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Adrenaline Induced Elevated Blood Pressure in Wistar Rats is not Reversed by Oral Administration of *Archachatina marginata* Hemolymph

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Authors' contributions

This work was carried out in collaboration between all authors. Author ABO designed the study and wrote the manuscript in conjunction with authors PIJ and ADW. Authors UA, PIJ and JAO conducted the wet lab analyses including the histological. Author ADW performed the statistical analyses and plotted all graphs. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: The increasing incidence of high blood pressure, its complications and associated fatalities has led to an upsurge in the use of alternate forms of medicaments in its management. The oral ingestion of the hemolymph of *Archachatina marginata* is commonly used as an antihypertensive by the Yoruba people of South West Nigeria. This study investigated the effect of oral administration of *Archachatina* hemolymph on normotensive and adrenaline induced hypertensive wistar rats.
Methods: The hemolymph of *Archachatina marginata* was orally administered at doses of 22.8 and 45.6 mg/kg body weight to normotensive and adrenaline induced hypertensive rats for 7 days. Blood pressure parameters were measured via a polygraph. Histopathological assessment of the heart tissue was conducted. Data gathering and analysis were done in 2014 (February – August).

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Results: In this study, the orally administered hemolymph had no significant (p<0.05) lowering effect on the systolic/ diastolic pressure, pulse pressure, mean arterial pressure or heart rate of either the normotensive or adrenaline induced hypertensive rats. Histopathological assessment of the cross section of the heart tissues shows the hemolymph had no adverse effect on the examined cross section of the heart tissue.
Conclusion: Based on the data from this study, there is no justifiable reason for the use of the hemolymph of *Archachatina marginata* as a antihypertensive.

Keywords: Archachatina marginata; haemolymph; wistar rats; hypertension; heart rate; pulse pressure.

1. INTRODUCTION

The incidence of high blood pressure related mortalities is globally on the increase, with the Nigerian nation not exempted. Hypertension is a common denominator in sudden unexpected natural deaths, among senior executives, army recruits and others in Nigeria [1-3]. Traditional medicine plays an important role among the population of most developing countries [4], as it is used by over half of the population. There are quite a number of folkloric therapies for high blood pressure in Nigeria, the oral ingestion of *Archachatina marginata* (Fig. 1) hemolymph is one of such.

Snails are gastropods and belong to the phyllum *Mollusca*. The name snail applies to members of the Gastropod family having a large external shell capable of completely covering the soft inner delicate parts when withdrawn into it. Snails have been eaten by mankind from time immemorial. The African land snails (*Archachatina marginata*) like other members of the snail family are not only eaten for gastronomic reasons, but also for different pharmacological/medicinal culturally alluded values. Snails are considered a delicacy in most parts of Nigeria where they are consumed. The meat of the African snail is very rich in proteins, low in both total carbohydrates, fats, and dense in minerals [5-8]. The use of the snail as a medicament is an old practice, where it is used whole, or as a component of various traditional recipes for different ailments, [9,10] such as restoring fertility, virility labour pains, blood loss in pregnancy etc [11-14]. A documented example is the Italian garden slug (*Arion hortensis*) is reported to cure gastritis (stomach ulcers) when swallowed whole [15]. The slime (haemolymph) from snails is a component used in the cosmetic industry, where it is used to treat acne, combat wrinkles, reduce pigmentation and scarring, treat dermatitis, inflammations, calluses and promote healing. The shell [16] and meat [7] of *Archachatina marginata* is reportedly used for treating gonorrhea and hypertension. There is presently a dearth of scientific information on the use of *Archachatina marginata* hemolymph [8,17]. This study is a pilot study aimed at finding scientific justification (if any) for the folkloric use of snail hemolymph in the treatment of hypertension among the Yoruba speaking people of South west Nigeria.

Fig. 1. Picture of live *Archachatina marginata* (to scale)

2. MATERIALS AND METHODS

2.1 Animal Studies

Forty (40) male wistar rats (120-200g) were purchased from an animal house in Lagos, South west Nigeria. The rats were housed in animal cages at the animal enclosure of the Department of Biochemistry, Lagos State University Ojo and allowed to acclimatize for 7 days with unrestricted access to clean water and rat chow under a 12 hours light/dark cycle. The temperature of the animal house during the experimental period was 27±4ºC.

2.2 Preparation of Haemolymph Sample

Snails (*Archachatina marginata*) were purchased from a snailery in Lagos. The snails were identified, thoroughly washed, and the guts of the snail were removed with a sharp object from the cracked cone [18]. The haemolymph was drained into a clean sterile container. Hemolymph extracted from the snail was filtered to remove debris and particulate matter. The protein concentration of the hemolymph filtrate was determined using the Folin Ciocalteau method. [19].

2.3 Sds Page

The filtered heamolymph (filterate) was centrifuged using a refrigerated TGL-16 centrifuge (5000rpm @ 4ºC) before 12 noon in the laboratory. The protein concentration of the resulting supernatant and pellet were determined and the samples (10µL) run on denaturing 12% polyacrylamide gel electrophoresis (SDS-PAGE) [20].

2.4 Experimental Design and Treatment

Experimental protocols were conducted in accord with guidelines of the Institutional Animal Care and Use Committee and were approved by the Animal Ethical Committee of the Department of Biochemistry, Lagos State University, Ojo, Lagos, Nigeria. The rats were randomly divided into four groups, with 5 rats in each group. The animals were orally administered with the hemolymph at 22.8 mg/kg body weight. Nifedipine was used as a positive control in this experiment. Administration route and dosage used in this study were based on oral and documented literature [8,17], the dosage used in this study is extrapolation from convention and oral interviews with people alternate medical practitioners and people already consuming the hemolymph. The age long traditional administration route for the hemolymph by its users is oral, which is simulated in this study.

Two sets of experiments were carried out using the filtered hemolyph of *Archachatina marginata*:

a. Effect of hemolymph on normotensive rats.
   Group C1: Rats administered distill water (control group).

   Group C2: Rats administered with standard antihypertensive (Nifedipine) drug.
   Group U1: Rats administered with 22.8 mg/kg body weight of hemolymph filterate.
   Group U2: Rats administered with 45.6 mg/kg body weight of hemolymph filterate.

b. Effect of hemolymph on adrenaline induced hypertensive rats: High blood pressure was induced in all groups of animals used in this experiment via administration with adrenaline.

2.5 Blood Pressure Measurement

Blood pressure was measured under urethrane anaesthesia by transduction polygraphy and heart rate by tachography. Briefly, the animals were administered intra-peritoneal injections of a mixture of 25% (w/v) urethrane and 1% α-chlorase in water. To reduce respiratory dead space and improve ventilation during the recordings, the animals had endotracheal intubation carried out on them. The trachea was exposed through a supra-sternal incision and a rubber cannula of suitable diameter was inserted into it and secured with ligatures.

After this, the left femoral artery was exposed through a groin incision and cannulated with a catheter filled with heparinised saline solution. The cannula was then connected through a Statham pressure transducer to a Grass 7D Polygraph. The transducer was previously calibrated with a mercury manometer. The Polygraph was set at a speed of 10mm/sec with a sensitivity of 1mv/cm. The Polygraph had a flat frequency response up to 30 Hz. Mean arterial pressure (MAP) was obtained from the blood pressure signal displayed on the chart recorder, while heart rate was calculated via tachographic beat-to-beat conversion [21].

2.6 Histology

At the end of the recordings the animals were sacrificed by high dose ketamine injections and x the heart was dissected out and processed for histological examination. Briefly, the heart was

washed in buffered saline and fixed in 10% formal saline solution for 72 hours. It was then cleared in Xylene, dehydrated in graded alcohol solutions and embedded in molten paraffin at 57ºC and blocked out. 5-µm sections were cut out of these blocks, rehydrated and stained with Heamatoxylin and Eosin, and examined in phase contrast microscopy at 100 and 400 magnifications [22].

2.7 Statistics

All data were collected and analysed in 2014.Data are expressed as mean ± SEM. One way analysis of variance (ANOVA) was carried out in all experiments. Data were analyzed using SPSS version 19.

3. RESULTS AND DISCUSSION

Generally, the management of hypertension is via change in lifestyle habits (feeding, exercise) or medicaments (synthetic or natural). One of the established mechanisms of anti-hypertensives like nifedipine (used in this study) is as a calcium channel blocker, [23] though there are other antihypertensives with other mechanisms. The folkloric use of a number of naturally occurring matter of plant and animal origin as a form of therapy or medicament is most times by virtue of oral tradition and culture. The management of hypertension via alternate/traditional or complementary medicines like the use of honey is in the public domain, where it exerts its action possibly via kidney protection against oxidative damage [24]. This study investigated the scientific rationale for the use of the hemolymph of *Archachtina marginata* in the management of high blood pressure. A previous study [7] had earlier suggested that the proximate composition of *Archachatina marginata* may account for its therapeutic use as antihypertensive. The results from this study (Figs. 2 – 6) indicate that the oral administration of 22.8 and 45.6mg/kg body weight of *Archachatina marginata* hemolymph had no significant (*P*=0.05) lowering effect on the diastolic, systolic, pulse pressure, mean arterial blood pressure and heart rate of both the normotensive and adrenaline induced hypertensive rats when compared with nifedipine (standard antihypertensive drug). This data is at variance with an earlier study, [25] that reported a lowering effect of the mucin of the giant African snail on the heart functions of toads (*Bufo regularis*). Another study [17] had also reported a lowering effect of the snail body fluid on blood pressure of normotensive dogs. Some proteins have been reported to possess hypotensive potentials, viz, [26-28] Denaturing SDS-PAGE of *Archachatina marginata* filtered hemolymph and supernatant indicate a protein profile from 5 - >225kDa, certainly with none of those proteins having hypotensive potentials. A positive observation noted in this study is the lack of any adverse effect on the heart tissues (Fig. 7) of the animals administered with the hemolymph. A reduction of high blood pressure can be achieved by either decreasing cardiac output or lowering vascular resistance [23].

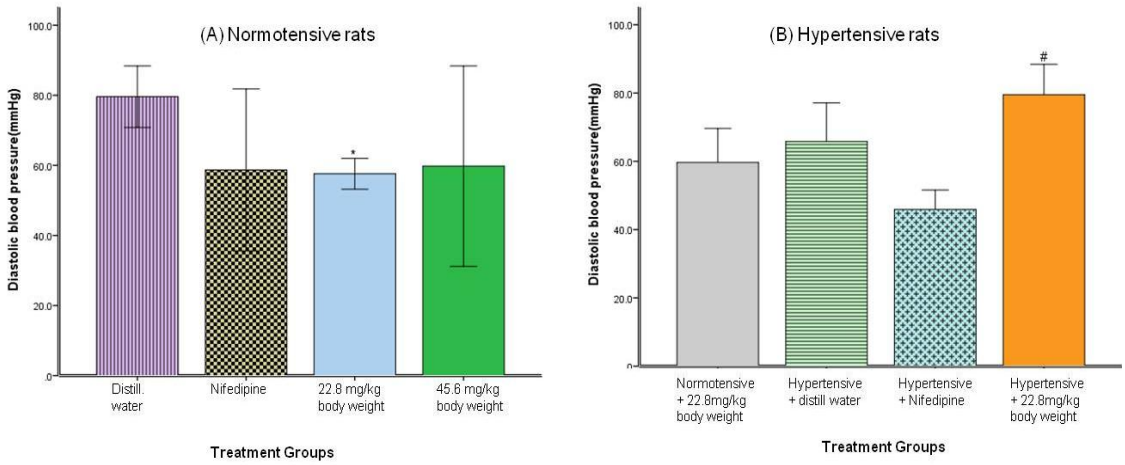

Fig. 2. Bar charts showing the effect of *Archachatina marginata* hemolymph on the diastolic blood pressure of (a) Normotensive and (b) Hypertensive rats

*Each bar represents the means ± S.E.M. of 6 rats. Bars with # are significantly different compared with nifedipine treated rats, # P = 0.05, while bars with * are significantly different compared with distilled water treated rats, * P = 0.05*

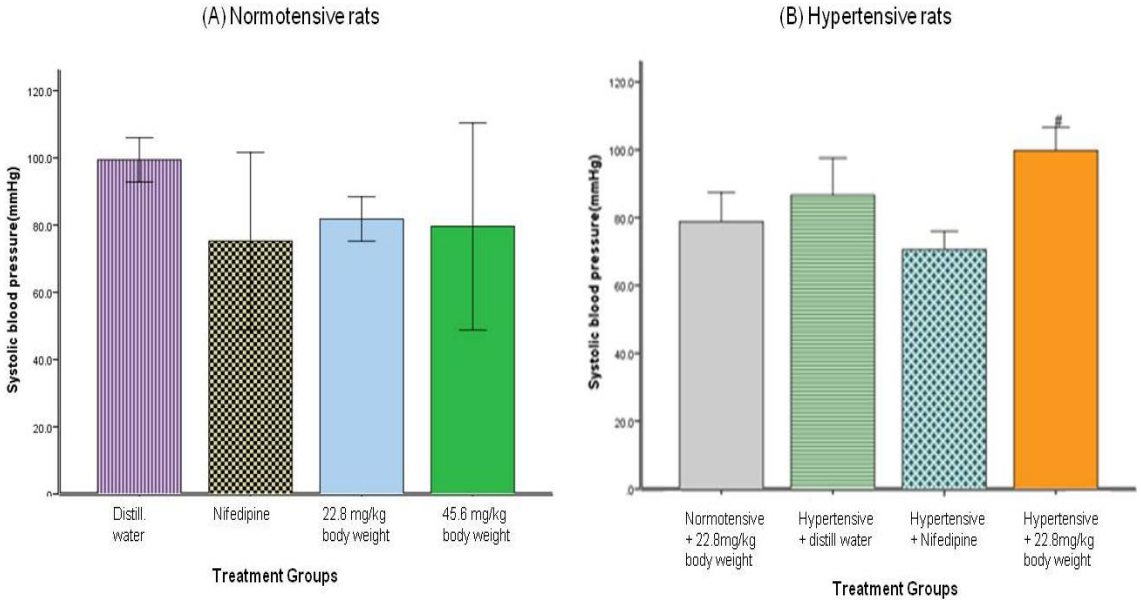

Fig. 3. Bar charts showing the effect of *Archachatina marginata* hemolymph on the systolic blood pressure of (a) Normotensive and (b) Hypertensive rats

*Each bar represents the means ± S.E.M. of 6 rats. Bars with # are significantly different compared with nifedipine treated rats, # P = 0.05, while bars with * are significantly different compared with distilled water treated rats, * P = 0.05*

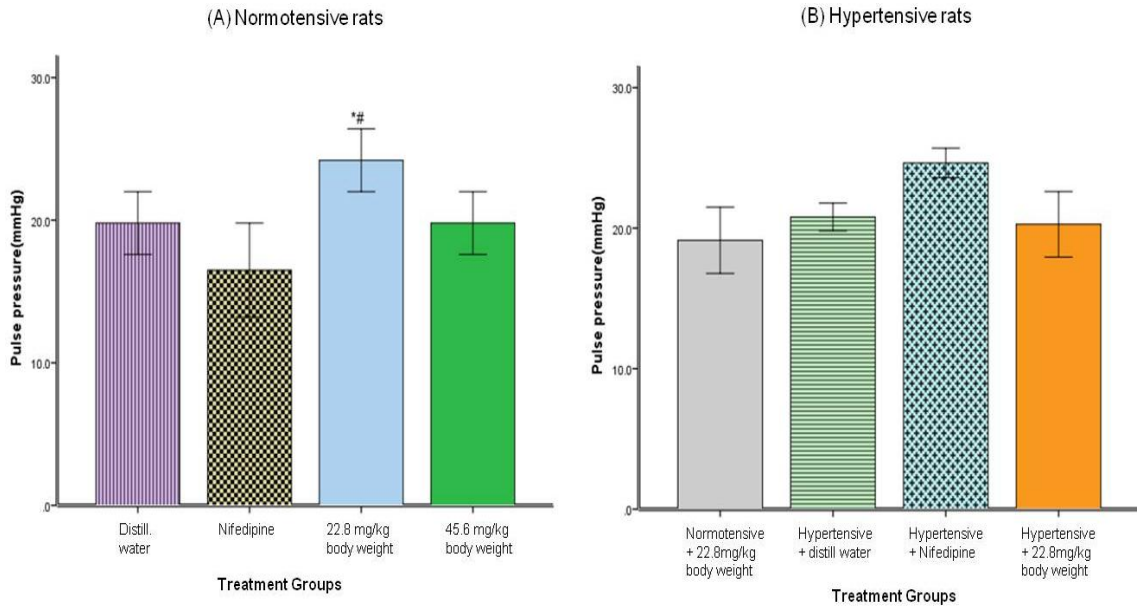

Fig. 4. Bar charts showing the effect of *Archachatina marginata* hemolymph on the pulse pressure of (a) Normotensive and (b) Hypertensive rats

*Each bar represents the means ± S.E.M. of 6 rats. Bars with # are significantly different compared with nifedipine treated rats, # P = 0.05, while bars with * are significantly different compared with distilled water treated rats, * P = 0.05*

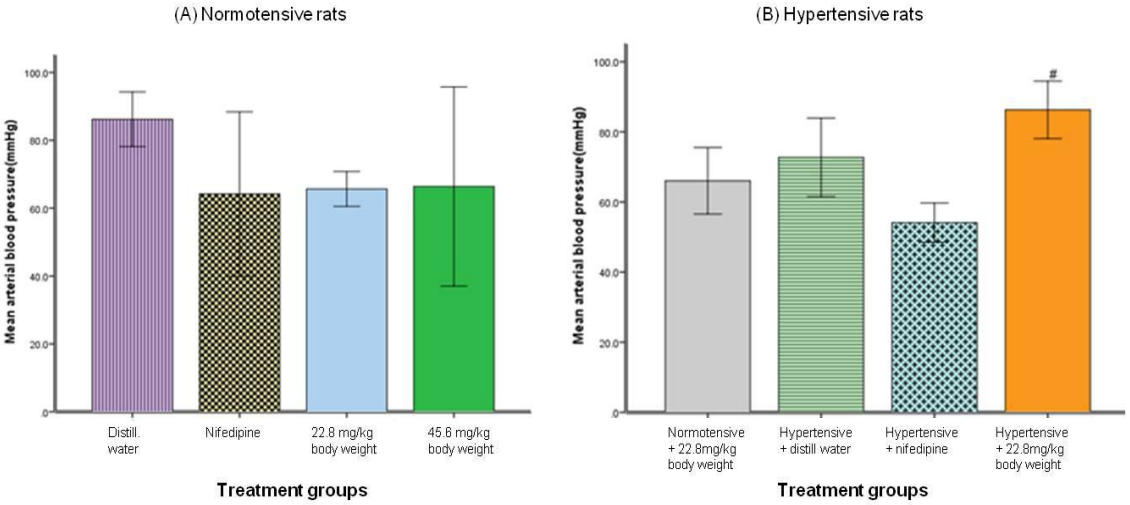

Fig. 5. Chart showing the effect of *Archachatina marginata* hemolymph on the mean arterial blood pressure of (A) Normotensive and (B) Hypertensive rats

*Each bar represents the means ± S.E.M. of 6 rats. Bars with # are significantly different compared with nifedipine treated rats, # P = 0.05, while bars with * are significantly different compared with distilled water treated rats, * P = 0.05*

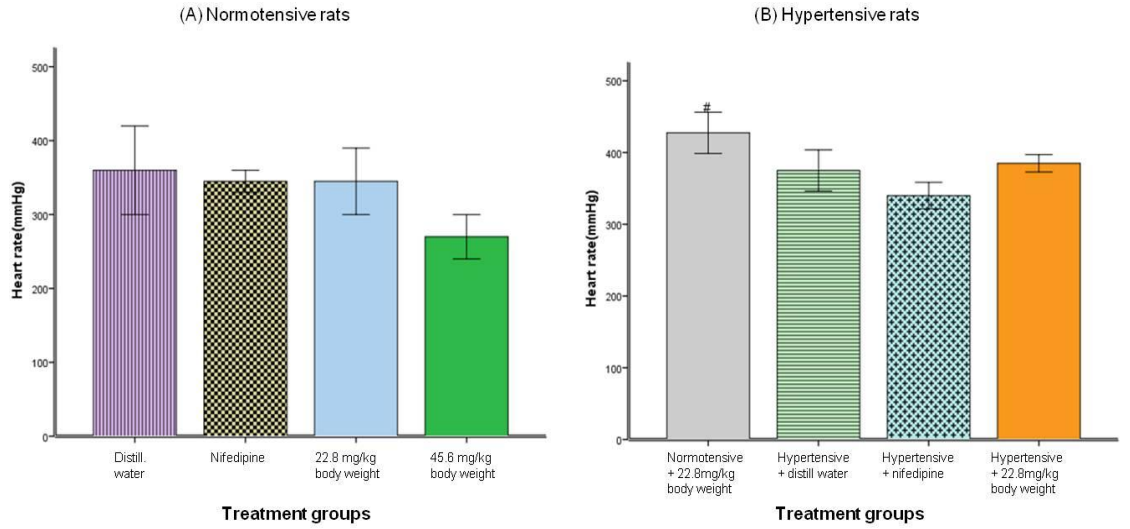

Fig. 6. Chart showing the effect of *Archachatina marginata* hemolymph on the heart rate of (A) Normotensive and (B) Hypertensive rats

*Each bar represents the means ± S.E.M. of 6 rats. Bars with # are significantly different compared with nifedipine treated rats, # P = 0.05, while bars with * are significantly different compared with distilled water treated rats, * P = 0.05*

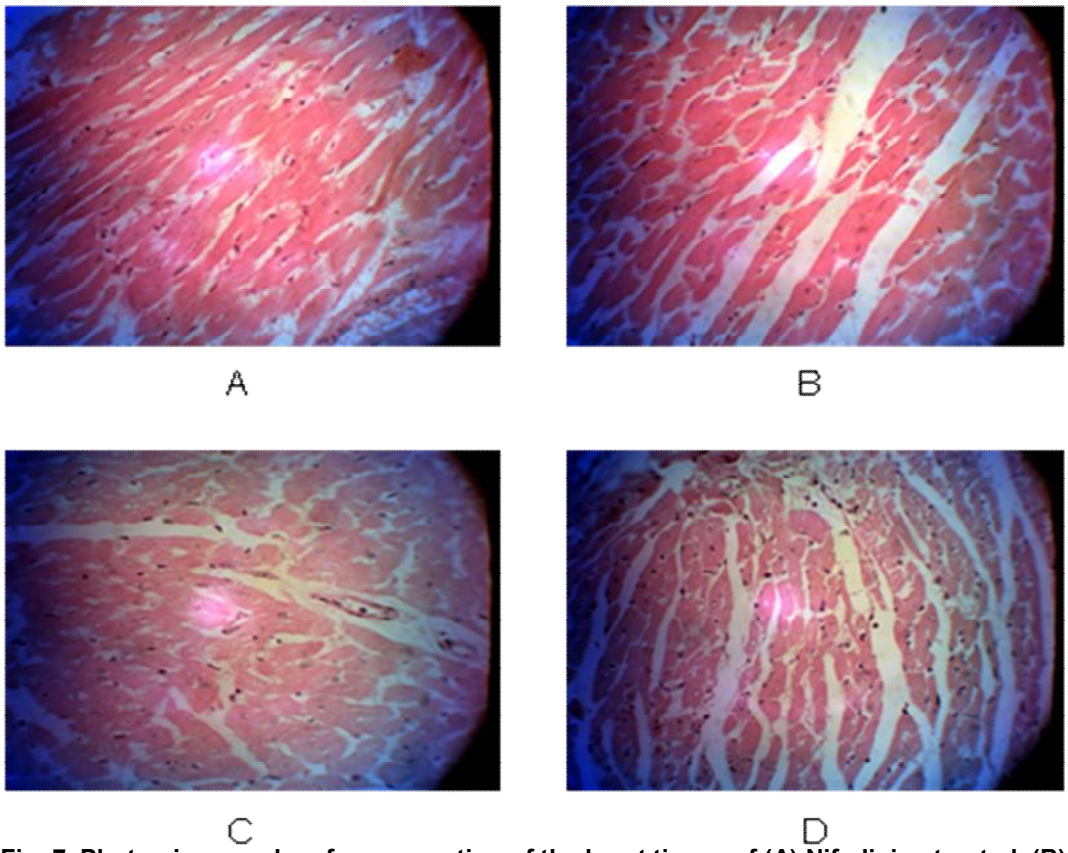

Fig. 7. Photomicrographs of cross section of the heart tissue of (A) Nifedipine treated, (B) Distill water treated, (C) 22.8 mg/kg body weight hemolymph treated and (D) 45.6mg/kg body weight hemolymph treated. H&E × 400

5. CONCLUSION

Taken together, the data from this study shows no blood lowering potentials consequent upon the oral administration of the hemolymph of *Archachatina marginata* in rats.

CONSENT

It is not applicable.

ETHICAL CONSIDERATIONS

Experiments were performed with strict adherence to global ethical standards as approved by the institution.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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