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Safety Evaluation of Giant African Land Snails *(Archachatina maginata*) Haemolymph on Hematological and Biochemical Parameters of Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors LB and OKS designed the study, wrote the protocol, performed the statistical analysis and undertook the initial and final editing of the manuscript. Authors LB, MBB, SS and MIA carried out the laboratory work, did the literature search and wrote the first draft of the manuscript. Author OKS supervised the work. All authors read and approved the final manuscript.

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ABSTRACT

The safety of *Archachatina maginata* haemolymph on biochemical and hematological indices of albino rats was investigated. Twelve white albino rats were grouped into 3 (A-C) of 4 animals each.

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Group A rats serve as control groups and received no treatment, while groups B and C received 2 and 4 ml/kg of the haemolymph respectively, for ten days. Haemolymph administration resulted in a significant increase in the serum activities of aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), total protein, albumin, total bilirubin and creatinine but significant decrease in the level of urea and chloride than the control rats. While serum bicarbonate, sodium, potassium, body weight gain, Relative organ weight were not significantly altered. However, there was a significant decrease in the relative kidney weight when compared with the control values. Haemolymph also produce significant dose dependent changes in erythrocyte, leucocyte and platelet indices when compared with control values. However, clinical observations for toxicological studies shows that the haemolymph did not produce any grossly negative behavioral changes, but increase water consumption was observed during the experimental periods. The results indicated changes in the investigated hematological and some biochemical parameters showing a more pronounced effect on the liver function than the renal function tests and at a higher dose than the lower dose. Hence, caution should be taken when using the haemolymph of *Archachatina maginata* for therapeutic purpose because it may also have some liver or tissue membrane damaging effect.

Keywords: Archachatina marginata; haemolymph; biomarker enzyme; liver; kidney; hematological; biochemical parameters.

1. INTRODUCTION

Medicinal uses of natural product are gaining popularity in developing countries as has been estimated that 80%of people on this planet still rely on their traditional material medica (medicinal plants and other materials) for their primary health care needs. Emphasizes however, has been laid that safety should be overriding criteria in the selection of herbal medicine for use in health care [1].

Scientific validations are being made globally to get evidences for traditionally used natural products. The active ingredients of natural products are chemicals that are similar to those in purified medications, but natural products lack defined dose and potency data and have the same potential to cause serious adverse effects [2]. Whilst the literature documents severe toxicity resulting from the use of herbs, the potential toxicity of wild animal and their products has not been recognized. One of the wild animals claimed to be used in folklore medicine without recourse to its safety is *Archachatina maginata* and it haemolymph.

The Giant African land snails (*Archachatina marginata*) are soft bodied animals that belong to the phylum Mollusca, class Gastropoda, family *Achatinidae* and order Pulmonata [3]. They are distributed throughout the world in the tropical and sub-tropical regions where most of the genera of the family are confined to Africa [4]. They serve as wildlife dietary protein sources in Nigeria and some parts of Africa [5].

According to Abere and Lameed [6], snails are useful in the treatment of arteriosclerosis and other heart-related diseases due to their low cholesterol level. They also have pain reducing effect and prevent loss of blood during labour as well as in the treatment of small pox [7]. Previous biological studies reported that the isolated mucin motifs from *A. marginata* exhibited rectal absorption enhancing properties for the administration of insulin in rats [8] and produce marked and consistent blood glucose lowering effect [9]. There are also reports that the haemolymph of *A. marginata* exhibit pharmacological values for hypertensive patients, in the preparations of vaccines, for treatment of kidney diseases, tuberculosis, diabetes, circulatory and stomach disorders [10]. Recently, we also reported that *A. marginata* haemolymph exhibited a significant protective effect against CCL4 induce liver damage [11].

Despite these pharmacological reports and wide spread use of the snail and its haemolymph, information on the safety or toxicity appears scanty. Therefore, this study was set out to assess the safety of the *Archachatina maginata* haemolymph on the biochemical parameters of organ damage. The organs (liver and kidney) have been carefully selected because of their role in the detoxification, excretion and absorption of the haemolymph. The hematological study was also carried out to determine it effect on blood component and corroborate it with alterations in the biochemical parameters.

2. MATERIALS AND METHODS

2.1 Snail Collection

African giant snails (*Archachatina maginata)* within weight ranges of 150-210 g were purchased from Kure market, Minna, Niger State. They were housed in a plastic vessel containing a layer of moist humus soil. The container was covered with a wire mesh for proper ventilation and also to prevent the snails from crawling out. The snails were fed *ad libitum* on fresh pawpaw leaves (*Carica papaya*).

2.2 Assay Kit

The assay kits for AST, ALT, ALP total protein and albumin were products of Randox Laboratories Ltd., United Kingdom. The urea and assay kits were obtained from Quinica Clinica Aplicada S.A., Spain. All other reagents used were of analytical grade and were prepared in distilled water.

2.3 Experimental Animals

A total of twelve (12) white albino rats (*Rattus novergicus*) of both sex weighing between 120 and 200 g was obtained from the Small Animal Holding Unit of the Department of Biochemistry, Federal University of Technology Minna. The rats were kept in clean plastic cages and maintained under standard laboratory conditions (temperature: 22±3ºC; photoperiod: 12 h natural light and 12 h dark; humidity: 40-45%).The animals were maintained on standard animal feeds (Bendel feeds and flour mills, Edo state, Nigeria) and tap water *ad libitum*. The principles governing the use of laboratory animals as laid out by the Federal university of Technology, Minna Committee on Ethics for Medical and Scientific Research and also existing internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review [12], were duly observed.

2.4 Haemolymph Collection

The apex of the snails was opened by the method adopted from Akinloye and Olorode [13]. The haemolymph was drained into a clean conical flask and stored in refrigerator.

2.5 Experimental Design

Twelve (12) were randomly divided into three groups of 4 rats each as follows:

Each group was separately housed.

(i) Group A— serve as control groups and received no treatment.
(ii) Group B— was intraperitoneally administered with 2 ml/kg of African Giant Snails (*Archachatina maginata)* haemolymph for 10 days.
(iii) Group C— was intraperitoneally administered with 4 ml/kg of African Giant Snails (*Archachatina maginata)* haemolymph for 10 days. All the animals were observed for clinical observations of toxicological studies throughout the periods of the experiment.

2.6 Collection of Blood, Serum and Organs

The collection of sample for hematological and biochemical analyses was as described by Yakubu et al. [14]. At the end of the ten days treatment, the animals were denied their feeds but still had water *ad libitum* for 24 h before they were sacrificed under ether anesthesia. The blood was collected in sample bottles containing EDTA for hematological analyses. Another 4 ml of the blood was collected in a clean, dry centrifuge tubes. The blood sample was allowed to stand for 10 minutes at room temperature and then centrifuged at 1000 rpm for 15minutes to get the serum. The animals were thereafter quickly dissected and the liver, kidneys spleen and heart were removed, cleaned and weighed.

2.7 Determination of Hematological Parameters

The hematological components including haemoglobin (Hb), haematocrite (HCT), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBC), Granulocyte count (GRA) lymphocytes (LY), platelate count (PLT) Mean platelate volume (PCT) Platelet ecritand platelete distribution weight were determined using the automated haematologic analyzer SYSMEX KX21, a product of SYSMEX Corporation, Japan employing the methods described by Dacie and Lewis [15].

2.8 Determination of Biochemical Parameters

The biochemical analyses were determined for alkaline phosphatase (ALP) based on methods of

Tietz, [16], Aspartate transaminase (AST) and alanine transaminase (ALT) as described by Reitman and Frankel, [17]. The serum total protein concentration was estimated by biuret method as described by Gornall et al. [18], albumin as described by Doumas et al. [19]. The procedure of Blass et al. [20], was used to determine serum creatinine concentration while the serum urea concentration was determined by the method of Veniamin and Vakirtzi [21]. The method of Evelyn and Malloy [22], was employed to determine the serum bilirubin concentration of the samples. While Na+, K+, bicarbonate and chloride ion concentrations were determined by flame photometry [16].

2.9 Determination of Body Weight and Relative Organ Weight

The body weights of the rats were determined on day 1 and 10 of the experiment and the weight gains were computed. Relative organ weights were computed by expressing the absolute weight of the organs to the body weight of the animals as described below.

Weight gain= Final weight of rat (g)-Initial weight of rat (g)

Relative organ weight= organ weight (g)/body weight (g) x 100

2.10 Statistical Analysis

Data were analyzed using statistical package for social science (SPSS) version 16 and presented as means±SEM. Comparisons between different groups was done using Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT). Values of $P<0.05$ were considered as statistically significant as described by Mahajan, [23].

3. RESULTS

3.1 Hematological Parameters

Table 1 illustrates the changes in hematological indices in rats following the repeated administration of *Archachatina maginata* haemolymph. The RBC and HCT were significantly lowered in rats administered 4 ml/kg haemolymph than the control rats; however, rats administered 2 ml/kg showed no significant difference in RBC and HCT as compared with control. Furthermore all haemolymph treated rat showed a significant increase in the HGB, MCV, MCH, RDH and decrease MCHC than the control rats. However, HGB, MCV and PDW count were higher in 2 ml/kg haemolymph administered rats than those administered 4 ml/kg.

The haemolymph also produce a dose dependent significant increase in WBC, LY and GRA as compared with the control values. While no significant difference was observed in PLT, MPV, PCT and PDW in rats administered 2 ml/kg haemolymph as compared with the control value, rats administered 4 ml/kg show a significant decrease in these parameters than the control rats.

3.2 Biochemical Parameters

The effects of administration of 2 and 4 ml/kg of haemolymph on the activities of some biomarker enzymes, the level of total protein, albumin and total bilirubin of rats are shown in Table 2. There were significant increases in serum activities of biomarker enzymes of rats administered with the haemolymph at a dose of 2 and 4 ml/kg except for ALP whose activity was significantly lower in 2 ml/kg haemolymph administered rat as compared with control rats. The serum activities of the ALP and ALT were significantly higher in rats receiving 4 ml/kg of haemolymph than those receiving 2 ml/kg. However, no significant difference was observed in AST activities between the two treatment doses.

The *Archachatina maginata* haemolymph also significantly increased the level of total protein, albumin and total bilirubin as compared with the control rats. There was no significant difference in the level of albumin and total bilirubin between the 2 doses of the haemolymph administered, however the level of total protein was significant higher at dose of 4 ml/kg than 2 ml/kg.

3.3 Serum Electrolyte, Urea and Creatinine

Table 3 illustrates the changes in the serum electrolyte, urea and creatinine in rats following the administration of *Archachatina maginata* haemolymph. The level of urea and chloride were significantly lower while the level of creatinine increased in animals treated with 2 and 4 ml/kg of haemolymph than the control rats. However, serum bicarbonate sodium and Potassium level was not significantly altered.

3.4 Body Weight and Relative Organ Weight

The effects of administration of 2 and 4 ml/kg of *Archachatina maginata* haemolymph on body weight and Relative organ weight of albino rat are shown in Table 4 and 5 respectively. Body weight gain of the rats administered *Archachatina maginata* haemolymph for 10 days were not significantly different from those of the control rats (Table 4). Similarly the computed liver, spleen and heart body weight ratios of the rats were not significantly different from those of the control rats (Table 5). However, there was significant decrease in the kidney/body weight ratio as compared with the control values.

3.5 Clinical Observations

Clinical observations for toxicological studies show that the haemolymph did not produce any grossly negative behavioral changes such as excitement, restlessness, respiratory distress, convulsions or coma, however, increase water consumption was observed during the experimental periods.

Table 1. Haematological parameters of rats administered *Achachatina maginata* haemolymph

Parameters	Control rats	Rat dose with 2 ml/kg haemolymph	Rats dose with 4 ml/kg haemolymph
White Blood Cells (x10^9/L)	23.80±2.01^a	85.10±4.05^b	120.06±3.52^c
Granulocyte (%)	29.30±3.15^a	69.00±3.15^b	73.30±4.06^b
Lymphocytes (x10^9/L)	14.30±1.14^a	25.40±1.22^b	30.90±1.10^c
Red Blood Cells (x10^{12}/L)	2.54±0.21^b	2.44±0.23^b	1.97±0.02^a
Hematocrite (L/L)	0.20±0.11^b	0.26±0.05^b	0.16±0.05^a
Hemoglobin (g/L)	126.00±2.07^a	370.06±7.02^c	296.03±5.10^b
Mean corpuscular haemoglobin (pg)	49.80±1.11^a	151.70±2.59^b	150.40±6.61^b
Mean Corpuscular Hemoglobin Concentration (g/L)	646.86±23.01^b	41.70±0.00^a	33.01±0.00^a
Mean Corpuscular Volume (FL)	77.05±4.31^a	105.96±2.17^c	84.00±2.17^b
Red cell distribution width (FL)	42.60±1.46^a	74.00±2.17^c	47.30±5.19^a
Platelate count (x10^9/L)	295.45±3.12^b	288.98±2.11^b	173.04±4.22^a
Mean platelate volume (FL)	23.76±2.11^b	24.06±1.10^b	12.90±0.42^a
Plateletecrit (L/L)	0.41±0.05^b	0.39±0.005^b	0.21 005^a
Platelete distribution weight (%)	2.54±0.21^b	2.44±0.23^b	1.97±0.02^a

Values are mean ± SEM of 4 determinations. The values along the same row with different superscripts are significantly different (p<0.05)

Table 2. Liver function indices of rats administered *Archachatina maginata* haemolymph

Parameters	Control rats	Rat dose with 2 ml/kg haemolymph	Rats dose with 4 ml/kg haemolymph
Total bilirubin (umol/l)	8.80±0.37^a	11.80±0.21^b	10.43±0.20^b
Albumin (g/dl)	2.90±0.05^a	3.30±0.11^b	3.80±0.01^b
Total protein (g/L)	60.90±0.15^a	68.80±1.23^b	71.90±2.05^c
AST(U/L)	36.0±0.27^a	67.00±2.23^b	67.00±0.34^b
ALT(U/L)	12.0±0.15^a	25.20±0.05^b	67.00±1.09^c
ALP(IU/L)	75.30±1.08^b	56.40±0.98^a	90.30±1.08^c

Values are mean ± SEM of 4 determinations. The values along the same row with different superscripts are significantly different (p<0.05)

Table 3. Kidney function indices of rats administered *Archachatina maginata* haemolymph

Parameters	Control rats	Rat dose with 2 ml/kg haemolymph	Rat dose with 4 ml/kg haemolymph
Urea (mmol/L)	4.36±0.20^c	2.30±0.44^a	3.90±0.01^b
Creatinine (µmol/L)	104.90±0.11^a	118.97±2.34^b	125.98±2.05^c
Sodium (MEq/L)	162.76±3.23^a	168.97±3.98^a	163.09±3.64^a
Potassium (mmol/L)	8.60±0.41^a	8.20±0.19^a	7.90±0.97^a
Chloride (mmol/L)	101.97±4.59^c	65.45±2.35^a	95.05±4.56^b
Bicarbonate (mmol/L)	22.03±0.31^a	21.60±0.09^a	23.80±1.05^a

Values are mean ± SEM of 4 determinations. The values along the same row with different superscripts are significantly different (p<0.05)

Table 4. Weight changes of rats administered *Archachatina maginata* haemolymph

Parameters	Initial weight (G)	Final weight (G)	Weight gain (G)
Control rats	112.70±3.47	126.10±3.59	13.45±2.12[a]
Rat dose 2 ml//kg haemolymph	172.40±3.03	186.14±4.54	13.74±1.03[a]
Rats dose 4 ml//kg haemolymph	200.40±4.21	214.50±4.40	14.10±2.10[a]

Values are mean ± SEM of 4 determinations. The values along the same row with different superscripts are significantly different (p<0.05)

Table 5. Relative organ weightof rats administered *Archachatina maginata* haemolymph

Parameters	Control rats	Rats dose with 2 ml/kg haemolymph	Rat dose with 4 ml/kg haemolymph
Liver	3.330±0.110[a]	3.688±0.001[a]	3.418±0.201[a]
Kidney	0.703±0.001[b]	0.5931±0.031[a]	0.607±0.015[a]
Heart	0.321±0.003[a]	0.376±0.004[a]	0.359±0.00[a]
Spleen	0.309±0.015[a]	0.399±0.012[a]	0.338±0.010[a]

Values are mean ± SEM of 4 determinations. The values along the same row with different superscripts are significantly different (p<0.05)

4. DISCUSSION

Hematological parameters generally provide information on the deleterious effects of foreign components on the blood and also explain blood-related functions of chemical compounds [24]. White blood cells defend the body against infections or any foreign body. The significant increase on WBC and factors relating to it (WBC, LY and GRA) recorded in this research work may indicate immunological response to the constituents of the haemolymph and this augmented the production of more WBC, LY and GRA to improve the health status of the animals. The decreases in RBC and HCT observed in haemolymph treated rats indicate anemic condition which could be attributed to the destruction of the erythroblast, or, limiting their synthesis. The decrease in the MCHC in this study was an indication of erythrocytes swelling. However, the increase RDW and MCV reflect hemolytic anemia and cytotoxic effect the haemolymph [25]. This indicates that the haemolymph has no antianaemic potentials at the dose studied contrary to the traditional belief and previous report that it has ability to cure anaemia.

The biochemical indices monitored in the serum of rats are useful 'markers' for assessment of tissue damage. The measurement of activities of various enzymes in the body fluids plays a significant role in disease investigation and diagnosis [26], assault on the organs/tissues and to a reasonable extent the toxicity of the drug [27]. Biomarker enzymes can also indicate tissue cellular damage caused by chemical compounds long before structural damage that can be picked by conventional histological techniques [28]. The transaminase (ALT and AST) are 'markers' of liver damage and can thus be used to assess liver cytolysis with ALT being a more sensitive biomarker of hepatotoxicity than AST [29]. Consequently, in the present work marked increase in ALT and AST observed after treatment with haemolymph may have occurred as a result of metabolism of the haemolymph by the liver and hepatic tissue turn-over, as response by the body system towards overcoming stress induced by the test substance.

Alkaline phosphatase is a "*marker*" enzyme for the plasma membrane and endoplasmic reticulum [30]. It is often employed to assess the integrity of plasma membrane [28] and endoplasmic reticulum. The reduction in the enzyme activities after 2 ml/kg administration of the haemolymph may be attributed to either loss of membrane component (including alkaline phosphatase) into the extracellular fluids [31] or inactivation of the enzyme molecule in situ [32] or depletion of important molecules required by the enzyme for maximum activity [27].This reduction in ALP activities can constitute threat to the life of cells that are dependent on a variety of phosphate group for their vital process e.g. synthesis of major membrane phospholipids, (phosphatidylethanolamine and phosphatidylcholine) since there may be less availability of the phosphate group which consequently affect membrane fluidity and

decrease the permeability of the epithelial cells [33]. The increase in the activity of ALP after 4 ml/kg administration of the haemolymph could be a consequence of activation of the enzyme or an increase in the rate of the synthesis of the enzyme induced by the higher dose of components of the haemolymph. This increase could also indicate an enhanced functional activity of the organs (kidney and small intestine) [34], since there may be an increase in the rate of ion transport across the cell membranes in these tissues.

The concentrations of total protein, albumin, total bilirubin, urea, creatinine and electrolytes are useful 'markers' of secretory, synthetic and excretory functioning of the liver and kidney [35] The observed increase in the total protein, albumin and total bilirubin content suggests a compromise of the synthetic ability of the liver arising from the administration of the haemolymph. The haemolymph might have increased the functional activity of the liver by interfering with the equilibrium in the rate of synthesis and destruction, removal or clearance of total protein, albumin and total bilirubin from the system of the animals. A similar finding has been previously reported in rats administered aqueous extract of *Crateva adansonii* [36]. Such increase in total protein could, however, lead to dehydration which is detrimental to cellular homeostasis. This will negatively affect the metabolic activities of the liver and consequently the health of the animals.

The kidneys regulate the excretion of urea and reabsorption of electrolytes into the blood. Filtration occurs at the glomeruli while reabsorption takes place in the renal tubules [37]. When there is compromise of normal glomerular function, substances normally cleared by the kidneys such as urea and creatinine accumulate in the biological fluid. The significant decrease in serum urea following the administration of the haemolymph for 10 days may be due to decreased protein catabolism (as observed in Table 2) or renal dysfunction [38], while the significant increase in creatinine content of the serum may be attributed to compromise of the renal functional capacity. The haemolymph might have either interfered with creatinine metabolism leading to increased synthesis or the tissue might have compromised all or part of its functional capacity of tubular excretion [39]. This therefore suggests that the continuous administration of the haemolyph of *A. marginata* could cause renal damage.

The absence of an effect on the levels of serum bicarbonate sodium and potassium suggests that the normal excretion of this electrolytes by the kidney was not been adversely affected by the haemolymph. Furthermore, the significant decreases in the levels of chloride ions concentration following the administration of haemolymph suggest that some aspects of tubular functioning as it relates to chloride have been compromised.

The treatment of the rats with 2 and 4 ml/kg of the haemolymph for 10 days in this study does not affect the body weight gain of the animals (Table 4). Organ body weight ratios are normally investigated to determine whether the size of the organ has changed in relation to the weight of the whole animal. The absence of an effect on the computed liver/body, spleen/body and heart/body weight ratios suggest that the haemolymph did not cause any form of swelling, atrophy and hypertrophy on the organs [40]. However, the significant reduction in kidney/body weight ratio following the 10 days administration of the haemolymph may be attributed to tissue necrosis since the organ is concerned with the excretion of foreign substances [27].

Furthermore, clinical observations for toxicological studies show that the haemolymph did not produce any grossly negative behavioral changes such as excitement, restlessness, respiratory distress, convulsions or coma, however, increase water consumption was observed during the experimental periods, this increase in water intake could be attributed to dehydration of the animals that results from an increase in total protein.

5. CONCLUSION

In conclusion, the results of this study indicate that administration of *Archachatina maginata* haemolymph at the doses of 2 and 4 ml/kg caused changes in the hematological profile and some biochemical parameters of liver and kidney dysfunction with a more pronounced effect on the liver function than the renal function tests and at a higher dose than the lower dose. Hence, caution should be taken when using the haemolymph of *Achachatina maginata* for therapeutic purpose because it may also have some liver or tissue membrane damaging effect. However, this study represents a preliminary toxicological investigation or human risk assessment, further studies on the effects of

prolong haemolymph administration on vital organs are recommended.

CONSENT

It is not applicable.

ETHICAL CLEARANCE

Ethical Clearance was given by Federal University of Technology, Minna/Nigerian Ethical Review Board (CUERB) in accordance with internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review**.**

COMPETING INTERESTS

Authors have declared that no competing interests exist

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