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Research Article

Toxicological Evaluation of the Aqueous Leaf Extract of *Spermacoce princeae* (Rubiaceae): A Traditional Antibacterial Preparation

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ABSTRACT

Spermacoce princeae (Rubiaceae) is a medicinal plant used in the South-West region of Cameroon to treat some bacterial infections. The possible toxicological risks of aqueous leaf extract upon consumption were assessed in mice and rats. Acute and sub-chronic toxicity test was carry out using standard methods. after treatment, the on organs, as well as hematological and biochemical parameters was assessed using standard methods or kits. At doses above or equal to 16 g/kg, mice showed reduced locomotion, reduced reaction to noise and absence of faeces. A weight loss was noted in females that received 16 g/kg of extract during the first three days. The median acute toxicity value (LD50) of the extract were 13.20 g/kg and 9.60 g/kg for female and male mice, respectively. The extract at doses above or equal to 570 mg/kg raised serum ALT and AST activity, triglycerides, HDL-cholesterol and creatinine in male rats. In femele rats, and increase in serum total cholesterol level and red blood cell count was equally noted at all doses. Despite this increase, arteriosclerosis index was increases only in groups that received this extract at 1000 mg/kg and above. We noted and At all doses, the extract lowered urinary creatinine in males while in females treated at 1000 mg/kg and above, a decrease was also noted in heart and liver total protein for female rats and in kidneys for male rats. These data suggest that the aqueous extract of *Spermacoce princeae* leaves may be practically non toxic. However, precaution should be taken when high doses of this extract are to be used, and for a long period, as this may affect the central nervous system, induce constipation, hepatotoxicity, nephrotoxicity, and cardiovascular diseases.

Keywords: *Spermacoce princeae*, antibacterial, leaf extract, arteriosclerosis index.

INTRODUCTION

Plants and plant-based derivatives constitute an important part of the human health care system since ancient civilization[1]. Studies carried out by Raskin et *al*.[2] have shown that 30% of the commercialized drugs contain active principles that were isolated for the first time from medicinal plants. These plants can bring about a solution to certain diseases, mostly in developing countries where synthetic drugs are relatively expensive and not very accessible to the underprivileged social strata[3].

Whenever a chemical or natural substance is administer to a biological system, we are expected to have different types of interactions and a series of dose-related responses[4]. This dose-respond relationship can sometime be harmful to the body, depending on the toxicological power of the compound. They are many types of toxicity tests which are routinely performed and aim to study the adverse effect that short or long time administration of a pharmaceutical substance can caused to human. These include acute, sub-acute and chronic toxicity test.

Spermacoce princeae (Rubiaceae) is a medicinal plant used in the South-West region of Cameroon to treat some bacterial infections. It is an herbal plant that produces white flowers at maturity. It belongs to the Rubiaceae family which contains more than 100 000 species divided into 600 classes. Most of the plants of this family grow in tropical, subtropical and temperate regions. Water extracts from leaves and roots are used for Chronic asthma, cancer, wounds, eye problems, mastitis in cows, venereal, skin diseases, pneumonia, typhoid, caterpillar bites and diarrhoea[5-7]. The water macerate of leaves and stem is used for the treatment of female infertility in Baham, Cameroon[8]. Leaves, when warmed on fire are pulverized and mixed with salt and red oil, are taken orally for kidney diseases[9].

From our ethnobotanical survey, the entire plant is used in West and South-West Region of Cameroon to treat wounds and infectious diseases respectively. It also freshly grewn and the pate is applied on the wound or is mixed with palm oil and consumed for the treatment of typhoid fever. Despite the traditional uses of this plant, no report has been given on the eventual side effect that administration of this plant extract can induced. In the purpose to preserve the security of the consumers, possible toxicological risks of aqueous leaf extract of *Spermacoce princeae* upon consumption in the treatment of some bacterial infections were assessed in mice and rats.

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MATERIALS AND METHODS

Plant material

Spermacoce princeae leaves were collected in Fontem, South West region of Cameroon, in the month of May 2008 and authentification was carried out at the National Herbarium of Cameroon (NHC) where a voucher specimen (N° 19837/SRF/CAM) was deposited.

Experimental animals

A total of 60 healthy adult *Swiss* albino mice (30 males and 30 females) aged 11 to 12 weeks and weighing between 30 and 40 g were used for the acute toxicity study, and 50 *Wistar* albino rats (25 males and 25 females) weighed between 130 and 140 g and aged 8 to 9 weeks were used in sub-chronic toxicity experiment. All the animals were grown in the Animal House of the Department of Biochemistry (University of Dschang), Cameroon, and were given food and water *ad libitum*. The bioassay was conducted in accordance with the welfare of animals, as recommended by WHO[10].

Preparation of the plant extract

The leaves of *S. princeae* were dried at room temperature until constant weight and powdered to coarse particles. One hundred grams (100 g) of powder were soaked in 1 L of distilled water for 3 days with frequent stirring and filtered using Whatman No. 1 filter paper. The filtrate was concentrated in a drying oven at 45°C to obtain aqueous macerate.

Acute toxicity study

Acute toxicity assay was performed using standard method as described by Gatsing et *al.*[11]. Sixty adult mice (thirty of each sex) were acclimatize for seven days to the laboratory condition before the experiment during which standard normal diet and water were provided ad libitum. They were then randomly divided into six groups of five animals each and housed in separate cages. All animals were fasted for 18 hrs prior to the administration of the plant extract. Mice of groups 2, 3, 4, 5 and 6 were orally given graded doses of the extract, that is 2, 4, 8, 16 and 32 g/kg body weight, respectively, while those of group 1, served as control and received only distilled water (1 ml per 30 g of body weight). They were continuously observed for 3 hours to detect any signs of toxicity such as: changes in autonomic or behavioral responses (locomotion, aggressiveness), spontaneous activity (reaction to tail pinch and to noise), social interactions, corneal reflex, aspect of feces and mortality. When animals are gathered together, it is an indicator of communication (i.e. gathering); they are said to be in activity when they are roaming in the cage; they are say to be reactive when any attempt to touch them, they react by biting; normal reaction to noise is when the mice are unsettled on hearing a noise; the cries of mice when pinched on their tail is an indicator of normal reaction to pinch; the tail is normal when it is flexible (i.e. no rigid); rigid tail is a sign of anger. After this period, the animals were supplied with food and water *ad libitum*. The deaths were counted within the first 48 hours and LD_{50} value was determined in both sexes using the Behrens and Karber's formula[12]. The surviving animals were monitored for 14 days for changes in body weight, food and water intake.

Subchronic toxicity study

Repeat-dose oral toxicity study was carried out according to OECD guideline 407[13]. Fifty albino rats of both sexes were used. During acclimatization (seven days) they were given standard normal diet and tap water ad libitum. Animal were divided into 10 groups (5 males and 5 females) of 5 rats each. Groups 2 to 5 received 1.5 ml of extract at doses of 287, 570, 1000 and 2000 mg/kg body weight respectively during 28 day. Group 1 received 1.5 ml of distilled water and served as control. The test stand for 28 days during which extract was administered daily at the same time and observed at least twice daily for morbidity and mortality. Food and water intake as well as body weights of the animals were recorded daily.

Sample collection

On the 28th day of experiment, animals were subjected to overnight fasting during which their urine was collected[14]. Blood samples were collected by cardiac puncture from chloroform vapors anaesthetized rats into heparinised and non heparinised tubes. The non heparinised tubes were allowed to clot and were centrifuged at 3000 rpm for 5 minutes to obtain the serum. Animals were further dissected and gross pathological examinations were done including weighing of different organs (liver, kidney, lung, heart and spleen). Fifteen percent homogenate of these organs were prepared in normal saline solution[11], then centrifuged at 3.000 rpm for 15 minutes and the supernatant was used for protein quantification.

Biochemical analysis

The serum was assayed for creatinine, aspartate amino transferase (AST), alanine amino tranferase (ALT), total cholesterol, high density lipoprotein (HDL) and triglycerides using commercial kits (DIALAB.). Urine was assayed for total protein and creatinine using Bradford's[15] method and commercial kit (DIALAB) respectively. Serum and tissue protein's quantification was done by Biuret method as described by Gornall et *al.*[16].

Haematological analysis

Malassez chamber was used to quantify the total red blood cells (RBCs) and white blood cells (WBCs). Haematocrit was estimated using the method described by Ekaidem et *al.*[17].

Statistical analysis

Statistical analysis were done with the aid of SPSS for Windows software program (Release 12.0). Data were expressed and presented as mean ± SD. Group comparisons were done using the Post Hook test of Waller-Duncan. A p value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Acute toxicity

The behavioural changes observed during acute toxicity are summarised in Table 1. Mice were observed for gathering, reactivity, activity, state of tail and excrement, reaction to pinch and noise, mortality (within 48 hours).

Table 1: Effect of aqueous leaf extract of *S. princeae* on some physiological parameters in males and females mice

Parameters	Doses (g/kg) Females						Doses (g/kg) Males				
	0	2	4	8	16	20	0	2	4	8	16
Gathering	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
Activity	A	A	A	A	A-	A-	A	A	A	A	A-
Reactivity	R-	R-	R-	R-	R-	R-	R-	R-	R-	R-	R-
Reaction to noise	N	N	N	N	N	N	N	N	N	N	N-
Reaction to pinch	N	N	N	N	N	N	N	N	N	N	N-
State of the tail	N	N	N	N	N	N	N	N	N	N	N
State of excrement	g	g	g	g	Ab	Ab	g	g	g	g	Ab
Mortality (within 48 h)	0	0	0	0	4	5	0	0	0	2	5
DL_{50}	13.20 g/kg						9.60 g/kg				

NG: No gathering; A; activity; A-: decreased activity; N: normal; R-: No reactivity; g: granular; Ab: Abs

Table 2: Effect of aqueous leaf extract of *S. princeae* on food and water intake in males and females mice.

Sex	Doses (mg/kg)	Food intake (g)		Water intake (ml)	
		1st week	2nd week	1st week	2nd week
Female	0	34.32 ± 1.73^{a}	32.22 ± 2.09^{a}	47.80 ± 2.22^{a}	52.00 ± 2.07^{a}
	2	42.72 ± 4.88^{ab}	35.62 ± 2.21^{ab}	44.00 ± 7.19^{ab}	33.80 ± 1.59^{bc}
	4	37.04 ± 0.56^{ab}	41.70 ± 3.78^{b}	$36.20 \pm 2,13^{a}$	36.40 ± 2.11^{b}
	8	35.44 ± 4.81^{ab}	41.84 ± 2.54^{b}	37.60 ± 4.43^{a}	37.20 ± 2.24^{b}
	16	45.60 ± 0.00^{b}	43.70 ± 0.00^{b}	37.00 ± 0.00^{a}	31.00 ± 1.29^{c}
Male	0	39.46 ± 4.24^{a}	38.70 ± 1.49^{a}	$35,20 \pm 5.75^{a}$	43.40 ± 1.63^{a}
	2	50.20 ± 4.28^{a}	44.64 ± 5.12^{ac}	$35,40 \pm 2.60^{a}$	59.20 ± 1.28^{b}
	4	35.92 ± 2.24^{a}	34.02 ± 2.71^{a}	$36,80 \pm 2.39^{a}$	59.00 ± 1.41^{b}
	8	37.38 ± 5.57^{a}	51.24 ± 1.59^{c}	$46,00 \pm 1.40^{b}$	54.20 ± 1.56^{c}

The values that have different letters in the same column are significantly different ($p < 0,05$) Values are expressed as Means ± SD of five animals except the groups of females and males that received atleast 16 and 8 g/kg respectively. Values with different letters in the same column are significantly different ($p < 0.05$).

Table 3: Effect of aqueous maceration of *S.princeae* on the relative weight of the organs (g/kg).

Sex	Doses (mg/kg)	Relative weight (%)				
		Liver	Lungs	Heart	Spleen	Kidney
Female	0	38.60 ± 0.72^{a}	9.77 ± 0.63^{a}	4.08 ± 0.12^{a}	6.36 ± 0.71^{a}	7.21 ± 0.25^{a}
	285	38.30 ± 1.90^{a}	9.33 ± 0.57^{a}	3.84 ± 0.09^{a}	4.89 ± 0.29^{a}	7.23 ± 0.09^{a}
	570	37.45 ± 1.72^{a}	9.78 ± 0.72^{a}	4.04 ± 0.09^{a}	5.10 ± 0.55^{a}	7.04 ± 0.11^{a}
	1000	40.53 ± 4.50^{a}	9.49 ± 0.77^{a}	3.96 ± 0.22^{a}	5.34 ± 0.48^{a}	7.65 ± 0.58^{a}
	2000	41.75 ± 2.02^{a}	9.92 ± 0.55^{a}	4.22 ± 0.30^{a}	5.90 ± 0.21^{a}	7.14 ± 0.35^{a}
Male	0	28.66 ± 0.93^{a}	6.63 ± 0.22^{a}	3.12 ± 0.09^{a}	4.25 ± 0.72^{a}	7.05 ± 0.14^{a}
	285	30.72 ± 1.86^{ab}	7.82 ± 1.56^{ab}	$3.64 \pm 0,11^{ab}$	3.06 ± 0.17^{a}	8.14 ± 0.50^{ab}
	570	32.12 ± 0.70^{ab}	8.99 ± 0.46^{ab}	3.63 ± 0.15^{ab}	4.02 ± 0.22^{a}	8.31 ± 0.22^{bc}
	1000	33.26 ± 2.03^{ab}	8.97 ± 0.48^{ab}	3.90 ± 0.21^{b}	4.01 ± 0.28^{a}	9.03 ± 0.51^{bc}
	2000	34.70 ± 2.23^{b}	9.75 ± 0.58^{b}	4.05 ± 0.09^{b}	4.01 ± 0.28^{a}	9.37 ± 0.44^{c}

Values of this table are expressed as Means ± SD of five determinations. Values with different letters in the same column are significantly different ($p < 0.05$).

From the results obtained, no change was observed in all animals that were treated with the aqueous extract of *Spermacoce princeae* compared to the control concerning reactivity and state of the tail in both sexes. Also no change of reaction to pinch and noise was observed in all female mice that received the extract and in males that received at most 8 g/kg of extract. At doses less or equal to 16 g/kg, decreases of reactivity and absence of faeces were observed in mice of both sex compared to the control. In males that received at least 16 g/kg, reduced sensitivity to noise and pinch was noted. At doses less or equal to 8 g/kg and 4 g/kg in females and males respectively, no mortality was observed. LD_{100} (dose that causes 100 % mortality) values were 20 and 16 g/kg for females and males respectively, while LD_{50} was 13.20 g/kg for females and 9.60 g/kg for males. Female mice that received 16 g/kg of extract lost weight during the first three days (Figure 1). Furthermore, a weight loss was

Table 4: Effect of aqueous maceration of *S.princeae* on some blood parameters, total proteins in serum and urinary proteins.

Sex	Doses (mg/kg)	Hematocrit (%)	RBCs ($\times 10^6$/mm^3)	WBCs (per mm^3)	Serum total proteins (mg/ml)	Urinary pro-teins (mg/ml)
Female	0	35.50 ± 1.46^a	26.91 ± 3.49^a	732.00 ± 62.48^a	192.56 ± 8.68^a	4.99 ±1.05^a
	285	34.60 ± 4.14^a	56.31 ± 9.14^b	848.00 ± 98.30^a	175,20 ± 14.15^a	3.52 ± 0.55^a
	570	34.22 ± 1.81^a	49.71 ±5.87^b	796.00 ± 124.00^a	94.48 ± 12.38^a	2.89 ± 0.84^a
	1000	38.41 ± 1.74^a	52.21 ± 7.35^b	900.00 ± 90.99^a	177.12 ± 12.12^a	2.84 ± 0.00^a
	2000	36.56 ± 0.75^a	41.93 ± 5.08^b	1123.00 ± 174.49^a	154.00 ± 12.79^a	2.44 ± 0.27^a
Male	0	35.91 ± 1.90^a	23.89 ± 2.13^a	356.00 ± 67.94^a	259.52 ± 4.97^a	3.40 ± 0.56^a
	285	37.51 ± 0.65^{ab}	29.18 ± 3.39^a	352.00 ± 23.32^a	262.64 ± 3.80^{ab}	1.76 ± 0.27^{ab}
	570	33.87 ± 1.65^{ab}	30.95 ± 4.72^a	440.00 ± 160.26^a	278.00 ± 6.35^b	1.34 ± 0.31^{ab}
	1000	30.58 ± 2.85^{ab}	29.82 ± 6.19^a	728.00 ± 153.04^a	255.16 ± 6.39^a	1.30 ± 0.44^{ab}
	2000	30.33 ± 2.46^a	25.25 ± 4.16^a	474.80 ± 52.55^a	230.08 ± 10.63^c	1.18 ± 0.20^{ab}

Values of this table are expressed as Means ± SD of five determinations. Values with different letters in the same column are significantly different ($p < 0.05$).

Table 5: Effect of aqueous maceration of *S. princeae* onsome lipid compounds in serum

Sex	Doses (mg/kg)	Total cholesterol (mg/dl)	HDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)	Triglycerides (mg/dl)	Arteriosclerosis Index
Female	0	65.60 ± 3,72^a	48.43 ± 1.04^a	13.37 ± 2.94^a	38.99 ± 5.23^a	0.35 ± 0.05^a
	285	86.00 ± 3.89^b	57.58 ± 2.97^a	14.95 ± 4.06^a	67.78 ± 3.78^a	0.50 ± 0.10^{ab}
	570	81.60 ± 2.99^b	53.70 ± 3.12^a	14.04 ± 3.52^a	69.27 ± 17.11^a	0.53 ± 0.09^{ab}
	1000	96.00 ± 4.38^c	55.51 ± 8.11^a	27.21 ± 6.09^a	76.39 ± 17.72^a	0.97 ± 0.30^b
	2000	86.00 ±3.89^b	47.90 ± 3.60^a	25.03 ± 5.72^a	65.27 ± 8.20^a	0.82 ± 0.18^b
Male	0	181.60 ± 14.62^a	88.73 ± 13.23^a	72.90 ± 5.21^a	99.91 ± 25.13^a	1.19 ± 0.25^a
	285	144.00 ± 14.08^a	86.45 ± 12.07^a	37.57 ± 10.80^a	99.82 ± 12.01^a	0.73 ± 0.14 ^{a}
	570	176.00 ± 12.13^{ab}	104.19 ± 4.03^{ab}	51.04 ± 12.35^a	103.76 ± 14.73^a	0.69 ± 0.10^a
	1000	200.00 ± 15.59^b	118.71 ± 5.11^b	66.79 ± 12.46^a	111.96 ± 22.62^a	0.67 ± 0.09^a
	2000	160.80 ± 13.39^{ab}	80.64 ± 4.08^a	63.37 ± 14.83^a	152.88 ± 65.57^a	1.05 ± 0.22^a

Values of this table are expressed as Means ± SD of five determinations. Values with different letters in the same column are significantly different ($p < 0.05$).

Table 6: Effect of aqueous maceration of *S. princeae* on serum and urinary creatinine, serum ALT and AST level

Sex	Doses (mg/kg)	Serum creatinine (mg/dl)	Urinary creatinine (mg/dl)	ALT (IU/L)	AST (IU/L)
Female	0	2.06 ± 0.27^a	375.00 ± 45.92^a	26.17 ± 2.86^a	85.50 ± 5.32^a
	285	1.87 ± 0.16^{ab}	303.12 ± 32.20^a	40.13 ± 4.90^a	69.79 ± 8.84^a
	570	1.35 ± 0.15^b	277.50 ± 75.56^{ab}	42.92 ± 11.27^a	67.00 ±13.08^a
	1000	0.97 ± 0.22^c	180.00 ± 40.03^b	24.02 ± 5.78^a	128.43 ± 19.04^a
	2000	1.31 ± 0.08^{bc}	196.87 ± 38.19^b	35.24 ± 2.78^a	112.72 ± 38.82^a
Male	0	2.10 ± 0.43^a	600.00 ± 117.39^a	39.78 ± 5.47^a	99.91 ± 25.13^a
	285	1.57 ± 0.21^a	365.62 ± 24.80^b	125.64 ±13.82^b	99.46 ± 8.0 ^{a}
	570	2.32 ± 0.58^a	382.50 ± 30.00^b	116.21 ± 24.73^b	134.36 ± 12.09^a
	1000	2.17 ± 0.24^a	118.71 ± 5.11^b	106.79 ± 12.46^b	117.96 ± 16.82^a
	2000	1.50 ± 0.00^a	225.00 ± 26.51^b	133.70 ± 2.78^b	136.11 ± 16.55^a

Values of this table are expressed as Means ± SD of five determinations. Values with different letters in the same column are significantly different ($p < 0.05$).

observed in those that received 8 g/kg during the first week. Male mice that received this extract showed increased weight compared to the control (Figure2). This increase was inversely proportional to the doses administered (Figures 1 and 2). Significant increase ($p < 0.05$) of food intake was observed in female mice that received 16 g/kg during the first week and in animals of both sexes that received the extract during the second week (Table 2). Water intake decreased significantly in females and increased significantly ($p < 0.05$) in males that received the extract compared to the control during the second week (table 2). These results suggest that at doses above or equal to 16 g/kg, aqueous maceration of *S. princeae* can excite the central nervous system or can induce an increase in the neurotransmitters able to excite the central nervous system concerning the decrease of activity and sensitivity to noise (males) noted during the test[18]. The absence of faeces observed at these doses may be due to the extract that prolonged the intestinal transit; this increases the contact of excrements with the colon

epithelium, thus increases reabsorbtion of water and electrolytes and then provokes constipation[19]. This result corroborate those of Juruto et al.[7] which suggest that water extracts of leaves and root of *S. princeae* is used for many discomfort amount diarrhoea. The administration of the extract to male mice caused decrease reaction to noise at doses 16 g/kg. This may be due to the decrease in the biosynthesis of algonenic substances such as prostaglandins, histamines which function in regulating the perception of pain, or to inhibit the central level of transmission of painful messages[20,11,21]. The decrease of body weight observed in female mice may be related to the decreased of water intake whereas the increase of body weight observed in males may be due to the increase of food intake. LD_{50} values obtained showed that aqueous leaf maceration of *S. princeae* can be classified as practically non toxic according to Hodge and Sterner's scale[22].

Subchronic toxicity

Albinos Wistar rats were treated during 28 days, sacrificed and some organs were collected and weighed. The relative weight of the organs is presented in Table 3. These results showed that apart from the significant ($p < 0.05$) increase of heart and kidneys relative weight in male rats treated at 1000 mg/kg and above, no other significant variation was observed concerning this parameter either in male or in female rats. These results suggest that the aqueous maceration of *S. princeae* at high doses may cause hypertrophy of liver and heart in male rats.

Hematocrit, red blood cells, white blood cells, total serum proteins and urinary proteins were evaluated and results are presented in Table 4. These result showed no significant changes in hematocrit, white blood cells level and urinary proteins in both males and females. However, the number of red blood cells increased significantly ($p < 0.05$) in female rats receiving different doses of *S princeae* extract as compared to the control; suggesting that the extract may induce haematopoiesis. The significant ($P<0.05$) decrease in serum total proteins observed in males that received 2000 mg/kg and urinary proteins although not significant, may be due to the reinforcement of liver cells member and thus reduced their permeability and to increase in glomerular filtration. This main that, aqueous extract os *S. princeae* possess hepatoprotective and nephroprotective properties. This result corroborate those of Focho et al.[9] which showed that Leaves, when warmed and mixed with salt and red oil, are taken orally for kidney diseases.

Results of serum level of total cholesterol, HDL-

Table 7: Effect of aqueous maceration of *S. princeae* on tissue level of proteins. (mg/g of organs)

Sex	Doses (mg/kg)	Liver	Lungs	Heart	Spleen	Kidneys
Female	0	259.36 ± 13.39^{a}	242.07 ± 15.46^{a}	230.54 ± 6.87^{a}	282.89 ± 13.53^{a}	24.75 ±1.74^{a}
	285	190.22 ± 13.39^{bc}	242.07 ± 15.46^{a}	176.75 ± 11.39^{b}	292.02 ± 11.54^{a}	36.00 ±2.84^{ab}
	570	152.15 ± 13.83^{c}	207.48 ± 21.87^{a}	159.84 ± 15.04^{b}	328.52 ± 23.08^{a}	34.20 ± 4.40^{ab}
	1000	165.98 ± 16,94^{bc}	207.48 ± 48.90^{a}	153.69 ± 29.16^{b}	321.22 ± 13.65^{a}	61.20 ± 12.53 ^{c}
	2000	224.77 ± 33.70^{b}	242.07 ± 46.39^{a}	199.80 ± 11.90^{ab}	273.77 ± 18.25^{a}	49.50 ± 4.50^{bc}
Male	0	248.98 ± 20.16^{a}	338.90 ± 41.50^{a}	152.16± 23.45 ^{a}	211.71 ± 7.30^{a}	56.27 ± 5.11^{a}
	285	271.13 ± 40,69^{a}	297.40 ± 20.75^{a}	11,49± 1,11^{bc}	226.31 ± 49.5^{a}	22.50 ± 3.29^{c}
	570	276.65 ± 37.88^{a}	298.84 ± 22.87^{a}	12,33± 0,63^{b}	237.27 ± 8.16^{a}	32.39 ± 3.19^{bc}
	1000	228.23 ± 25.87^{a}	235.15 ± 45.61^{a}	10,82± 1,25^{c}	240.41 ± 14.60^{a}	32.39 ± 3.19^{bc}
	2000	276.65 ± 24.45^{a}	242.07 ± 32.80^{a}	11,61± 0,27^{bc}	246.39 ± 26.76^{a}	39.21 ± 3.41^{b}

Values of this table are expressed as Means ± SD of five determinations. Values with different letters in the same column are significantly different ($p < 0.05$).

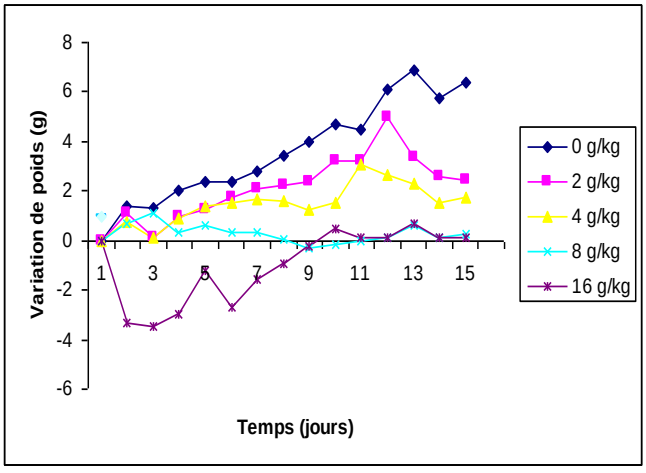

Figure 1: Effect of aqueous leaf extract of *S. princeae* on female mice body weight.

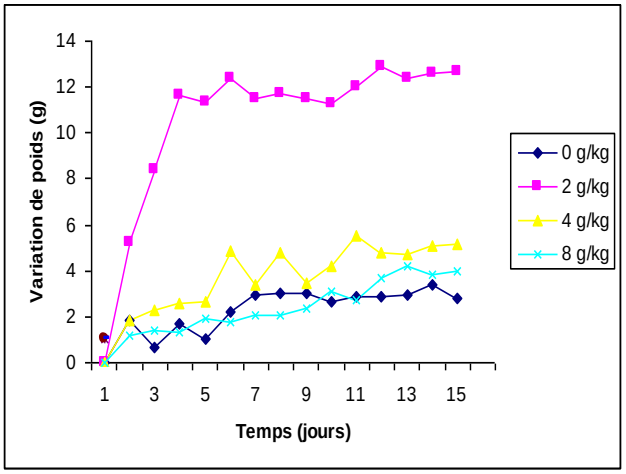

Figure 2: Effect of aqueous leaf extract of *S. princeae* on male mice body weight.

cholesterol, LDL-cholesterol and triglycerides and Arteriosclerosis Index are reported in Table 5. Apart from the significant ($p < 0.05$) increase of total cholesterol noted in female rats that received different doses of extract, no other significant modification was noted for these parameters compared to the control. The increase level of total cholesterol in these animals may be due to the stimulation of their production by the liver follow by the inhibition of their assimilation by the genital organs as oestrogen precursors[23,24]. Despite The significant increase of total cholesterol, the arteriosclerosis index was not significantly affected either in male or in female, and thus indicated that administration of this extract may not induce cardiovascular diseases.

The level of creatinine in serum and urine, and transaminases in serum was also evaluated using commercial kits. The results (Table 6) showed significant ($p < 0.05$) decrease of urinary creatinine The significant decrease of cratinine level in both serum and urine in female rates may no not be due to kidney defects, but to the inhibition of its production at the level of muscles. These results suggest that the aqueous maceration of *S. princeae* may not be nephrotoxic. Indeed, according to Schaffler and Menche[24], and; Aliyu et *al.*[25], decreases in urinary creatinine can be considered as sign of nephrotoxicity it is follow by increases of it's blood level.

The level of proteins in tissues is presented in (Table 7). This table showed a significant decrease of heart's and kidney's proteins at all doses in males compared to control; while in female rats at these same doses, a significant ($p < 0.05$) decrease of heart and liver's protein level was noted. The decreasing of the protein level in the tissues may be due to the inhibition of their production by the extract.

CONCLUSION

From these investigations it is clear that the aqueous leaves maceration of *Spermacoce princeae* used by traditional healers to treat some bacterial infections is practically non-toxic. However, it contains some compounds that may induce second effects. It may prolong the intestinal transit, excite directly or indirectly the central nervous system. It possesses nephroprotective and hematoprotective effect when administered at middle doses.

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