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TWENTY EIGHT DAYS ORAL ADMINISTRATION ASSESSMENT OF *HYDNORA AFRICANA* THUNB. AQUEOUS ROOT EXTRACT ON KEY METABOLIC MARKERS OF WISTAR RATS

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ABSTRACT: *Hydnora africana* Thumb (Hydronaceae) is a parasitic flowering plant with significant global ethnomedicinal value. This study evaluated the toxicological effects of the acute and sub-acute dosing treatments of the aqueous root extract of Ha in male and female wistar rats using OECD guidelines 423 and 407. For the acute study, the extract was administered to the animals at single oral graded doses of 1000, 2000 and 5000 mg/kg body weight (b.w.) and subsequently observed for 14 days, while it was given at 250, 500, and 1000 mg/kg b.w. once daily for 28 days in the sub-acute testing. Clinical toxicity signs, behavioral changes, hematological and biochemical parameters were then monitored and evaluated. The result revealed that, at a limit dose of 5000 mg/kg b.w., the extract neither had treatment - mediated symptoms of toxicity nor mortality over the 14 days observation period. In the sub-acute study and particularly for the male rats, the extract significantly increased the serum levels of the erythrocytes and haemoglobin, while the platelet counts increased dose-dependently in both male and female treated animals. The non-significant ($p>0.05$) effect on other evaluated parameters and the no treatment-induced abnormalities in the relative organ weights of the animals indicated that it is unlikely to be toxic to the investigated organs and suggests that its oral median lethal dose is approximately higher than 5000 mg/kg. From the data presented in this study, it could be logically inferred that aqueous root extract of *H. africana* is non-toxic at the tested doses and within the period of exposure.

INTRODUCTION: Medicinal plants are endowed with bioactive compounds from which new lead drugs to treat degenerative ailments are discovered. These plants have been well studied and their efficacy as pharmacological agents in the treatment of various diseases is undoubtable.

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In recent times, the swift focus on the pharmacological significance of medicinal plants could either be due to the decreased potency or increased contradictions, non affordability and non-availability of the synthetic drugs [1]. These factors (presenting the plants as relatively affordable, more efficacious, easily accessible and with minimal side effects) have made many users of herbs believe that they are toxic free [2]. However, newer and emerging research based reports have rebutted this claim and are advocating for the need to ascertain the toxicological profiles of plants on key metabolic tissues of animals including humans [3].

Such advocation is aimed at establishing the safety dosages of plant formulations as normally done with the conventional drugs that have been properly researched and developed. It may also be imperative to labelling and classifying the botanicals as either toxic or practically safe for consumption [4].

Hydnora africana Thumb (Hydronaceae) is a parasitic flowering plant growing in the order Piperales. It contains two genera, Hydnora and Prosopanche [5]. Prosopanche is native to Central and South America, while Hydnora can be found in the desert regions of Africa, the Arabian Peninsula, and Madagascar [5]. It comprises between 10 and 15 species in South Africa. The plant, similar to about ten species in the world, lives a greater part of its life cycle underground and is unable to photosynthesis due to lack of chlorophyll pigments [5]. While preliminary GC-MS analysis of the plant revealed substantial amounts of classes of compounds including; carboxylic acids (30.68%), terpenes (10.70%), alkyl aldehydes (4.86%) and esters (0.82%) [6]. Previous reports on the phytoconstituents of extracts of *H. africana* showed the presence of alkaloids, saponins, tannins, flavonoids, phenolics and steroids as major adaptogenic compounds [7, 8].

In the traditional systems of medicine, the roots, tuber, fruits, leaves and fruit pulps of Hydnora species are used to treat diseases including microbial infections [8]. Besides being used as a tanning agent in fishing nets, the pharmacological importance of *Hydnora africana* in the treatment of diarrhoea, swollen glands, inflamed throat, dysentery, acne, kidney and urinary tract infections have been well documented [7, 8, 9]. In view of the potential health benefits of this plant and coupled with the lack of scientific information in literature on its toxicological profile, the present study seeks to enrich biochemical information on the safety of acute and sub-acute oral administration of *H. africana* aqueous root extract on key metabolic markers in Wistar rats.

MATERIALS AND METHODS:

Collection and Identification of Plant Material: Fresh matured roots of *Hydnora africana* was collected in May, 2016 at Ntselamanzi area of the Eastern Cape Province of South Africa. The plant material was initially authenticated by Prof. DS Grierson, a botanist at the University of Fort Hare, Alice, South Africa. A voucher specimen (Win 2014/1) as formally deposited was prepared and deposited at the Giffen's herbarium of the Institution.

Sample Extraction: The fresh roots of *H. africana* were screened of bad ones, washed and oven-dried to constant weight (40 °C) and milled to a homogeneous powder. The powdered sample (300 g) was extracted in sterile distilled water (3 L) with continuous agitation on a shaker (Orbital Incubator Shaker, Gallenkamp) maintained at 140 rpm for 24 h. The resulting solution was filtered using Whatman no. 1 filter paper and the filtrate obtained was subsequently freeze-dried (Vir Tis benchtop K, Vir Tis Co., Gardiner, NY). This gave the aqueous root extract of *H. africana* (REHA) that was kept air-tight and refrigerated prior to use.

Experimental Animals: Eighty (80) healthy Wistar rats (*Rattus norvegicus*: n = 40 males, 40 females) with mean weight of 125.0 ± 15.0 g were purchased from the South African Vaccine Producers (Pvt.,) Ltd. They were kept in clean polypropylene cages placed in a well ventilated house with optimum condition (temperature: 22 ± 2ºC; photoperiod: 12 h light and 12 h dark cycle; humidity: 40 - 45%). The animals were fed *ad libitum* with rat pellets and tap water freed of contaminants. The cages were cleaned on a daily basis and treatments were in accordance with the guidelines of the ethics committee on the use and care of experimental animals of University of Fort Hare, Alice, South Africa. The study was approved (REC-270710-028-RA WIN001) prior to commencement.

Acute Toxicity Evaluation: This was carried out following the adopted organization for economic co-operation and development (OECD) guideline 420 OECD10, with slight modifications. Forty rats were divided into four groups of 10 animals each (5 males and 5 females). Following an overnight fasting period, the control group received only sterile distilled water while the other 3 groups were given respective single oral graded doses (1000, 2000 and 5000 mg/kg body weight (b.w.) of REHA. The animals were observed individually during the first 30 min and thereafter 24 hourly for

a period of 14 days. Clinical signs of toxicity, body weight changes, feed and water intake for each group was observed daily for 14 days. On the 15th day and subsequent to 18 h fasting period, all the rats were humanely sacrificed under halothane anaesthetization. The blood sample was collected in non-heparinized and EDTA bottles for the evaluation of biochemical and hematological parameters respectively. Parameters including red blood cells (RBC), heamoglobin (Hb), hematocrit (HCT), white blood cell (WBC), platelets, aspartate amino transferase (AST), alkaline phosphatase (ALP), alanine amino transferase (ALT), total protein, albumin, blood urea nitrogen, creatinine and serum electrolyte concentrations were assayed. The liver, kidney and heart of each animal were also excised and weighed.

Sub - Acute Toxicity Testing: The sub-acute toxicity testing was conducted according to the OECD guideline 407 for testing of chemicals [11]. Forty rats were randomly assigned into 4 groups of 10 animals each (5 females and 5 males). The animals in group 1 were given 1 ml sterile distilled water and served as control. Groups 2 - 4 comprised animals administered with 1 ml graded doses (250, 500 and 1000 mg/kg/day b.w.) of REHA, respectively. Treatments with REHA were done once daily via oral intubation for 28 days. The rats were weighed at 24 h interval and subjected to thorough observations for mortality, behavioral changes and possible symptoms of humane end point during the 28 days experimental period. While the body weight changes of the animals were measured on weekly basis, the adapted method of Ajani *et al*., [12] was employed in the determination of the daily feed and water intakes of the animals throughout the experimental period.

Blood Collection and Organs Isolation: After 28 days of extract administration, rats were fasted overnight, anesthetized using halothane and subsequently humanely sacrificed on the 29th day. The blood sample collection and isolation of the organs were done as earlier reported [13]. The rats were quickly dissected and the liver, kidney and heart were excised, freed of fat, bloated with clean tissue paper and weighed. Relative organ body weight ratios (ROW) were also calculated. The organs were also prepared for histopathological examinations for probable gross pathological features [6].

Data Analysis: Data were expressed as mean ± standard error of mean (SEM) of t the replicates. The results were subjected to one way analysis of variance (ANOVA) followed by Duncan multiple range test to determine significant difference in all the parameters. Values were considered statistically significant at p values of less than 0.05.

RESULTS:

Acute Toxicity: Treatment with the single oral graded doses of REHA up to 5000 mg/kg b.w. and subsequent observation for 14 days produced no clinical adverse effect of substance related toxicity on both the male and female animals. All the rats behaved adequately normal and neither morbidity nor mortality was observed at all the tested doses **Table 1**. The results obtained with respect to the weight gained, ROW, hematological and bio-chemical indices showed that there were no significant differences ($p>0.05$) between the extract-treated animals and the respective control **Tables 2, 3** and **4**.

28 Day Toxicity Study: The 28-day repeated dose oral t treatment with REHA elicited no obvious symptoms or adverse effect on the rats of both sexes. There was neither mortality nor prominent abnormal clinical signs of toxicity in all the treated groups throughout the period of investigation. Cage side observation of the animals revealed no changes in their behavioural pattern and there was no evidence of lethargy, convulsion, salivation, diarrhoea and coma. Compared with the control, dose-dependent significant increases were observed in body weights of the REHA - administered rats **Table 5**.

TABLE 1: OBSERVATIONS FOLLOWING REHA ACUTE DOSING ON RATS

Dose (mg/kg)	Male		Female		Symptoms
	Dead/Total	Mortality (h)	Dead/Total	Mortality (h)	
Control (0)	0/5	0	0/5	0	None
1000	0/5	0	0/5	0	None
2000	0/5	0	0/5	0	None
5000	0/5	0	0/5	0	None

TABLE 2: EFFECT OF ORAL ACUTE DOSING OF AQUEOUS ROOT EXTRACT OF *HYDNORA AFRICANA* ON THE BODY AND ROW OF MALE AND FEMALE WISTAR RATS

	Extract (mg/ kg body weight)							
	Male animals				Female animals			
Parameters	**Control**	**1000**	**2000**	**5000**	**Control**	**1000**	**2000**	**5000**
IBW (g)	177.46±1.02[a]	199.83±0.01[a]	169.24±0.25[a]	181.27±1.00[a]	195.48±1.06[a]	197.68±1.09[a]	211.22±2.00[a]	181.52±1.05[a]
FBW (g)	187.65±0.99[a]	209.03±0.25[a]	179.24±1.03[a]	192.20±1.02[a]	206.59±0.09[a]	210.01±1.10[a]	221.11±0.25[a]	193.93±1.02[a]
RHW (g/100 g)	0.62±0.02[a]	0.61±0.01 [a]	0.59±0.02 [a]	0.60±0.01 [a]	0.59±0.01 [a]	0.60±0.01 [a]	0.61±0.02 [a]	0.62±0.03 [a]
RKW (g/100 g)	0.85±0.01 [a]	0.84±0.03 [a]	0.85±0.02 [a]	0.86±0.01 [a]	0.89±0.02 [a]	0.87±0.02 [a]	0.85±0.03 [a]	0.89±0.01 [a]
RLW (g/100 g)	3.45±0.75[a]	3.43±0.36[a]	3.46±0.25[a]	3.50±0.22[a]	3.46±0.41[a]	3.46±0.25[a]	3.52±0.45[a]	3.49±0.33[a]

Values are expressed as Mean ± SEM n = 5. [a]Not significantly different (p>0.05) across the same row for each parameter. IBW = initial body weight, FBW = final body weight, RHW = relative heart weight, RKW = relative kidney weight, RLW = relative liver weight.

TABLE 3: EFFECT OF ORAL ACUTE DOSING OF AQUEOUS ROOT EXTRACT OF *HYDNORA AFRICANA* ON HAEMATOLOGICAL PARAMETERS OF WISTAR RATS

Parameters	Extract (mg/ kg body weight)							
	Male animals				Female animals			
	Control	**1000**	**2000**	**5000**	**Control**	**1000**	**2000**	**5000**
RBC(x10^{12}/L)	8.48±0.30[a]	8.63±0.01[a]	8.32±0.02[a]	8.55±0.02[a]	8.01±0.01[a]	8.35±0.03[a]	8.25±0.02[a]	8.45±0.02[a]
Hb (g/dL)	14.45±0.71[a]	14.45±0.25[a]	15.20±0.64[a]	15.40±0.32[a]	14.20±0.32[a]	14.50±0.43[a]	14.70±0.45[a]	14.85±0.71[a]
HCT (1/L)	0.44±0.00 [a]	0.43±0.04 [a]	0.45±0.04 [a]	0.47±0.02 [a]	0.43±0.02 [a]	0.43±0.15 [a]	0.45±0.00 [a]	0.43±0.03 [a]
MCV (fl)	51.50±2.12[a]	52.33±1.53[a]	55.33±1.15[a]	53.50±0.71[a]	52.12±0.02[a]	52.00±0.01[a]	53.00±1.05[a]	52.50±2.02[a]
MCH (pg)	17.00±0.00[a]	17.33±2.08[a]	17.67±1.53[a]	18.01±0.58[a]	17.02±0.01[a]	17.01±0.01[a]	17.00±0.05[a]	17.02±0.02[a]
MCHC(g/dL)	33.00±1.41[a]	33.00±1.00[a]	33.50±0.70[a]	34.17±0.15[a]	33.01±0.20[a]	33.01±0.10[a]	33.00±0.02[a]	33.02±0.01[a]
RCDW (%)	15.50±0.99[a]	14.99±0.33[a]	15.09±0.12[a]	14.86±0.30[a]	15.30±0.30[a]	15.00±0.12[a]	14.90±0.10[a]	15.01±0.22[a]
WBC(x10^{9}/L)	7.80±1.56[a]	7.60±2.83[a]	7.60±2.08[a]	7.90±0.30[a]	6.70±0.01[a]	6.30±0.01[a]	6.80±0.02[a]	6.80±0.03[a]
Lymphocytes (%)	6.51±1.62[a]	6.58±3.01[a]	6.62±0.02[a]	6.89±0.02[a]	6.69±0.09[a]	6.92±0.05[a]	6.67±0.0[a]	6.59±0.04[a]
Platelets (10^{9}/L)	761.00±4.36[a]	763.01±2.08[a]	768.00±2.00[a]	756.50±1.13[a]	750.23±2.00[a]	754.01±1.11[a]	765.01±1.0[a]	765.00±1.05[a]

Values are expressed as Mean ± SEM n=5 a Not significantly different (p>0.05) across the same row for each parameter. RBC = red blood cell, Hb = haemoglobin, HCT = haematocrit, MCV= mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC= mean corpuscular haemoglobin concentration, RCDW= red blood cell distribution width, WBC= white blood cell.

TABLE 4: EFFECT OF ORAL ACUTE DOSING OF AQUEOUS ROOT EXTRACT OF *HYDNORA AFRICANA* ON THE BIOCHEMICAL PARAMETERS OF MALE AND FEMALE RATS

Parameters	Extract (mg/ kg body weight)							
	Male animals				Female animals			
	Control	**1000**	**2000**	**5000**	**Control**	**1000**	**2000**	**5000**
Total protein (g/L)	60.75±1.26[a]	60.25±1.33[a]	61.00±1.71[a]	60.25±1.15[a]	62.00±2.01[a]	60.00±0.99[a]	61.02±2.01[a]	62.05±1.00[a]
Albumin (g/L)	33.25±1.71[a]	32.75±2.59[a]	33.50±2.38[a]	32.00±1.83[a]	32.23±0.18[a]	33.21±1.05[a]	32.00±1.34[a]	33.09±0.95[a]
ALP (µ/L)	332.00±4.36[a]	333.07±3.01[a]	339.00±4.00[a]	337.00±3.11[a]	335.00±5.12[a]	334.03±4.02[a]	337.01±4.01[a]	335.00±4.04[a]
ALT (µ/L)	68.00 ±2.01[a]	65.00±1.08[a]	63.00±1.68[a]	64.25±1.99[a]	63.02 ±1.15[a]	65.05±1.05[a]	69.00±1.05[a]	64.33±1.25[a]
AST (µ/L)	177.50±4.01[a]	169.00±2.99[a]	169.33±3.05[a]	174.00±4.02[a]	165.00±5.00[a]	166.08±4.90[a]	164.05±3.00[a]	167.25±4.11[a]
Sodium (mmol/L)	138.75±0.96[a]	138.33±0.58[a]	140.00±0.82[a]	139.00±1.63[a]	140.02±0.25[a]	139.00±0.05[a]	141.00±0.25[a]	138.02±1.23[a]
Potassium (mmol/L)	5.93±0.25[a]	6.00±0.82[a]	6.25±0.35[a]	6.40±0.11[a]	6.40±0.25[a]	6.31±0.35[a]	5.99±0.22[a]	6.00±0.22[a]
Calcium (mmol/L)	2.41±0.07 [a]	2.42±0.08 [a]	2.46±0.12 [a]	2.50±0.09 [a]	2.50±0.18 [a]	2.46±0.25 [a]	2.43±0.10 [a]	2.50±0.45 [a]
Urea (mmol/L)	6.65±0.72[a]	6.60±0.93[a]	6.80±1.48[a]	6.60±0.67[a]	6.90±0.32[a]	6.60±0.10[a]	6.60±0.22[a]	6.80±0.15[a]
Creatinine (mmol/L)	30.75±1.50[a]	35.00±1.73[a]	32.00±1.73[a]	35.07±0.09[a]	33.30±0.25[a]	34.00±0.01[a]	30.70±0.11[a]	34.00±1.35[a]
Cholesterol (mmol/L)	1.85±0.30[a]	1.85±0.45[a]	1.90±0.18[a]	2.10±0.27[a]	1.90±0.25[a]	1.88±0.01[a]	1.89±0.12[a]	1.99±0.25[a]
Triglycerides (mmol/L)	0.93±0.30[a]	0.95±0.17[a]	1.05±0.52[a]	1.07±0.30[a]	1.20±0.25[a]	0.99±0.10[a]	1.01±0.25[a]	0.95±0.01[a]
HDL-C (mmol/L)	1.05±0.13 [a]	1.08±0.17 [a]	1.10±0.11[a]	1.20±0.29[a]	1.09±0.11[a]	1.07±0.23[a]	1.05±0.20[a]	0.99±0.12[a]

Values are expressed as Mean ± SEM n=5. Values across each row for each parameter are not significantly different (p>0.05). T. bilirubin = Total bilirubin, ALP = Alkaline phosphatase, ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, HDL-C = High density lipoprotein cholesterol.

TABLE 5: EFFECT OF ORAL SUB-ACUTE DOSING OF AQUEOUS ROOT EXTRACT OF *HYDNORA AFRICANA* ON THE BODY AND ROW OF MALE AND FEMALE WISTAR RATS

Parameters	Extract (mg/ kg body weight)							
	Male animals				Female animals			
	Control	**250**	**500**	**1000**	**Control**	**250**	**500**	**1000**
IBW (g)	135.98±2.76	120.47±4.46	154.26±2.27	141.84±2.74	125.87±6.45	114.27±1.14	110.29±6.41	115.56±2.77
FBW (g)	234.42±1.05	230.97±1.79	269.11±1.33	256.95±2.76	220.82±3.92	220.37±2.12	225.57±2.44	233.04±1.84
RHW (g/100 g)	0.36±0.02	0.36±0.03	0.37±0.04	0.38±0.03	0.37±0.02	0.43±0.06	0.43±0.05	0.40±0.04
RKW (g/100 g)	0.65±0.05	0.68±0.03	0.69±0.01	0.64±0.15	0.74±0.04	0.71±0.05	0.70±0.01	0.74±0.01
RLW (g/100 g)	2.98±0.30	2.72±0.48	2.79±0.38	3.68±0.24	2.59±0.43	2.60±0.37	2.61±0.26	3.99±0.42

Values are expressed as Mean ± SEM n=5. Values across each row for each parameter are not significantly different (p>0.05). IBW = initial body weight, FBW = final body weight, RHW= relative heart weight, RKW = relative kidney weight, RLW = relative liver weight.

However, except with the marginal increase in the relative liver weight of the 1000 mg/kg b.w. REHA-treated rats (both sexes), there were no significant ($p>0.05$) differences in this parameter for other organs when compared with the control group **Table 5**. At necropsy, the histoarchitectural integrity of the investigated organs remained essentially normal with no obvious pathological gross observations **Fig. 1a** and **1b**.

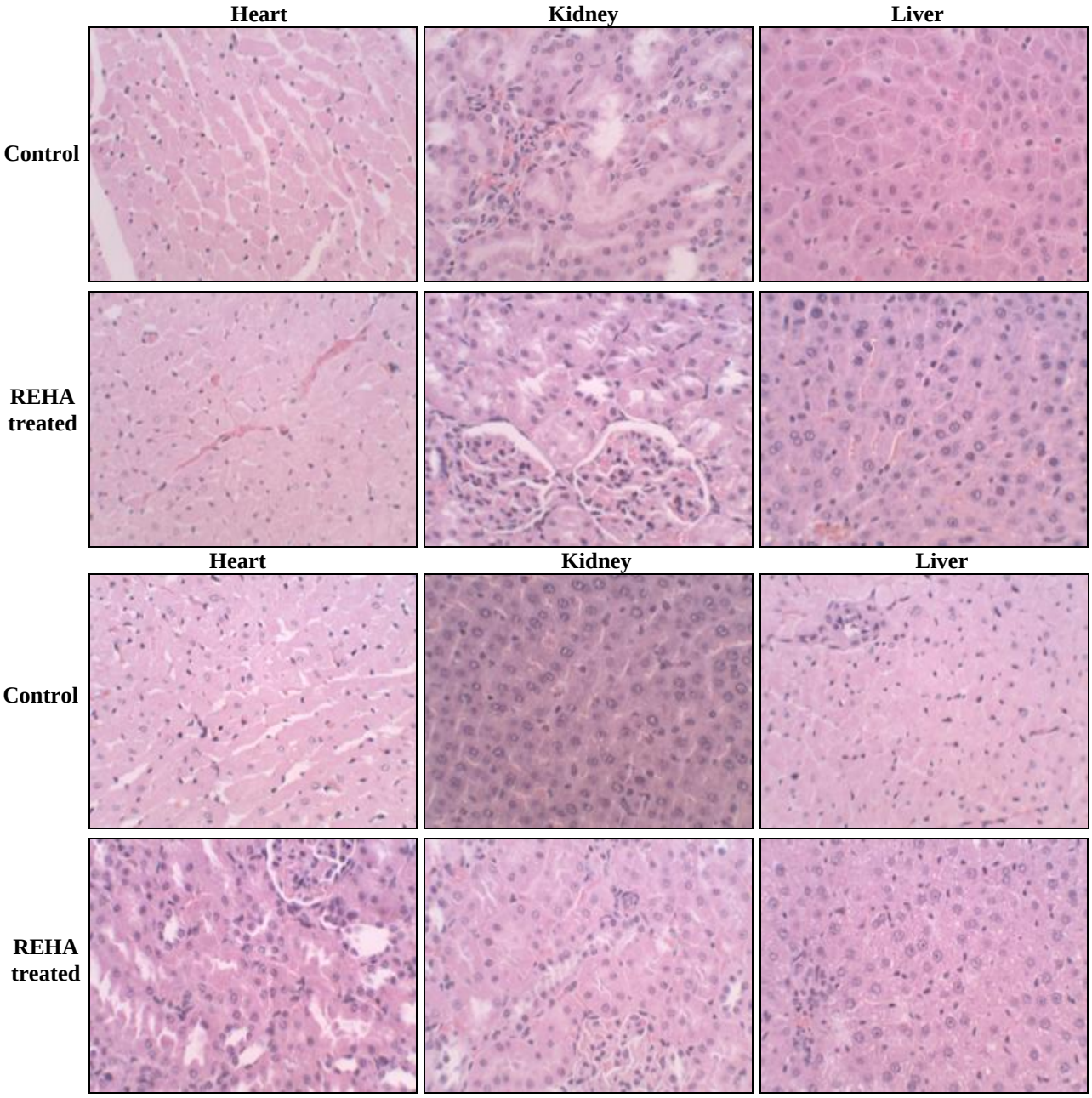

FIG. 1: PHOTOMICROGRAPHS (400×, HEMATOXYLIN + EOSIN STAINED) OF TISSUE SECTIONS OF THE HEART, KIDNEY AND LIVER OF CONTROL AND HIGHEST DOSE (1000 mg/kg BODY WEIGHT) AQUEOUS ROOT EXTRACT OF *HYDNORA AFRICANA* TREATED MALE AND FEMALE RATS FOR 28 DAYS SHOWING WELL PRESERVED HISTOARCHITECTURAL FEATURES OF THE ORGANS

Fig. 2 and **3** present the results of the feeding patterns of the animals. Although, when compared with the control, the food and water intakes by the extract-treated animals increased significantly throughout the 2nd and 3rd weeks of the experiment, a fairly steady consumption pattern was maintained during the 4th week **Fig. 2a**, **2b**, **3a** and **3b**. It is however worthy of note, that the most prominent effects on the feeding pattern of the animals were observed on the 500 and 1000 mg/kg b.w. REHA-treated male rats.

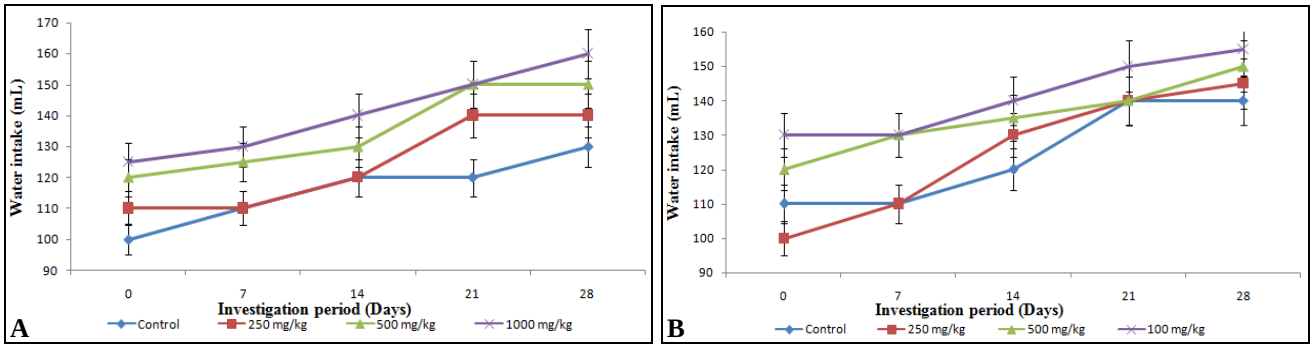

FIG. 2: EFFECT OF AQUEOUS ROOT EXTRACT OF *HYDNORA AFRICANA* ON WEEKLY WATER INTAKES OF THE MALE (A) AND FEMALE (B) RATS

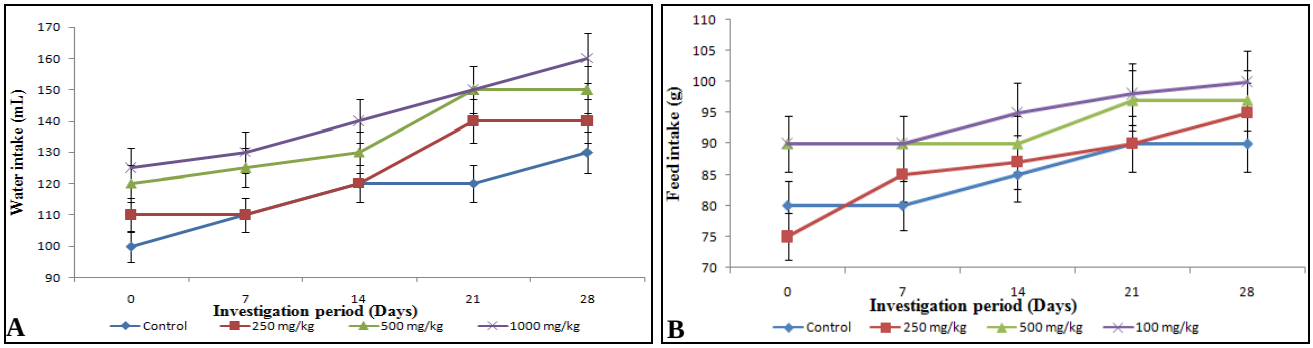

FIG. 3: EFFECT OF AQUEOUS ROOT EXTRACT OF *HYDNORA AFRICANA* ON WEEKLY FEED INTAKES OF THE MALE (A) AND FEMALE (B) RATS

The effects of repeated dose administration of REHA on the hematopoietic systems, and other biochemical indices of the male and female rats are presented in **Table 6** and **7**. With the exception of the dose-related significantly ($p<0.05$) increased platelet counts in the extract-treated animals as well as the significantly higher RBC and Hb levels in the extract-treated male rats across the investigated doses when compared with the control, the extract elicited no significant ($p>0.05$) effect on the blood levels of other hematological indices of both male and female rats within the 28 days period of evaluation **Table 6**. The data obtained with respect to the kidney and liver function parameters evaluated in this study revealed that repeated daily dose treatment with the extract at all the investigated doses produced no significant ($p>0.05$) alteration in the serum levels of all the indices of all the animals when compared with the respective control **Table 7**. At all the concentrations of the extract investigated, the serum levels of TC, TG and HDL-C of all the animals were not affected **Table 7**.

TABLE 6: EFFECT OF ORAL SUB-ACUTE DOSING OF AQUEOUS ROOT EXTRACT OF *HYDNORA AFRICANA* ON HAEMATOLOGICAL PARAMETERS OF WISTAR RATS

Parameters	Extract (mg/ kg body weight)							
	Male animals				Female animals			
	Control	250	500	1000	Control	250	500	1000
RBC($x10^{12}$/L)	8.57 ± 0.01^{a}	8.92 ± 0.07^{a}	10.73 ± 0.42^{b}	10.97 ± 0.04^{b}	8.08 ± 0.04^{a}	8.03 ± 0.35^{a}	8.03 ± 0.35^{a}	8.29 ± 0.14^{a}
Hb (g/dL)	14.0 ± 0.01^{a}	14.35 ± 0.21^{a}	16.0 ± 0.71^{b}	16.4 ± 0.14^{b}	14.40 ± 0.2^{a}	14.17 ± 0.15^{a}	15.01 ± 0.45^{a}	15.03 ± 0.59^{a}
HCT (1/L)	0.58±0.03	0.59±0.01	0.57±0.02	0.58±0.01	0.55±0.01	0.56±0.03	0.55±0.00	0.58±0.01
MCV (fl)	65.70±0.21	65.78±0.07	64.90±0.28	64.65±1.27	63.30±0.49	62.90±0.28	63.00±1.05	63.13±0.51
MCH (pg)	18.70±0.28	18.35±0.07	18.35±0.07	18.05±0.21	17.89±0.21	17.85±0.49	17.90±0.05	17.87±0.38
MCHC (g/dL)	28.40±0.21	27.90±0.14	28.50±0.42	28.85±0.07	28.90±0.14	28.8±0.99	28.00 ± 0.02^{a}	29.27±0.67
RCDW (%)	10.90±0.21	10.95±0.07	11.50±0.99	11.2±0.28	10.89±0.01	10.55±0.21	10.90±0.10	10.47±0.46
WBC ($x10^{9}$/L)	10.82±0.21	10.08±1.20	10.47±2.67	10.58±0.01	10.52±0.01	10.63±1.45	10.80±0.02	10.39±0.85
Lymphocytes (%)	6.67±0.02	6.58±0.24	6.43±1.48	6.51±0.03	6.57±0.01	6.56±1.28	6.67±0.0	6.48±3.58
Platelets (10^{9}/L)	538.05 ± 9.71^{a}	626.50 ± 9.45^{b}	664±2.83c	764.5 ± 10.42^{d}	677.5 ± 0.71^{a}	765.01 ± 1.09^{b}	812.5 ± 1.61^{c}	887.00 ± 5.85^{d}

Values are expressed as Mean ± SEM n=5. a Not significantly different ($p>0.05$) across the same row for each parameter, b, c, d Significantly ($p<0.05$) from the control. RBC= red blood cell, Hb = haemoglobin, HCT = haematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular haemoglobin concentration, RCDW = red blood cell distribution width, WBC = white blood cell.

TABLE 7: EFFECT OF ORAL SUB-ACUTE DOSING OF AQUEOUS ROOT EXTRACT OF *HYDNORA AFRICANA* ON THE BIOCHEMICAL PARAMETERS OF MALE AND FEMALE RATS

Parameters	Extract (mg/ kg body weight)							
	Male animals				Female animals			
	Control	250	500	1000	Control	250	500	1000
Total protein(g/L)	51.00±1.83	54.00±1.83	54.00±1.83	52.50±2.38	50.75±0.96	51.75±1.6	53.00±2.45	52.50±2.38
Albumin (g/L)	31.50±0.58	31.75±0.50	31.30±0.96	31.00±0.82	31.75±0.50	31.50±0.58	31.67±0.58	32.75±0.50
ALP (µ/L)	265.75±6.06	262.50±4.53	265.75±1.53	264.25±2.55	253.00±8.04	253.50±5.61	265.67±2.58	258.0±16.09
ALT (µ/L)	57.00±1.83	58.00±1.48	57.50±3.11	60.00±2.76	61.25±1.14	62.75±1.75	63.0±1.79	61.25±2.45
AST (µ/L)	160.50±8.66	163.00±6.58	164.75±1.97	163.75±1.09	218.11±3.27	218.50±4.89	214.25±3.22	218.67±2.73
Sodium(mmol/L)	140.25±1.89	140.00±1.00	140.25±1.50	139.50±1.50	140.00±1.41	139.25±0.96	139.75±1.71	139.75±0.50
Potassium(mmol/L)	5.99±0.11	6.02±0.32	5.88±0.10	5.79±0.25	6.00±0.01	6.01±0.01	5.99±0.05	6.21±0.01
Calcium (mmol/L)	2.40±0.03	2.42±0.05	2.38±0.03	2.44±0.07	2.39±0.03	2.38±0.04	2.40±0.04	2.39±0.06
Urea (mmol/L)	4.13±0.30	4.13±0.39	4.28±0.05	4.28±0.54	4.45±0.37	4.43±0.82	4.53±0.68	4.93±0.51
Creatinine (mmol/L)	36.25±5.56	35.25±4.57	36.0±4.24	34.5±2.89	37.75±6.70	36.50±4.65	38.75±8.30	36.25±1.50
Cholesterol(mmol/L)	1.00±0.08	0.98±0.13	1.03±0.06	1.10±0.22	0.88±0.17	1.03±0.10	1.00±0.08	1.00±0.08
Triglycerides(mmol/L)	0.99±0.25	1.53±0.30	1.51±0.42	1.51±0.47	1.34±0.42	1.36±0.14	1.46±0.36	1.45±0.54
HDL-C (mmol/L)	1.11±0.25	1.21±0.10	1.10±0.20	1.20±0.05	1.01±0.01	1.19±0.01	1.05±0.01	1.03±0.02

Values are expressed as Mean ± SEM n=5. Values across each row for each parameter are not significantly different ($p>0.05$). T. bilirubin = Total bilirubin, ALP = Alkaline phosphatase, ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, HDL-C = High density lipoprotein cholesterol.

DISCUSSION: Toxicological studies are not only significant to the labelling and classification of a new drug but also provide useful hint on whether the drug should be adopted for clinical use or not. In this study, the fact that the single oral graded doses (1000, 2000 and 5000 mg/kg body weight (b.w.) of REHA produced no treatment-mediated clinical signs of toxic tendencies on both the male and female experimental rats could suggest that it may not be acutely toxic to the animals. This could also predicts that its median lethal dose is well above the limit dose of 5000 mg/kg b.w. This may present REHA as non-acutely toxic when orally administered in Wistar rats. Additionally, the non-significant effect of the limit dose (5000 mg/kg b.w) treatment of the extract on the ROW, histopathological, hematological and biochemical parameters of the treated animals is another tenable fact supporting the non-toxic potential of REHA. These findings are in agreement with the report of Oladipupo *et al*., [3] Sabiu and Ashafa [4] and Afolayan *et al*., [14] where plant extracts were reported to be relatively safe and non-acutely toxic to experimental animals.

Subsequent to the no treatment-induced toxico-logical effect of REHA in the oral acute toxicity evaluation, further study was carried out to assess the repeated dose safety profile of the extract over a 28 day observation period. This was done with a view to providing comprehensive toxicological data on this ethnomedicinally valued plant. That the repeated dosing of the animals with REHA also had no adverse symptoms of toxicity and recorded neither morbidity nor mortality across all the treatment groups may be indicative of its unlikely toxic tendency with the investigation period. Changes in the body weight of rats may serve as sensitive indication of their general well-being and also constitute an important index in toxicological studies [15]. The pro rata body weight gained by the rats in all the treatment groups could suggest that the extract had no deleterious influence on their normal metabolic processes relating to growth and development when compared with animals in the control group. This could be attributed to the increased feeding habits of the treated animals that may be ascribed to the palatability of REHA which in turn enhanced their appetite as evidently shown in this study. These findings are in agreement with the report of Ajani *et al*., [3].

The authors linked weight gained following treatment with an aqueous extract of tiger nut with enhanced appetite in rats. In addition to defining toxicity as pathological changes observed in organs of interest, the ROW may also be suggestive of organ swelling, atrophy or hypertrophy [16]. In this study, except for the marginally increased liver body weight of the animals placed on 1000 mg/kg b.w. of the extract, the non-significant differences in the ROWs of the rats may suggest that the investigated organs were neither adversely impacted nor was their metabolic functions compromised throughout the investigation period. Although, the increased relative weight of the liver of the treated animals administered with 1000 mg/kg b.w. dose may indicate inherent toxic effect

of the extract on the liver cells, however, it could be regarded as been biochemically and toxicologically irrelevant as it was not consistent with data from the hematological, biochemical and histopathological analyses.

The haematological parameters assayed in this study are useful indices to establish the toxic effects of chemicals and plant extracts in metabolically active experimental animals [17]. With the exception of the increased RBC and Hb levels in male rats, the non-significant difference in these parameters for the female rats following the 28 day repeated dosing treatment with the extract could be indicative of its non-toxic effect on the hematopoietic system. This implies that erythropoietin was not released in the kidneys of the female rats and as such keeping a balance in the rates of production and destruction of blood corpuscles [18]. This non-significant effect on the Hb and RBC counts may further indicates that, the morphology as well as either incorporation of Hb into the RBCs or osmotic fragility of the RBCs was not altered sequel to treatment with REHA in the female rats [19]. Interestingly, sex difference in the Hb levels and RBC count in animals have been attributed to a direct stimulatory effect of androgen in the bone marrow of men in association with erythropoietin, a stimulatory effect of androgen on erythropoietin production in the kidney, and an inhibitory effect of oestrogen on the bone marrow in women [4]. Hence, the significantly increased RBCs and Hb in the REHA-administered male animals relative to the female rats in this study could be a consequence of the stimulatory and inhibitory tendency of REHA on the sex hormones of the male and female animals, respectively. This resultantly aided the sex difference in the Hb levels and RBC count consequential to modulatory influence of the respective hormones on erythropoietin.

Furthermore, the non-significant effect of REHA on other parameters (HCT, RCDW, MCV, MCHC and MCH) relating to the status of RBCs in both the male and female rats was an indication that Hb weight per RBCs and RBCs' microcytes (defining anaemic condition) were not adversely perturbed by treatment with the extract. This, therefore, means that the 28 day repeated daily dose treatment with the extract did not predispose the rats to anaemic condition within the evaluation period. Other authors Afolayan *et al*., [14] and Sabiu *et al*., [17] have also given similar assertions, while evaluating the toxicological implications of treatment with plant extracts on the blood systems of Wistar rats. The WBC count is an important index to the defensive capability of an organism against pathogens. In this study, the unaltered level of the WBCs subsequent to treatment with REHA may be another justifiable submission that the vascular permeability and immune system of the treated rats were essentially maintained.

In addition, that REHA produced no alteration in the levels of lymphocytes is a further attestation that the inherent immunity of the animals was not challenged and corroborated the probable non-haematotoxic effect of the extract at the tested regimen. Also, the observed dose-related increases in the platelet count across the treatment groups in both male and female animals during the 28 days experimental period could mean that the extract has the potential to stimulate thrombopoietin. This indicates that the extract may enhance thrombopoiesis and manage thrombocytopenia in animals [18].

The kidney function parameters (electrolytes, urea, uric acid and creatinine) are important biomarkers and any damage to the kidney has often been associated with alteration in their serum concentrations [20]. Similarly, the evaluation of serum activities of AST, ALT and ALP coupled with the concentrations of bilirubin, globulin, albumin and total protein have been described as very important tools to know the nature of pathological damage to the liver [12].

Therefore, the non-significant effects observed in all the renal and liver function parameters investigated in this study following treatment with REHA for 28 days at the tested doses is a further confirmation of its probable non-toxic effect. Hence, it could be logically inferred that the extract is unlikely to be toxic to the liver and the kidney at the tested doses and within the period of investigation. Alterations in the serum levels of TC, TG and HDL-C may suggest tentative predisposition of the heart to cardiovascular complications and the overall state of lipid metabolism in animals [17] HDL-C and TG are

closely associated with atherosclerotic tendency and lipolysis, respectively Oyedemi *et al*., [21].

The non-significant effect of the extract on the serum levels of TC and TG may indicate that the process of β-oxidation of fatty acids and concurrent energy metabolism were not affected by its repeated daily dosing treatment on all the animals Sabiu *et al*., [17] and thus, suggesting that it is not likely to be toxic to lipid metabolism. Similarly, its non-significant effect on the HDL-C levels across the tested doses for both male and female rats may be indicative of adequately and well regulated processes involving metabolism of cholesterol in the liver, which consequently keep the risks of cardiac myofraction and other lipid-related complications under proper checks as previously reported by Oladipupo *et al*., [3].

Apart from complementing the results of biochemical investigations, the histopathological examination of the organs of interest is also germane in assessing the safety of a potential therapeutic agent [22]. The well preserved and no treatment-related assaults, inflammations and other pathological features as revealed in the microscopic examination of all the organs from the extract-treated groups is also supportive of its tendency to preserve the architectural integrity of the organs.

CONCLUSION: Overall, the results from this study have demonstrated that the median lethal dose of REHA is above the limit dose of 5000 mg/kg b.w. in male and female Wistar rats. Following its repeated dosing treatment for 28 days, it may be logically concluded that it elicited no symptoms of clinical toxicity. Hence, aqueous root extract of *Hydnora africana* may be considered non-toxic when orally administered and could be adjudged practically safe at the tested regimens and within the period of exposure in this study.

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