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IN-VIVO TOXICOLOGICAL PROFILE OF THE AQUEOUS EXTRACT OF THE ROOTS OF *SCHUMANNIOPHYTON MAGNIFICUM* (K. SCHUM.) HARMS (RUBIACEAE) IN WISTAR RATS

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Keywords:

Schumanniophyton magnificum (K. Schum.), Acute toxicity, Subchronic toxicity, Aqueous extract, Wistar rats

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ABSTRACT: Background: Also known as Gogologo, *Schumanniophyton magnificum* (K. Schum.) Harms is a medicinal plant used by the "Baka" Pygmies in the South region of Cameroon for the management of erectile dysfunction, but less information is known on its potential toxicity. **Objective:** This study aimed to assess the safety and toxicological potential of the aqueous extract of the plant's roots through acute and subchronic studies. **Materials and Methods:** The aqueous extract of the plant was administered *per os* once a day to 20 rats for the acute study (2000 mg/kg) and 40 rats for the subchronic study (100, 300 and 533 mg/kg) according to the guidelines 420 and 408 of the Organization for Economic Cooperation and Development. Then, animals were observed on general behavior, signs of toxicity, body weights, food and water intake. At the end of each experiment, biochemical, hematological and histopathological analyses were performed. **Results:** For the acute study, the single dose of 2000 mg/kg of the aqueous extract of *Schumanniophyton magnificum* caused no deaths, no change in the general appearance of the animals, suggesting that the fifty lethal dose is greater than 2000 mg/kg. The sub-chronic toxicity analysis showed that the extract significantly (P<0.05) lowered total cholesterol levels and the activity of aspartate aminotransferase in the serum. The extract induced a significant (P<0.05) increase in total protein, creatinine, and triglycerides levels compared to the control group. **Conclusion:** Results showed that the aqueous extract of *Schumanniophyton magnificum* could be safely used with doses up to 300 mg/kg body weight.

INTRODUCTION: Herbal treatments have long been used in traditional African societies to cure a wide range of illnesses in the decade.

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However, the lack of a defined chemical profile, a recommended dosage, and sufficient toxicity data to assess the safety of herbal medications has restricted their general appeal.

In addition, to acting as defensive systems against predators, plants can produce bioactive chemicals that are potentially harmful. It is now essential to evaluate the safety of plants used for medical purposes in order to rule out any potential toxicity [1]. Furthermore, the use of plant-based compositions

is particularly growing in developing countries of the world where up to 80% of the population can access [2]. So, medicinal plants remain the best alternative source for producing a variety of drugs from their bioactive principles (secondary metabolites). However, the administration of extracts from plants does not rule out their toxic effects. Studies have reported that plants are responsible for 3-5% of all reported intoxications, 17% associated with deaths [3].

Schumanniophyton magnificum (K. Schum.) Harms (Rubiaceae) is a tree species found in the tropical forests of West and Central Africa. The bark, leaves, wood and roots of the plant are widely used in African pharmacopoeia (Nigeria, Cameroon, and Gabon) to treat a range of pathologies, including otitis, diarrhea, skin diseases, subcutaneous parasitic infections, male sexual dysfunctions and venereal diseases [4]. In West Africa, the plant is used to treat snake bites. In Cameroon, *Schumanniophyton magnificum* (*S. magnificum*) is commonly used by Baka population (Pygmies) of the Southern region to treat erectile dysfunction [5]. Studies have shown that the plant has anti-hyperglycemic, antioxidant and organ-protective properties in dexamethasone-induced insulin-resistant in rats [6], and anti-venomous properties [7]. The aqueous extract of the plant has been shown to be effective on sexual maturation and fertility in immature female Wistar rats [8]. Keumedjio *et al.* [9] demonstrated that the extract of this plant stimulates the *in-vitro* production of testosterone. The phytochemical screening done with aqueous extract revealed that the plant contains alkaloids, phenols, flavonoids, glycosides, reducing sugars, saponosides, sterols and triterpenes that may be contributed to its pharmacological activities [9, 10].

Regarding the potential health benefits of the plant and lack of prior reports on its toxicological evaluation, the current investigation was conducted to determine the safety and toxicity of the aqueous extract of roots of *S. magnificumin* Wistar rats by assessing acute and sub-chronic toxicity studies.

MATERIALS AND METHODS:

Plant Collection and Extract Preparation: Fresh roots of *S. magnificum* were collected from Assock village in the Southern Region of Cameroon. The roots and leaves collected were identified and authenticated in the National Herbarium of Cameroon in Yaoundé, under a deposited voucher number 52761HNC. The plant name has been checked with http://www.worldfloraonline.org (accessed 30January 2024). The roots were air-dried in the laboratory and ground using an electric grinder. The plant extract was prepared according to the recommendations of the traditional healer. Briefly, 200 g of the dried powder of the roots were boiled for 30 min in2 liters of distilled water. After cooling, the obtained extract was filtered and the filtrate after concentration under vacuum, was stored in the fridge at 4°C.The extract was reconstituted daily in distilled water to prepare the different doses. The percentage yield of the plant extract was 12.01%.

Animal Material: Wistar albino rats of the either sex used in this study were obtained from the animal house of the Biochemistry Laboratory of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I. Animals weighing 120-200g and 90-110 g, were used for the acute toxicity and sub-chronic toxicity studies, respectively [11]. They were placed in clean, and sterilized plastic cages for a 14-day acclimatization period under laboratory environmental conditions (27±2°C), with free access to drinking water and food. The litter was changed daily and the cages were washed twice a month.

Ethical Approval: This work was carried out with respect for animal welfare, according to OECD recommendations. In accordance with academic standards, the study design was approved by the "Animal Ethics Committee", the Join Institutional Review Board of Animal and Human Bioethics in Cameroon under the following reference number: BTC-JIRB2022-038.

Evaluation of the Acute Oral Toxicity: Acute toxicity was assessed according to the Organization for Economic Cooperation and Development (OECD) guidelines 420 [12]. Twenty healthy rats 8-9 weeks-old rats (10 males and 10 females) weighing 120-200 g were randomly selected, and divided into four groups of 5 rats per sex each. After a 14-days of acclimatization period, the animals were kept in cages under laboratory conditions (standard temperature, relative humidity with a 12 hour

day/light cycle, fed *ad libitum*) and divided as follows:

1. A normal control group of male rats that received distilled water 1mL/100g, body weight (BW).
2. A test group of male rats treated with the aqueous extract of *S. magnificum* at a dose of 2000 mg/kg, BW.
3. A normal control group of female rats that received distilled water 1mL/100g, BW.
4. A test group of female rats treated with the aqueous extract of *S. magnificum* at a dose of 2000 mg/kg, BW.

All these groups of rats were administered *per os* once a day with either the extract or distilled water. The observation of signs of acute toxicity such as lethargy, off-feed, weight loss, diarrhea, restlessness, signs of neurological disorder was done daily during 14 days. The fifty lethal dose (LD_{50}) was determined according to the OECD guidelines 420. Thereafter, animals were fasted for 24 h and killed by decapitation under ether anesthesia. The blood from each animal was collected and centrifuged at 3000 g for 15 min. Then, the serum was collected and stored at -20°C for biochemical analysis. Vital organs (liver, kidney, heart, lungs, brain, spleen), and reproductive organs (ovaries, testis, epididymis and prostate) were collected and then fixed in Bouin's solution for histopathological examination.

Evaluation of the Sub-chronic Toxicity:

Experimental Design: The sub-chronic toxicity assessment was carried out in accordance with the repeated dose oral toxicity study guidelines 408 [13]. Forty male rats divided into four groups were treated daily with the extract for 52 days as follows: Rats in normal control group were administered 1 mL/ 100 g distilled water (DW); Test groups 1, 2 and 3 were given orally the aqueous extract (AE) of *S. magnificum* at the dose of 100, 300 and 533 mg/kg, BW respectively once a day for 52 days. The body weight of each rat was measured weekly. Throughout the study, animals were observed for signs of abnormalities including zoo technical parameters (food intake and weight gain), clinical condition of the animals (aggressiveness, somnolence, morbidity and mortality), sensitivity to sound, changes in skin color, hair appearance, eye color, tremors, and convulsions. After 52 days of treatment, rats were starved for 24 hours and killed by decapitation under ether anesthesia. The blood and organs from each animal were collected and processed as previously described.

This study was focused on male rats because the plant is used for the management of male infertility by the 'Baka' Pygmies of Cameroon.

Hematological Analysis: The blood samples were collected in EDTA tubes for hematological analysis using an automated blood analyzer (Cyan Hemato). The measured hematological parameters included white blood cell count(WBC), red blood cell count (RBC), hematocrit (HCT), hemoglobin (HGB), platelets (PLT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), Lymphocytes (L), mean corpuscular volume (MCV) and granulocyte concentration (GRA).

Measurement of Biochemical Parameters: Biochemical investigations of the liver and kidney were carried out by assessing gamma glutamyl transferase (GGT) (EC 2.3.2.2) activity using akit *BIOLABO* (81110) method described by Persijn *et al.* [14]. Alanine aminotransferase (ALAT)(EC 2.6.1.2) and Aspartate aminotransferase (ASAT) (EC 2.6.1.1) activities were determined by the colorimetric method of Reitman and Frankel [15]. The total cholesterol and HDL-cholesterol levels were determined by the enzymatic method described by Allain *et al.* [16] using a kit *BIOLABO* (Ref: 80106) while serum triglycerides levels were determined by the method of Fossati and Prencipe [17]. The LDL-cholesterol fraction was determined directly using the formula of Friedewald *et al.* [18]. The creatinine levels were assessed as described by Bartels *et al.* [19] and urea levels were determined by the modified colorimetric method of Berthelot. The total tissue and serum protein levels were determined by the method of Gornall *et al.* [20].

Histopathological Study: Histological analysis was performed by various steps including fixation, trimming, dehydration, inclusion, sectioning, staining, mounting and observation. A piece of each of organ (liver, kidney, brain, testis,

epididymis, and prostate) was fixed in a solution of 10% buffered formalin, picric acid and acetic acid; dehydrated in ascending grades of ethanol (70, 90, and 95%v/v), cleaned in xylene, and embedded in paraffin wax. Organs were thinly sectioned (5 µm) using a microtome and tissue sections were then prepared and stained with hematoxylin-eosinprior to the examination [21].

Statistical Analysis: Results obtained are expressed as mean ± standard deviation. Statistical analyses were performed using R software (version 4.2.3, Lyon). Data were analyzed using the Kruskal-Wallis test followed by the Dunn's post-hoc test. Differences were considered significant at $P<0.05$.

RESULTS:

Acute Toxicity Study: Oral administration of a single dose of the aqueous extract of the roots of *S. magnificum* (2000mg/kg, BW), did not induce any clinical signs of acute toxicity. In fact, after 14 days of treatment, no mortality or morbidity of animal was observed. Similarly, daily observations of trembling, convulsion, fecal appearance, changes in coat color, eye color, response to sound and altered gait revealed no abnormal changes in physical appearance in all the groups.

Determination of the 50% Lethal Dose (LD_{50}): No sign of toxicity was observed among the animals and no death was recorded during the 14-day experimental period. Hence, the fifty lethal dose 50 was estimated to be greater than 2000 mg/kg, BW. The extract can be classified in category 5, slightly toxic substances.

Evaluation of the Aqueous Extract of *S. magnificum* Roots on Biochemical Parameters: Biochemical analysis showed no significant difference ($P>0.05$) variation in the biomarkers of the liver (GGT, ALAT and ASAT) as well as kidney (creatinine, urea) in all the groups of treatment compared to the control groups **Table 1**.

TABLE 1: EFFECT OF AQUEOUS EXTRACT OF *S. MAGNIFICUM* ROOTS ON SERUM BIOCHEMICAL PARAMETERS AS A FUNCTION OF EXTRACT DOSES IN MALE AND FEMALE RATS

Sex	Male		Female	
	Plant extract (mg/kg)			
Biochemical parameters	**0**	**2000**	**0**	**2000**
GGT (U/L)	2.34 ± 0.86	1.82 ± 0.51	0.72 ± 0.42	0.90 ± 0.46
ALAT (IU/L)	66.62 ± 12.60	56.23 ± 15.73	40.79 ± 18.47	46.10 ± 10.11
ASAT (IU/L)	26.02 ± 6.59	23.15 ± 6.40	20.72 ± 3.99	14.10 ± 3.81
Urea (mmol/L)	1.09 ± 0.25	1.37 ± 0.37	1.59 ± 0.51	0.89 ± 0.32
Creatinine (µmol/L)	100.40 ± 29.71	73.03 ± 8.80	82.53 ± 18.25	59.43 ± 13.11

Values are expressed as mean ± SD (n=5). GGT: gamma glutamyl transferase; ALAT: alanine aminotransferase; ASAT: aspartate aminotransferase.

Histopathological Analysis of Liver and Kidneyl: Fig. 1 shows histological sections of the liver and kidney of female and male animals treated with the aqueous extract of *S. magnificum* for 14 days. Microscopic examination of these tissues showed no significant alterations, regardless of the group or sex considered.

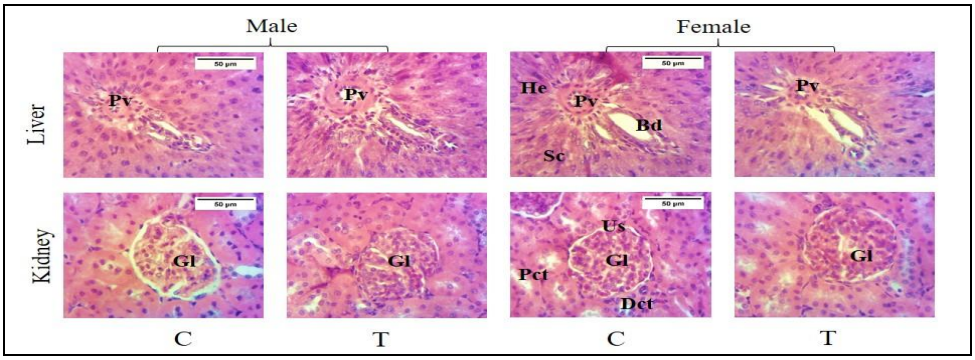

FIG. 1: PHOTOMICROGRAPHS OF THE LIVER AND KIDNEYS OF MALE AND FEMALE RATS AFTER 14 DAYS TREATED WITH THE AQUEOUS EXTRACT OF *S. MAGNIFICUM*. Pv= Portal vein; He = Hepatocytes; Bc= Bile canaliculus; Sc= Sinusoid capillaries; Gl= Glomerulus; Us= Urinary space; Dct= Distal convoluted tubule; Pct= Proximal convoluted tubule. C:control group,T: test group (2000 mg/kg).

Subchronic Toxicity Studies:

General Signs: As shown in **Table 2**, no toxicity sign was observed in the groups of rats treated with extract at 100 mg/kg and 300 mg/kg BW.

No death was recorded either in the control group or in the group of rats treated with extract at 100 mg/kg BW. However, we noted that 4 rats died in the group treated with extract at 300 mg/kg BW; where two were due to throat injuries.

More than 50% of successive deaths occurred in the group receiving the highest dose of extract (533 mg/kg BW), particularly from the 6th week onwards. The autopsy of these rats revealed a considerable loss of the adipose tissue.

TABLE 2: EFFECT OF REPEATED ADMINISTRATION OF AQUEOUS EXTRACT OF *S. MAGNIFICUM* ROOTS ON ANIMAL BEHAVIOR

	Groups			
	Plant extract (mg /kg)			
Observed symptoms	**0**	**100**	**300**	**533**
Mortality	A	A	Yes	Yes
Aggressively	A	A	A	Yes
Tremor	A	A	A	A
Convulsion	A	A	A	A
Handling reaction	N	N	N	N
Change in coat	A	A	A	A
Excessive agitation	A	A	Yes	Yes
Appearance of stools	N	N	N	N
Reaction to sound	N	N	N	N
Altered gait	A	A	A	Yes
Change in eyes	A	A	A	A

Values are presented as mean ± standard deviation (n=5). A: absent; N: normal.

Effect of Aqueous extract of *S. magnificum* Roots on Food Consumption: Fig. 2 shows a high food consumption in the test groups compared to the control group. In the treated-group with the extract at the dose of 533 mg/kg BW, the food consumption increased until day 26, and then it began to decrease. In the treated-group with the extract at the dose of 100 and 300 mg/kg BW, a saw tooth progression was observed, followed by an increase of food consumption from day 47.

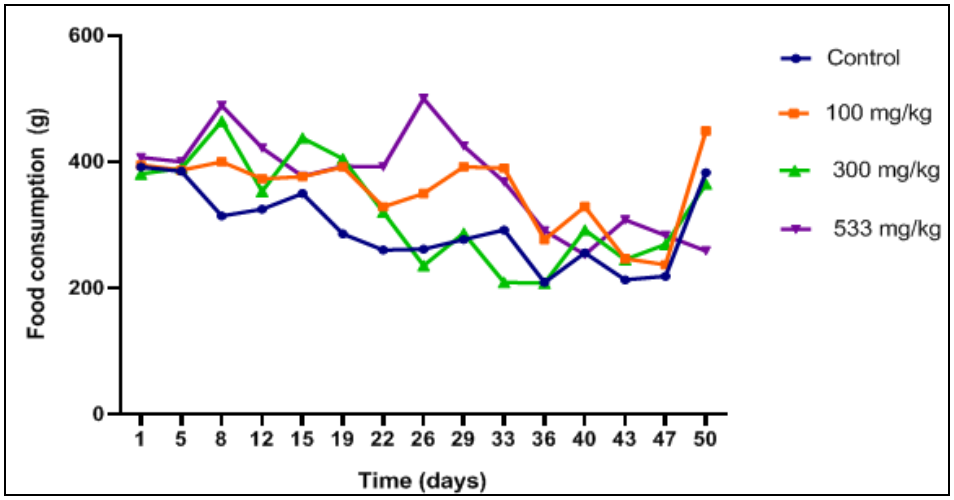

FIG. 2: CHANGES IN FOOD INTAKE IN MALE RATS AS A FUNCTION OF TREATMENT TIME. Values are presented as mean ± standard deviation (n=5).

Effect of Aqueous Extract of *S. magnificum* Roots on the Animal's Weight and Relative Organ Weight: The weight trends of the treated animals are shown in **Fig. 3**, which indicates a gradual body weight gain in all the groups. No significant differences (P>0.05) were observed in the relative weights of the isolated organs of animals in the test groups compared with the control group **Table 3**.

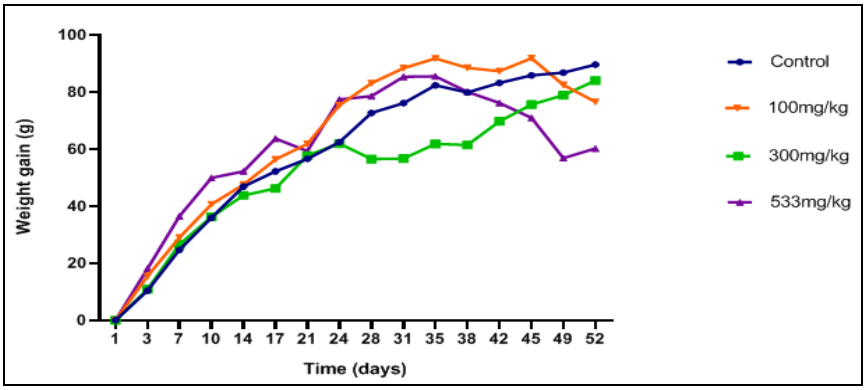

FIG. 3: WEIGHT GROWTH IN RATS TREATED WITH THE AQUEOUS EXTRACT OF *S. MAGNIFICUM* ROOTS AT VARIOUS DOSES. Values are presented as mean ± standard deviation (n=5).

TABLE 3: EFFECT OF REPEATED ADMINISTRATION OF THE AQUEOUS EXTRACT OF *S. MAGNIFICUM* ROOTS ON THE RELATIVE ORGAN WEIGHTS

	Relative organ weight (gper 100 g bodyweight)			
	Plant extract (mg /kg)			
Organs	**0**	**100**	**300**	**533**
Liver	3.10±0.62	2.99±0.39	3.23±0.05	4.01±050
Kidney	0.28±0.02	0.28±0.00	0.27±0.01	0.34±0.00
Lungs	0.72±0.22	0.81±0.10	0.82±0.10	1.01±0.08
Brain	0.83±0.08	0.88±0.21	0.84±0.10	0.91±0.06
Heart	0.36±0.10	0.37±0.02	0.34±0.02	0.33±0.01
Testes	0.42±0.00	0.43±0.02	0.40±0.01	0.38±0.01
Spleen	0.63±0.14	0.58±0.04	0.61±0.03	0.57±0.05
Prostate	0.09±0.06	0.08±0.01	0.13±0.03	0.07±0.00
Epididymis	0.14±0.03	0.17±0.01	0.18±0.00	0.18±0.01

Values are presented as means ± standard deviation (n=5).

Effect of Aqueous Extract of Roots of *S. magnificum* on Hematological Parameters: The hematological analysis **Table 4** revealed no-significantly increases in the WBC levels in rats treated with the extract at 100 and 300 mg/kg BW. Monocyte levels were relatively reduced by approximately 64.95% in these groups, while granulocyte levels were reduced by 29.89% in thetreated-group with the dose highest of the extract, compared to the control group. There was also a no-significantly increase in the erythrocyte lineage (P>0.05).

TABLE 4: EFFECT OF REPEATED ADMINISTRATION OF THE AQUEOUS EXTRACT OF THE ROOTS OF *S. MAGNIFICUM* ON THE LEUCOCYTE AND ERYTHROCYTE FORMULAS

	Groups			
	Plant extract (mg /kg)			
	0	**100**	**300**	**533**
WBC(10^9/L)	12.65 ± 0.93	13.01 ± 0.45	18.14 ± 6.20	4.65 ±0.41
LYM (%)	62.85 ± 8.06	71.25 ± 0.11	85.22 ± 1.93	73.37±0.41
MON (%)	16.70 ± 5.54	8.45 ± 0.27	8.72 ± 1.81	18.63±0.45
GRA (%)	20.44 ± 2.51	20.25 ± 0.35	6.11 ± 0.15	6.37±0.41
PLT (10^9/L)	455.30 ± 1.10	62.60 ± 0.79	520.05 ± 0.75	494.05±0.75
MPV(fL)	9.74 ± 0.22	9.10 ± 0.31	9.97 ± 0.34	897.00 ±0.34
RBC(10^{12}/L)	7.53 ± 1.16	8.36 ± 0.24	8.14 ± 0.21	7.02±0.41
HGB (g/dL)	13.46 ± 1.33	15.23 ± 0.43	15.07 ± 0.19	12.63±0.45
HCT (%)	43.66 ± 4.71	51.11 ± 2.05	48.50 ± 0.97	44.51±0.44
MCV (fL)	58.60 ± 3.71	61.30 ± 4.02	59.50 ± 1.11	59.90±0.07
MCH(pg)	18.02 ± 1.58	18.26 ± 1.04	18.51 ± 0.48	17.02±0.37
MCHC (g/dL)	30.82 ± 0.80	29.87 ± 0.82	31.02 ± 0.37	28.73±0.45

Values are presented as mean ± standard deviation (n=5). GRA: granulocyte concentration; HGB: hemoglobin; HCT: hematocrit; LYM: lymphocyte concentration; MCH: mean corpuscular hemoglobin; MCHC: mean cell hemoglobin concentration; MCV: mean corpuscular volume; MPV: mean platelet volume; MON: monocytes; PLT: platelets; RBC: red blood cell count; WBC: white blood cell count.

Effect of Aqueous Extract of Roots of *S. magnificum* on Serum Biochemical Parameters: As shown in **Table 5**, serum levels were elevated in the rats treated with highest dose (553 mg/kg BW) and in the middle dose (300 mg/kg BW).

Serum Gamma GT, ALAT and ASAT levels as a function of the different doses administered to the rats showed that there was no significant difference between GGT levels in the test and control groups (P>0.05).

There was no significantly difference for the value of ALAT in all the groups treated compared to the control. However, the results showed a significant decrease in ASAT activity in the group of animals treated with the extract at 300 mg/kg BW compared to the control group (P<0.05). But, no significant variation in the animals treated at 100 mg/kg or 533 mg/kg BW compared to the control group.

There was a non-significant variation (P>0.05) in serum protein concentrations in the test groups compared to the control group. The table shows the effect of our extract on serum urea and creatinine concentrations during treatment.

It can be seen that there was no significant difference (P>0.05) between the urea levels of animals in the test groups compared to those in the normal control group. On the other hand, a significant increase (P<0.05) in creatinine levels was observed in animals given the highest dose of extract (533 mg/kg BW).

Total cholesterol level was significantly (P<0.05) increased in the group of rats treated with the extract at 300 mg/kg BW. At the same concentration, there was no significant difference in HDL cholesterol (HDL-C) levels was observed between the groups.

However, results showed a significant increase (P<0.05) in the levels of HDL cholesterol in rats given EARS.m at 100 mg/kg and 533 mg/kg BW compared to the control. Also, we recorded a significant increase (P<0.05) in LDL cholesterol levels in animals in the test group where EARS.m was administered at 300 mg/kg BW.

Concerning atherogenic index, we noted a non-significant increase in the groups of animals treated with the extract, unlike the control group.

TABLE 5: EFFECT OF AQUEOUS EXTRACT OF *S. MAGNIFICUM* ROOTS ON SERUM BIOCHEMICAL PARAMETERS AS A FUNCTION OF EXTRACT DOSES

	Groups			
	Plant extract (mg /kg)			
Parameters	**0**	**100**	**300**	**533**
GGT(U/L)	0.68±0.11	0.69±0.17	0.43±0.07	0.81±0.22
ASAT(IU/L)	68.50 ±11.25	63.95±9.55	15.37±4.05[a]	46.12±1.11
ALAT(IU/L)	61.13±11.51	60.03±13.08	32.27±9.53	88.76±6.85
UREA (mmol/L)	4.58±0.33	5.04±0.63	5.07±1.08	4.62±0.65
CREA (µmol/L)	56.49±8.06	68.87±3.05	86.29±17.11[a]	108.60±7.14[a]
PROT (g/L)	54.43±4.31	57.69±2.07	57.43±0.58	60.67±0.19
HDL-C(mmol/L)	1.12±0.37	0.71±0.11	2.42±0.43	2.17±0.45
LDL-C (mmol/L)	0.53 ± 0.17	0.79 ± 0.24	1.45 ± 0.21[a]	0.90 ± 0.20
TG (mmol/L)	0.09 ± 0.04	0.22 ± 0.00[a]	0.19 ± 0.01	0.21 ± 0.05[a]
AI	0.35 ± 0.19	1.00 ± 0.60	0.78 ± 0.28	0.38 ± 0.12

Values are presented as mean ± standard deviation (n=5); [a]P<0.05: significant difference between the control and test groups; AI: Atherogenic Index; ALAT: Alanine aminotransferase; ASAT: Aspartate aminotransferase; CREA: Creatinine; GGT: Gamma glutamyl transferase; HDL-C: High-density lipoprotein-cholesterol; LDL-C: Low-density lipoprotein-cholesterol; PROT: Proteins; TG: triglycerides.

Effect of Aqueous Extract of *S. magnificum* Roots on the Liver, Kidney, Testis and Epididymis Histopathology: Histopathological changes **Fig. 4** were only observed in the group of rats treated with the aqueous extract at a dose of 533 mg/kg, BW (E3) which showed leukocytic infiltration of the liver, hemorrhage and mesangial expansion of the kidney, sperm agglutination in the epididymis and impaired spermatogenesis in the testes.

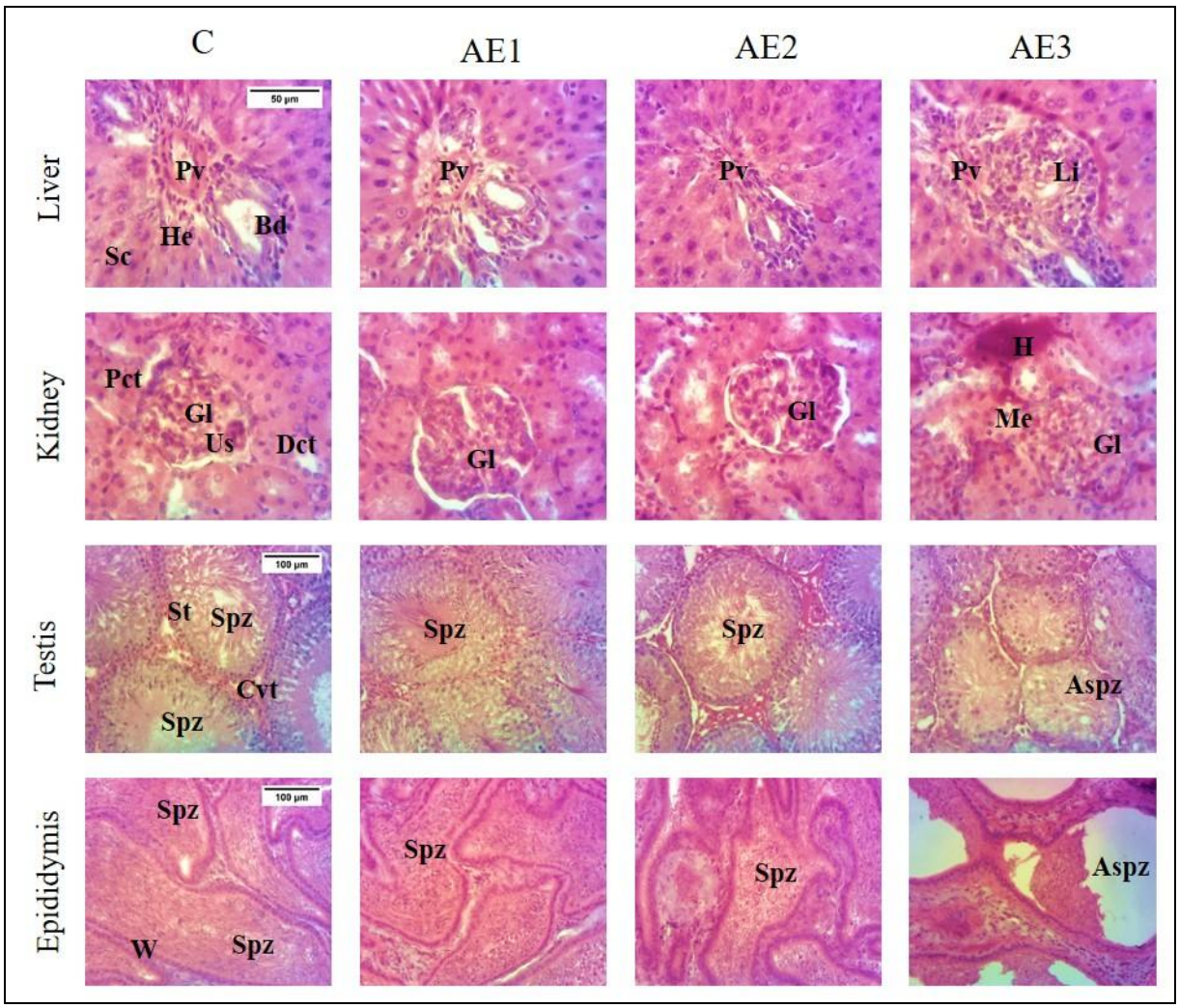

FIG. 4: PHOTOMICROGRAPHS OF HISTOPATHOLOGICAL CHANGES OF LIVER, KIDNEY, TESTIS AND EPIDIDYMIS; STAINED WITH HEMATOXYLIN-EOSIN. Aspz = Agglutinated spermatozoa; AE= aqueous extract; Bd = Bile duct; C= control; Cvt = Connective vascular tissue; Dct= Distal convoluted tubule; Gl = Glomerulus; H = Hemorrhage; He = Hepatocytes; Li = Leukocyte infiltration; Me = Mesangial expansion; Pct= Proximal convoluted tubule; Sc = Sinusoid capillaries; Spz = Spermatozoa; St = Seminiferous tubule; Us = Urinary space; Vp = Portal vein; W= Wall.

DISCUSSION: The uses of herbal remedies without a recommended or prescribed dosage and the dearth of sufficient scientific research on their safety have raised concerns on their toxicity [1]. In this study, we investigated the toxicological profile of the aqueous extract of the roots of *S. magnificum* through acute and sub-chronic toxicities assays. Recent studies have demonstrated the efficacy of EARS.m in improving male reproductive function by stimulating testosterone production [9], and enhancing the libido and sexual behavior in male rats. The screening of natural products for pharmacological activity requires prior assessment and evaluation of the toxicity of the extract or natural substance (one of the first steps), but this has not yet been done for this extract, hence the aim of our study.

The purpose of an acute toxicity study is to determine the lethal dose (LD_{50}) of a single dose of a test substance causing the death of 50 per cent of the animals when administered orally. The method used provides information that enables both hazard assessment and classification of substances in the Globally Harmonized Classification System (GHCS) for substances causing acute toxicity [12]. The results obtained after administration of a single dose (2000 mg/kg BW) of the aqueous extract of the roots of *S. magnificum* revealed no negative behavioral changes with regard to convulsion, reaction to sound, agitation or respiration. No deaths were recorded during treatment in animals of either sex. The lack of significance in food consumption, water intake and weight gain in treated animals compared to the control, and

similarly in terms of the relative weights of the target organs, leads us to believe that the extract exerts no visible adverse effects. So, the aqueous extract of the roots of *S. magnificum* administered *per os* at 2000mg/kg BWdisplayis relatively safe andnon-toxic. In accordance to OECD, this extract is assigned to category 5 (2000 mg/kg<LD_{50}≤5000 mg/kg). These results are similar to those referenced showing thatthe LD_{50} of the aqueous extracts of *Psychotria calceata* and *Heinsia crinita* is greater than 2000 mg/kg BW [22, 23].

In order to verify whether our extract, despite having an LD_{50}>2000 mg/kg cannot cause lesions on the main target organs of toxicity (liver and kidneys); we assayed some biochemical markers such as GGT, ALAT, ASAT, and urea, creatinine, as well as the histology of these organs. No significant (P>0.05) variations of the level of these enzymes and no histopathological changes were observed compared to the control. These results indicated that the plant extract is non-toxic at this dose.

Since there was no indication of treatment-related toxicity during the acute toxicity evaluation, additional testing was carried out to assess the effect of the aqueous extract of the roots of *S. magnificum* repeated daily dosage on some metabolic markers in rats for 52 days. The goal was to provide thorough toxicological information on this extract at different dosages (100, 300, and 533 mg/kg, BW). Generally, it is reasonable to conclude that the plant extract is unlikely to be hazardous at the tested doses over the observation period given that a 52-day daily dose therapy with the extract produced no clinical indicators of toxicity, morbidity, or death across all treatment groups. Nevertheless, we detected a few signs of toxicity in the animals given the highest dose of extract (533 mg/kg, BW) compared with those in the control group. Also, changes were observed in behavior, animals were more agitated, due to the effect of the aqueous extract of *S. magnificum*, known to induce the onset of puberty [8]; they overreacted to handling, and their gait was altered over time. From day 42 onwards, we began to record deaths. This could be explain by the presence of glucosides and other phytochemicals compounds present in higher quantity in our extract, which are toxic [24].

It was observed that oral administration of EARS.m for a period of 52 days influenced the body mass of animals treated with different doses, compared with those in the control group. This variation was not perfectly correlated with food consumption, suggesting that the extract may stimulate and facilitate the absorption of nutrient and permit to rats to eat more and gain the weight in the group treated with doses lower than 533 mg/kg BW. In fact, the plant roots containing the saponins [9] originally used to make soap turned out to be poisons that caused loss of appetite, apathy in the animals and weight loss. Also, the administration of plant extracts in high doses can metabolize toxicend products that effectively interfere with gastric function [25]. This is the case with our extract, which contains an isolated and identified molecule known as beta sitosterol which reduces blood cholesterol levels [10].

A significant shift in body weight is a sensitive indicator of toxicity and can be used to assess an animal's overall health. The non-significant change in this parameter when compared with the control group suggests that the extract did not interfere with the normal metabolism. Regarding relative organ weights, no significant difference was observed in the groups treated with the different doses compared to the control. This remains consistent with the results obtained in subacute toxicity on the aqueous extract of the bark of the same plant [6]. Assessment of function of liver and kidney is crucial in toxicological evaluation of plant extracts due to the implication of detoxification. An increase of serum activities of GGT, ALT, and AST are correlated with hepatic injury [26]. In the present study, no significant difference in the serum activities of these enzymes in rats treated with the extract at different doses of extract relative to normal control are informative to the fact that the extract does not affect hepatocytes function or the integrity of the liver cells. The significant reduction of GGT, ALT, and AST in rats treated at 300 mg/kg BW as would be due to the hepato-protective effect of our extract [27]. However, the increase of the level of tissue protein observed in this in the group treated with 533 mg/kg, BW suggest that the prolonged administration of this extract at this dose could cause significant liver damage.

This was confirmed by the histological examination of the liver where leukocytic infiltration was found in the group of animals receiving the extract at the highest dose (533 mg/kg, BW). These similar results were reported showing that the 70% hydroethanol extract of *Terminalia superba* bark at doses of 500 and 750 mg/kg caused steatosis and cellular infiltration [11].

In this study, we observed a significant increase in total cholesterol levels at 300 mg/kg BW and in triglycerides at 100 mg/kg and 533 mg/kg, BW compared to the control group. This suggests that EARS.m contributes to androgen production because cholesterol is a precursor of the synthesis of steroids confirming androgenic properties of the aqueous extract of *S. magnificum*. Similarly, there was a non-significant increase in HDL cholesterol and the atherogenic index, suggesting that treatment with extract from the roots of *S. magnificum* does not cause any disturbance in the lipid profile.

Analyzing the haematological parameters might help ascertain how much foreign chemicals in plant extracts are detrimental to blood components of animal. The hematopoietic system is known to be highly sensitive to toxic substances and remains a significant indicator of physiological and pathological conditions in humans and animals [28]. Thus, any damage to its organs can disrupt the synthesis of these cells. The present evaluated hematological parameters during repeated daily administration of the aqueous extract of the roots of *S. magnificum*. No significant change was observed compared to the group that received distilled water in either the leucocyte or erythrocyte lineage. The extract is not blood-toxic. This suggests that the shape, osmotic fragility were unaffected. Also, the ability of blood to carry oxygen and the quantity of oxygen that is supplied to the tissues after the extract is applied are both unaltered. The same results were obtained with the sub-chronic toxicity study of the aqueous extract of *Tiliacora triandra* and the aqueous extract of *Holarrhena floribunda* leaves [29]. In the present study, an increase in platelets count was observed in the test groups compared to the control group, suggesting the potential effect of the extract to stimulate thrombopoietin. The absence of abnormalities observed in the histological sections of the testis and epididymis of rats treated with 100 and 300 mg/kg of our plant extract confirm non-significant variation in the relative weight of the organs in these animals at those doses. These photomicrograph sections show normal spermatogenesis compared to the control, and these results suggest that *S. magnificum* extract has no harmful effect on the reproductive system, and consequently on the testicular and epididymis morphology. However, at 533 mg/kg, we noted alterations in spermatogenesis and agglutination of spermatozoa; the extract is thought to contain active compounds which may be toxic at highest dose. In our work, the NOAEL was set at 100 mg/kg, BW/day. Additional research work should be undertaken in order to assess the effects of the aqueous extract of on the some parameters of the antioxidant system.

CONCLUSION: In summary, the acute and sub-acute toxicity studies of the aqueous extract of the roots of *Schumanniophyton magnificum* revealed no toxicological symptoms or mortality in the Wistar rats. Daily treatments with the extract at the doses of 100, 300, and 533 mg/kg, BW had no adverse effect on body weight. The fifty lethal dose was higher than 2000 mg/kg. Therefore, caution must be taken with the medicinal use of the aqueous extract especially for a prolonged use at higher doses.

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