0.05$) reduced white blood cells at 10, 50 and 250 mg/kg and also decreased lymphocytes at 50 mg/kg (Table 4).

Table 2: Effect of subchronic ARTT treatment on vital organ weights of exposed rats

Relative organ weight (g/100 g)	Sex	Control	ARTT (10 mg/kg)	ARTT (50 mg/kg)	ARTT (250 mg/kg
Brain	M	2.49 ± 0.02	2.29 ± 0.08*	1.83 ± 0.02*	1.68 ± 0.03*
	F	1.77 ± 0.01	1.81 ± 0.02	1.86 ± 0.03	1.89 ± 0.05
Heart	M	0.77 ± 0.11	1.22 ± 0.12*	1.04 ± 0.02	1.40 ± 0.19*
	F	0.86 ± 0.02	1.47 ± 0.02*	1.46 ± 0.03*	1.17 ± 0.01
Lungs	M	2.09 ± 0.06	1.83 ± 0.20	1.83 ± 0.04	2.24 ± 0.15
	F	2.26 ± 0.08	2.67 ± 0.04	1.92 ± 0.01	2.60 ± 0.05
Liver	M	7.37 ± 0.19	11.10 ± 0.66*	9.74 ± 1.14	15.38 ± 0.85*
	F	7.51 ± 0.23	7.78 ± 0.23	9.39 ± 0.16	8.45 ± 0.12
Pancreas	M	1.28 ± 0.05	0.94 ± 0.05*	1.07 ± 0.12	1.44 ± 0.06
	F	0.67 ± 0.07	1.51 ± 0.05*	1.32 ± 0.02*	0.94 ± 0.05
Spleen	M	0.93 ± 0.05	0.93 ± 0.09	0.73 ± 0.04	1.08 ± 0.16
	F	1.20 ± 0.20	0.67 ± 0.04*	0.82 ± 0.01	0.83 ± 0.02
Kidneys	M	1.86 ± 0.16	2.21 ± 0.24	1.86 ± 0.16	1.84 ± 0.07
	F	1.76 ± 0.02	1.99 ± 0.03	1.64 ± 0.03	1.49 ± 0.04
Testes	M	2.30 ± 0.04	3.56 ± 0.10*	2.48 ± 0.12	3.28 ± 0.25*
Ovaries	F	0.53 ± 0.02	0.75 ± 0.02*	0.76 ± 0.01*	0.78 ± 0.06*

Values are mean ± S.E.M (n=5) *p < 0.05) vs control (One way ANOVA followed by Tukey's multiple comparison test). Control group received 10 ml/kg body weight of distilled water. M - male, F – female, ARTT –aqueous root extract of *T. triangulare*

3.2.4 Effect of subchronic ARTT treatment on serum biochemical indices

The extract significantly ($p < 0.05$) increased the level of aspartate transaminase at 250 mg/kg and alkaline phosphatase at 50 mg/kg, while it reduced albumin level at 10 mg/kg in male rats. For female rats, it decreased alanine transaminase at 10 - 250 mg/kg; alkaline phosphatase at 50 mg/kg; bilirubin at 10 and 250 mg/kg; albumin at 50 mg/kg and total protein at 10 mg/kg (Table 5). At 10 – 250 mg/kg, ARTT significantly ($p < 0.05$) reduced urea levels of male and female rats but at 250 mg/kg, it raised their creatinine levels (Table 6). The extract significantly ($p < 0.05$) increased sodium and chloride electrolytes at 10 and 250 mg/kg; bicarbonate at 250 mg/kg and calcium at 10 – 250 mg/kg for male rats. For female rats, it increased sodium and chloride at 50 and 250 mg/kg; calcium at 10 - 250 mg/kg (Table 7). Regarding serum lipid profile, ARTT increased total cholesterol at 10 - 250 mg/kg but reduced triglyceride at 250 mg/kg in female rats and dose-dependently increased triglyceride level of male rats at 50 and 250 mg/kg (Table 8). The extract decreased reduced glutathione levels of male and female rats at 10 – 250 mg/kg; catalase in male rats at 10 - 250 mg/kg, in female rats at 10 and 50 mg/kg; and superoxide dismutase in female rats at 10 and 50 mg/kg. However, increase in superoxide dismutase and catalase at 250 mg/kg as well as peroxidase at 10 and 250 mg/kg among female rats were noted. Lipid peroxidation marker, malondialdehyde, was increased in male rats at 50 and 250 mg/kg and in female rats at 10 and 250 mg/kg (Table

9).

Table 3: Effect of subchronic ARTT treatment on histology of selected organs of exposed rats

Organs	Sex	Distilled water (10 ml/kg)	ARTT (10 mg/kg)	ARTT (50 mg/kg)	ARTT (250 mg/kg)
Liver	**M**	Normal	Normal	Normal	Normal
	F	Normal	Normal	Normal	Normal
Kidney	**M**	Normal	Normal	Normal	Blood vessel congestion
	F	Normal	Normal	Interstitial inflammation	Interstitial inflammation
Lungs	**M**	Normal	Normal	Severe interstitial inflammation	Severe interstitial inflammation
	F	Normal	Normal	Interstitial inflammation	Interstitial inflammation
Heart	**M**	Normal	Normal	Normal	Normal
	F	Normal	Normal	Normal	Normal
Brain	**M**	Normal	Normal	Normal	Normal
	F	Normal	Normal	Normal	Normal
Pancreas	**M**	Normal	Normal	Normal	Normal
	F	Normal	Normal	Normal	Normal
Spleen	**M**	Normal	Normal	Normal	Normal
	F	Normal	Normal	Normal	Normal
Testes	**M**	Normal	Normal	Normal	Normal
	F	Normal	Normal	Normal	Normal

M - male, F – female, ARTT –aqueous root extract of *T. triangulare*

Table 4: Effect of subchronic ARTT treatment on haematological indices of exposed rats

Parameters	Sex	Control	ARTT 10 mg/kg	ARTT 50 mg/kg	ARTT 250 mg/kg
RBC (10^3/µL)	M	7.89 ± 0.33	7.22 ± 0.08	7.80 ± 0.25	8.12 ± 0.36
	F	8.17 ± 0.52	7.65 ± 0.16	5.91± 0.52*	6.65 ± 0.19*
WBC (10^3/µL)	M	15.66 ± 1.75	12.24 ± 0.43*	7.65 ± 0.48*	9.08 ± 0.13*
	F	14.08 ± 0.83	8.86 ± 0.15*	6.92 ± 0.32*	8.66 ± 0.35*
HGB (g/dL)	M	13.80 ± 0.34	13.86 ± 0.35	14.26 ± 0.40	13.82 ± 0.31
	F	14.86 ± 0.16	13.36 ± 0.42	8.06 ± 0.19*	12.28 ± 0.55*
HCT (%)	M	42.40 ± 1.06	41.00 ± 0.50	43.02 ± 1.11	41.58 ± 0.43
	F	44.52 ± 1.07	41.56 ± 0.50	27.12 ± 1.84*	35.24 ± 0.93*
PLT (10^4 /µL)	M	626.80 ± 74.92	567.20 ± 50.15	579.60 ± 60.58	619.60 ± 21.71
	F	660.60 ± 38.57	635.20 ± 14.60	418.80 ± 7.21*	449.80 ± 13.72*
LYM (%)	M	7.84 ± 1.44	7.82 ± 0.44	3.94 ± 0.15*	6.08 ± 0.28
	F	8.24 ± 0.13	4.99 ± 0.10*	3.68 ± 0.19*	4.266 ± 0.17*

Values are mean ± S.E.M (n-5). *p < 0.05 vs control (One way ANOVA followed by Tukey's multiple comparison test). Control group received 10 ml/kg body weight of distilled water. RBC - red blood cell, WBC - white blood cell, HGB - haemoglobin, HCT – haematocrit, PLT - platelet, DLC - differential leukocyte count, LYM - lymphocytes, M - male, F – female, ARTT –aqueous root extract of *T. triangulare*

Table 5: Effect of subchronic ARTT treatment on hepatic function indices of exposed rats

Parameters	Sex	Control	ARTT 10 mg/kg	ARTT 50 mg/kg	ARTT 250 mg/kg
AST (u/L)	M	138.60 ± 1.56	159.10 ± 13.21	136.00 ± 11.72	181.20 ± 8.03*
	F	167.20 ± 3.03	135.70 ± 1.34	141.00 ± 4.52	189.90 ± 6.59
ALT (u/L)	M	65.45 ± 4.78	55.80 ± 0.96	68.14 ± 9.22	55.94 ± 1.30
	F	80.00 ± 1.19	48.96 ± 1.16*	48.36 ± 0.97*	59.59 ± 0.91*
ALP (u/L)	M	132.70 ± 1.06	147.00 ± 23.83	206.70 ± 23.11*	152.30 ± 7.43
	F	165.30 ± 8.61	121.40 ± 9.71	98.20 ± 6.74*	164.30 ± 2.50
BIL (µmol/L)	M	5.14 ± 0.05	4.68 ± 0.08	5.54 ± 0.08	5.08 ± 0.35
	F	6.26 ± 0.13	5.16 ± 0.14*	5.54 ± 0.10	5.22 ± 0.07*
ALB (g/L)	M	42.36 ± 1.85	35.36 ± 1.51*	42.38 ± 0.34	39.58 ± 1.41
	F	47.34 ± 0.50	44.46 ± 0.60	42.30 ± 0.33*	43.68 ± 0.46
TP (g/L)	M	76.80 ± 0.67	70.98 ± 1.64	78.26 ± 1.58	78.82 ± 2.94
	F	86.14 ± 0.83	75.72 ± 1.52*	82.34 ± 1.26	85.02 ± 1.38

Values are mean ± S.E.M (n=5), *p < 0.05 vs control (One way ANOVA followed by Tukey's multiple comparison test). Control group received 10 ml/kg body weight of distilled water. AST - aspartate transaminase, ALT - alanine transaminase, ALP - alkaline phosphatase, BIL - bilirubin, ALB - albumin, TP - total proteins, M - male, F – female, ARTT–aqueous root extract of *T. triangulare*

Table 6: Effects of subchronic ARTT treatment on renal function indices of exposed rats

Parameters	Sex	Control	ARTT 10 mg/kg	ARTT 50 mg/kg	ARTT 250 mg/kg
CRT (µmol/L)	M	44.18 ± 1.99	47.71 ± 1.65	45.53 ± 0.63	51.68 ±1.81*
	F	60.86 ± 0.87	50.76 ± 2.33	50.12 ± 2.10	70.19 ± 1.60*
Urea (mmol/L)	M	9.18 ± 0.09	6.92 ± 0.45*	6.55 ± 0.14*	7.64 ± 0.20*
	F	12.86 ± 0.40	7.98 ± 0.13*	9.56 ± 0.13*	8.46 ± 0.14*
UA (µmol/L)	M	81.19 ± 2.80	106.90 ± 12.81	61.83 ± 4.53	95.81 ± 13.70
	F	77.06 ± 1.40	50.52 ± 0.71	51.90 ± 1.23	73.40 ± 12.50

Values are mean ± S.E.M (n = 5), *p < 0.05 vs control (One way ANOVA followed by Tukey's multiple comparison test). Control group received 10 ml/kg body weight of distilled water.
CRT – creatinine, UA – uric acid, M - Male. F – Female, ARTT –aqueous root extract of *T. triangulare*

Table 7: Effect of subchronic ARTT treatment on serum electrolytes of exposed rats

Electrolytes (mmol/L)	Sex	Control	ARTT 10 mg/kg	ARTT 50 mg/kg	ARTT 250 mg/kg
Na^+	M	101.00 ± 2.61	127.40 ± 1.20*	101.20 ± 1.72	139.40 ± 3.04*
	F	98.40 ± 2.32	115.00 ± 4.91	138.40 ± 3.03*	119.40 ± 1.72*
K^+	M	4.16 ± 0.11	4.40 ± 0.16	3.94 ± 0.12	5.48 ± 0.18
	F	4.02 ± 0.09	4.32 ± 0.13	4.64 ± 0.18	5.12 ± 0.14
Cl^-	M	77.00 ± 2.12	95.60 ± 1.89*	76.40 ± 1.63	95.80 ± 1.86*
	F	73.00 ±1.30	76.40 ± 1.89	83.60 ± 1.44*	91.60 ± 1.08*
HCO_3^-	M	19.00 ± 1.41	19.40 ± 1.08	14.40 ± 1.08	25.80 ± 1.56*
	F	21.60 ± 1.08	17.00 ± 1.00	23.00 ± 1.41	19.40 ± 0.87
Ca^{2+}	M	1.02 ± 0.10	2.08 ± 0.06*	2.59 ± 0.10*	3.65 ± 0.05*
	F	0.79 ± 0.08	1.68 ± 0.09*	2.36 ± 0.10*	3.16 ± 0.016*

Values are mean ± S.E.M. (n = 5) *p < 0.05 vs control. (One way ANOVA followed by Tukey's multiple comparison test). Control group received 10 ml/kg body weight of distilled water. Na^+ - sodium ion, K^+ - potassium ion, Cl^- - chloride ion, HCO_3^- - bicarbonate ion, Ca^+ - calcium ion, M – male, F – female, ARTT –aqueous root extract of *T. triangulare*

Table 8: Effect of ARTT on serum lipid profile of exposed rats

Parameters	Sex	Control	ARTT 10 mg/kg	ARTT 50 mg/kg	ARTT 250 mg/kg
TG (mol/L)	M	0.80 ± 0.03	0.74 ± 0.04	1.08 ± 0.04*	1.36 ± 0.09*
	F	1.04 ± 0.04	0.80 ± 0.06	0.86 ± 0.05	0.64 ± 0.04*
CHOL (mmol/L)	M	2.12 ± 0.04	2.00 ± 0.03	2.26 ± 0.06	2.28 ± 0.09
	F	1.62 ± 0.14	2.02 ± 0.05*	2.00 ± 0.03*	1.96 ± 0.04*
HDL (mmol/L)	M	0.86 ± 0.07	1.22 ± 0.07	1.04 ± 0.09	1.14 ± 0.05
	F	0.72 ± 0.11	0.60 ± 0.10	1.08 ± 0.07	1.06 ± 0.14
LDL (mmol/L)	M	0.54 ± 0.37	0.20 ± 0.01	0.28 ±0.02	0.14 ± 0.03
	F	1.62 ± 0.14	2.02 ± 0.05	2.00 ± 0.03	1.96 ± 0.04

Values are mean ± S.E.M (n = 5). *p < 0.05 vs control (One way ANOVA followed by Tukey's multiple comparison test). Control group received 10 ml/kg body weight of distilled water. TG – triglyceride, CHOL – cholesterol, HDL - high density lipoprotein, LDL - low density lipoprotein, M – male, F – female, ARTT –aqueous root extract of *T. triangulare*

Table 9: Effect of subchronic ARTT treatment on oxidative stress markers of exposed rats

Parameters	Sex	Control	ARTT 10 mg/kg	ARTT 50 mg/kg	ARTT 250 mg/kg
GSH (u/mg)	M	0.06 ± 0.03	0.02 ± 0.01*	0.02 ± 0.01*	0.02 ± 0.00*
	F	0.07 ± 0.03	0.02 ± 0.01*	0.02 ± 0.00*	0.03 ± 0.01*
SOD (u/mg)	M	1.50 ± 0.18	0.97 ± 0.15	0.77 ± 0.21	1.16 ± 0.09
	F	1.24 ± 0.03	1.02 ± 0.01*	0.85 ± 0.02*	1.51 ± 0.01*
CAT (u/mg)	M	10.31 ± 0.24	6.73 ± 0.13*	5.25 ± 0.03*	7.89 ± 0.08*
	F	8.24 ± 0.10	6.71 ± 0.09*	5.63 ± 0.05*	10.06 ± 0.07*
MDA (u/mg)	M	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.01*	0.03 ± 0.01*
	F	0.01 ± 0.00	0.04 ± 0.02*	0.02 ± 0.00	0.05 ± 0.02*
PRX (u/mg)	M	0.02 ±0.00	0.03 ± 0.01	0.03 ± 0.00	0.03 ± 0.01
	F	0.01± 0.00	0.04 ± 0.02*	0.03 ± 0.01	0.05 ± 0.02*

Values are mean ± S.E.M (n - 5). *p < 0.05 vs control (One way ANOVA followed by Tukey's multiple comparison test). Control group received 10 ml/kg body weight of water. GSH - reduced glutathione, SOD - superoxide dismutase. CAT - catalase. MDA – malondialdehyde, PRX – peroxidase, M – male, F – female, ARTT –aqueous root extract of *T. triangulare*

3.2.5 Effect of subchronic ARTT treatment on sperm count, motility and morphology

A significant (p < 0.05) reduction in sperm count, motility, and corresponding increase in percentage of abnormal sperm cells was observed among exposed male rats. Whereas the decreasing effect of the extract on sperm motility was only significant at 250 mg/kg, sperm count reduced at all tested doses. The increase in the percentage of abnormal sperm cells was significant at 50 and 250 mg/kg of ARTT (Table 10).

Table 10: Effect of subchronic ARTT treatment on sperm count, motility and morphology of exposed male rats

Parameters	Control	ARTT 10 mg/kg	ARTT 50 mg/kg	ARTT 250 mg/kg
Sperm motility (%)	49.20 ± 2.06	43.30 ± 1.56	42.40 ± 3.01	39.00 ± 2.21*
Sperm count (million/mL)	67.00 ± 2.42	52.00 ± 2.29*	53.50 ± 1.28*	55.00 ± 2.09*
Abnormality (%)	1.20 ± 0.58	2.00 ± 0.71	5.80 ± 1.53*	7.00 ± 1.40*

Values are mean ± S.E.M. (n-5) *p < 0.05 vs control. (One way ANOVA followed by Tukey's multiple comparison test). Control group received 10 ml/kg body weight of distilled water. In assessing sperm morphology, the percentage abnormality was determined. ARTT –aqueous root extract of *T. triangulare*

4. DISCUSSION

The extract's LD_{50} values, 5623.41 mg/kg and 398 mg/kg, obtained for oral and intraperitoneal acute toxicity tests respectively, are comparable to 5514 mg/kg and 403 mg/kg reported by Adeyemi *et al*. [9] for both tests respectively. According to Loomis and Hayes [21] and the United States National Archives and Records Administration [22], a substance with an LD_{50} value ≥ 5000 mg/kg is practically non-toxic, while one with LD_{50} within the range of 50 – 500 mg/kg is moderately toxic. The lower LD_{50} value of ARTT via intraperitoneal route indicates that routes that allow for availability of higher concentration of the extract in the body following acute exposure, predisposes the recipient to ARTT-induced acute toxicity. The relatively low rate of absorption following oral administration resulted in a concentration of the extract too low to induce the level of toxicity observed with intraperitoneal administration. In the subchronic toxicity study, the effects of accumulated relatively lower doses of the extract (being $1/5^{th}$ of the pharmacologically active dose, the pharmacologically active dose and 5 times the pharmacologically active dose) were observed. Alterations in body and organ weights as were observed in this study, are important, sensitive, yet simple indices of toxicity following exposure to potentially toxic substances [23]. The extract significantly reduced body weight, possibly via mechanism(s) that include reduction in food intake, which could be due to loss of appetite [24] induced by ARTT. Ajagbonna *et al.* [25] reported that plants containing high concentrations of saponins may induce weight loss in exposed animals due to the appetite-inhibiting effect of saponins, which have been identified in the extract [9]. The extract significantly altered the weights of several organs of exposed male and female rats. Organ weight change is associated with organ toxicity, as previously reported for known toxicants [23]. An increase in organ weight is an important indication of organ hyperplasia/hypertrophy or organomegally [26]. The extract may therefore be toxic to the organs in question. Histological examination revealed thicker air-blood barrier in lung tissues of ARTT treated rats, which could impair respiratory capacity of the lungs, an effect that can prove fatal. The extract suppressed haematological parameters especially among female rats. Reductions in red blood cells parameters could be due to lysis of the cells and/or suppression of their synthesis. The haemolytic effect of the ethanolic leaf extract of *T. triangulare* had earlier been reported by Ekpo *et al*. [27]. Ezekwe *et al*. [28] also reported the haematocrit reducing effect of the methanolic leaf extract of *T. triangulare* in rats. The extract's reduction of platelets may also indicate its potential to suppress platelet synthesis. Reduction of white blood cells by ARTT reveals its potential to induce immune suppression, which could increase the risk of infectious diseases among users. The extract produced an AST/ALT ratio greater than 3. Elevations in AST/ALT ratios are occasionally associated with liver disease pattern in patients with non-alcoholic steatohepatitis [29]. Increase in AST level has also been associated with damage to other organs such as the heart, kidney and muscles. Although significant reductions in the level of ALT, ALP bilirubin were observed among female rats, reduction in albumin and total proteins among them suggest some level of hepatic dysfunction. Changes in albumin and ALP as observed among male rats also demonstrate the potential of ARTT to induce hepatic dysfunction in male rats. The lack of dose-dependency in some of these findings suggests that the effects may not be directly due to ARTT exposure alone. While some other factors not examined in this study may be involved in these observations, the role of ARTT cannot be ignored. Although the extract significantly reduced urea level among male and female rats, it significantly increased their creatinine level at 250 mg/kg, revealing a nephrotoxic potential of the extract as also demonstrated by the histopathological findings. The significant increase in the concentration of electrolytes in this study, could have arisen from the loss of hypotonic fluid and electrolyte-free fluids. Electrolyte-free fluids loss could be due to hypermetabolic states, resulting in dehydration and consequent elevations of measured electrolytes. Loss of hypotonic fluids is known to occur with certain types of diuresis and in association with some intrinsic renal disorders. Renal dysfunction has also been associated with hypercalcemia [30], which was observed in this study. The extract's increase in serum triglyceride level for male rats and cholesterol for female rats indicates its potential to induce alterations in lipid metabolism and dyslipidemia. The findings with respect to oxidative stress biomarkers indicate the extract's potential to induce oxidative stress in exposed rats. The observed reduction in antioxidant enzymes is usually indicative of a situation in which the enzymes would have been used up for scavenging of excess free radicals. Under such circumstance, an increase in antioxidant enzymes (as observed among female rats for catalase at 250 mg/kg and for peroxidase at 10 and 250

mg/kg of ARTT) indicates that a compensatory mechanism is at play to make more enzymes available for the mop up of excess free radical due to an existing oxidative stress state. Semen analysis revealed that ARTT reduced sperm motility and count while also increasing sperm abnormality. These conditions are valid indices of male infertility or reduced fertility in laboratory animals [31]. This effect as well as enlargement of the testes also observed with ARTT in this study implies that its sub-chronic administration induces a compromising effect on the male reproductive system. Regarding the differences between male and female rats' response in this study, differences in genetic make-up; temperature; hormones and hormonal cycles; circadian rhythm; and metabolism among others, could be responsible [32]. In this respect, studies designed to closely examine how these and other factors contribute to variations in response to ARTT by male and female rats would provide needed insight.

5. CONCLUSION

The results of the acute toxicity test shows that the extract can be toxic following acute exposure especially if administered via a route with less barriers to absorption as in the case of intraperitoneal administration. Sub-chronic daily repeated oral dosing of ARTT for 90 days induces anorexia, weight loss, hepatotoxicity, nephrotoxicity, dyslipidemia, oxidative stress, electrolyte imbalance and inadequacy of the male reproductive system capability. The potential of the extract to induce haemolysis and immune suppression were also demonstrated in this study. These observations provide evidence in support of the expected effects of *T. triangulare* root extract when used as a pesticide for pesky rats' population control but also indicate the need to strongly discourage its use in humans in regions of the world where it is still used for traditional medicine. Where necessary, it calls for careful evaluation of its risk relative to benefit before consideration of its use in traditional medicine.

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Declaration of interest statement

The authors declare no conflict of interest regarding this study.

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