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Oral Administration of Archachatina Marginata Hemolymph Depresses Reactive Oxygen Species Scavenging Potentials in Wistar Rat

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Abstract
High blood pressure (hypertension) and its related complications and fatality are on the increase globally. Hypertension is conventionally managed with drugs and lifestyle adjustment. There exists a myriad of folkloric remedies to manage hypertension. The oral administration of the filtered hemolymph of Archachatina marginata is one of the more common ways of managing hypertension among the Yoruba speaking people (South-west) of Nigeria. This study investigated the effects of the oral administration of the hemolymph of Archachatina marginata on normotensive wistar rats. Different groups of normotensive wistar rats were orally administered with 22.8mg/kg and 45.6mg/kg body weight filtered hemolymph, and nifedipine daily for seven (7) days. The animals were subsequently sacrificed post peritoneal anesthesia with mixture of urethane and chlorase. Blood was collected, and the different organs removed and evaluated for the different types and concentrations of antioxidants. Glutathione and superoxide dismutase concentrations were reduced in the liver and kidney of rats administered with hemolymph compared with the control and nifedipine treated groups. Liver and kidney malonaldehyde concentrations were elevated compared with the distill water and nifedipine treated groups. Malonaldehyde and glutathione levels were significantly higher in the heart of the hemolymph treated rats compared with other groups. The circulating blood levels of total cholesterol, high density and low density lipoproteins and triglycerides were higher in the hemolymph treated groups compared with the distill water treated group. Taken together, all these data suggests an increased level of oxidative stress on the animals consequent upon the administration of the hemolymph of Archachatina marginata.
Keywords: *Archachatina marginata*, reactive oxygen species, hemolymph.

1. Introduction

Archachatina marginata commonly called the giant African snail is a common source of protein to a diverse (Busari Ahmed, Idris-Adeniyi et al.) group of people in Nigeria and other parts of the world. It is known to be rich in macronutrients such as proteins and minerals (Ebenso 2003, Eneji, Ogogo et al. 2008, Babalola and Akinsoyinu 2009, Hamilton-Amachree, Mepba et al. 2009, Adeyeye 2012, Engmann, Afoakwah et al. 2013, Offiong E. E. A. 2013). The consumption (or administration) of snail meat, and its hemolymph are not only consumed, but also alleged folklorically (some scientifically proven) to cure ailments like hypertension, antiulcerogenic (Marquis 1974, Nwandu 1999, Dede, Odia et al. 2003, Adikwu 2005, Adikwu and Nnamani 2006, Nj, Okonta et al. 2009, Ajibola, Rahman et al. 2013) etc, even though some reports counter some of these claims (Mabadeje 1974). The potentials of using mucin from *Archachatina marginata* in drug delivery has also been reported (Kenechukwu, Ibezim et al. 2013, Momoh, Kenechukwu et al. 2013, Momoh, Adedokun et al. 2014) and in modification of experimental osteoarthritis in dogs (Ajadi, Oladele et al. 2013). Oxidative stress is associated with ailments , with hypertension not excluded (Giugliano, Ceriello et al. 1995, Vaziri, Wang et al. 2000, Redón, Oliva et al. 2003, Vaziri and Rodríguez-Iturbe 2006). This study is a continuation of our studies aimed at evaluating the biological or health benefits (if any) in the consumption (oral administration) of *Archachatina marginata* hemolymph.

2. Materials and methods

2.1. Animals

Forty (40) male wistar albino 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± 4^{o} C.

2.2. Preparation of hemolymph 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 (Akinloye and Olorode 2000).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 (Lowry, Rosebrough et al. 1951).

2.3 Experimental design and treatment

The animal experiment was carried out in accordance with the guidelines for laboratory procedures laid down by the University ethics committee on research as well as internationally accepted principles regarding animal care and use of animals for laboratory experiments. The rats were randomly divided into four groups, with 3 rats in each group. The animals were orally administered with the hemolymph at 22.8mg/kg and 45.6mg/kg body weight daily for seven (7) days

The rats were grouped thus:

Group 1: rats administered distil water (control group)
Group 2: rats administered with standard antihypertensive (Nifedipine) drug.
Group 3: rats administered with 22.8mg/kg body weight of hemolymph filterate
Group 4: rats administered with 45.6mg/kg body weight of hemolymph filterate

2.3. Preparation of animals for experiment.

The rats (group) were anaesthetized with a mixture of urethane 25% (w/v) and alpha chlorase 1% (w/v) in distilled water. The anaesthesia was administered peritoneally at a dose 0.5ml/100g body weight. The anaesthesia was at a depth sufficient to produce surgical analgesia. After the administration of anaesthetic agent, the animal was kept in the cage for some minutes before use. Animals were used only when the anaesthesia had set in. This was when the degree of anaesthetic was suitable for dissection, i.e. when there is absence of muscular tone, absence of corneal reflex and lack of response to painful stimuli. Blood was collected via cardiac puncture and organs removed.

2.4 Statistics

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

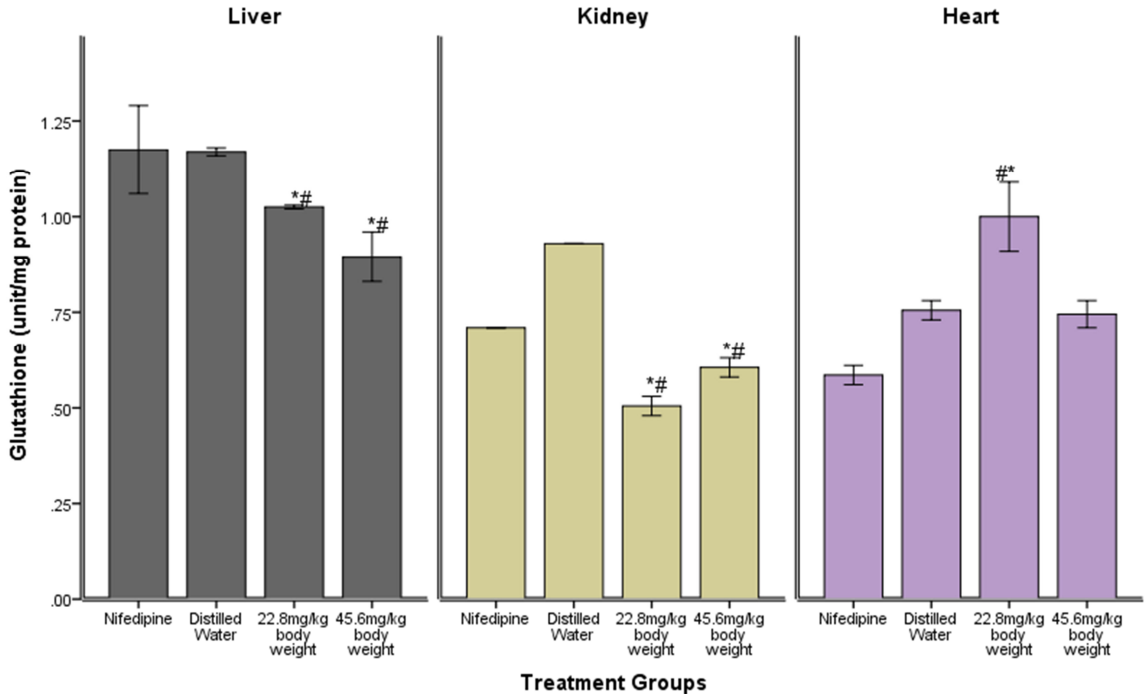

Figure 1: Graph showing levels of glutathione (unit/mg protein) in the liver, kidney and heart of the control (distil water), positive control (nifedipine) and oral hemolymph (22.8 and 45.6 mg/kg body weight) administered rats.

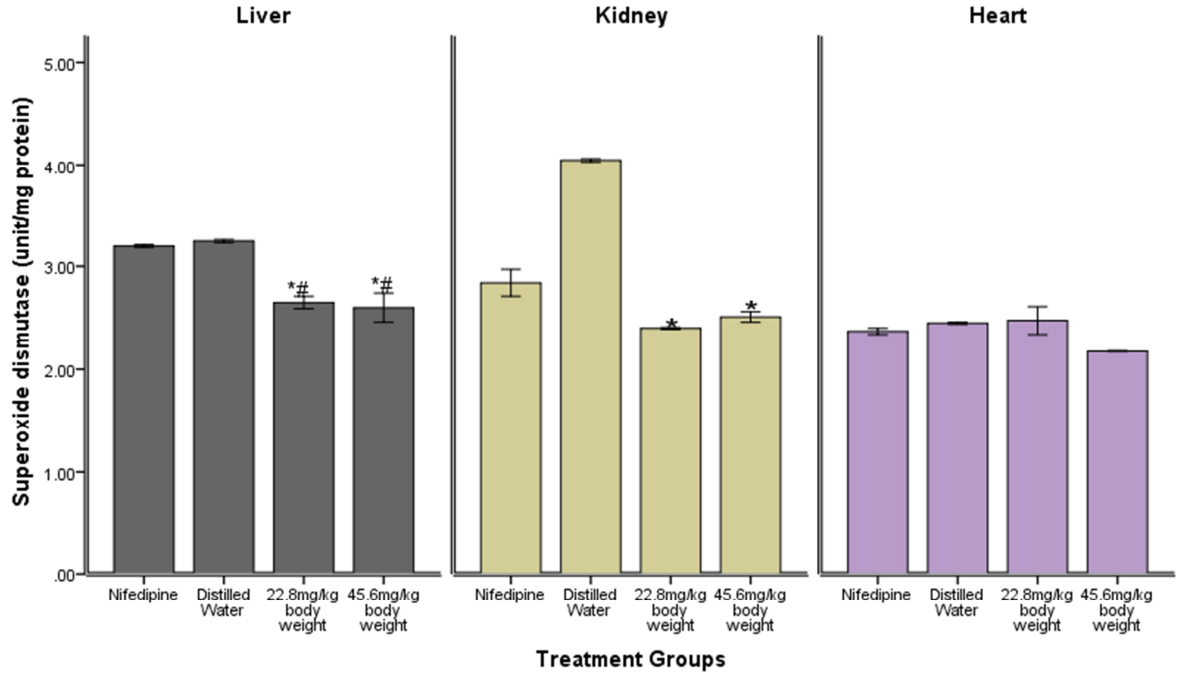

Figure 2: Graph showing levels of superoxide dismutase (unit/mg protein) in the liver, kidney and heart of the control (distil water), positive control (nifedipine) and oral hemolymph (22.8 and 45.6 mg/kg body weight) administered rats.

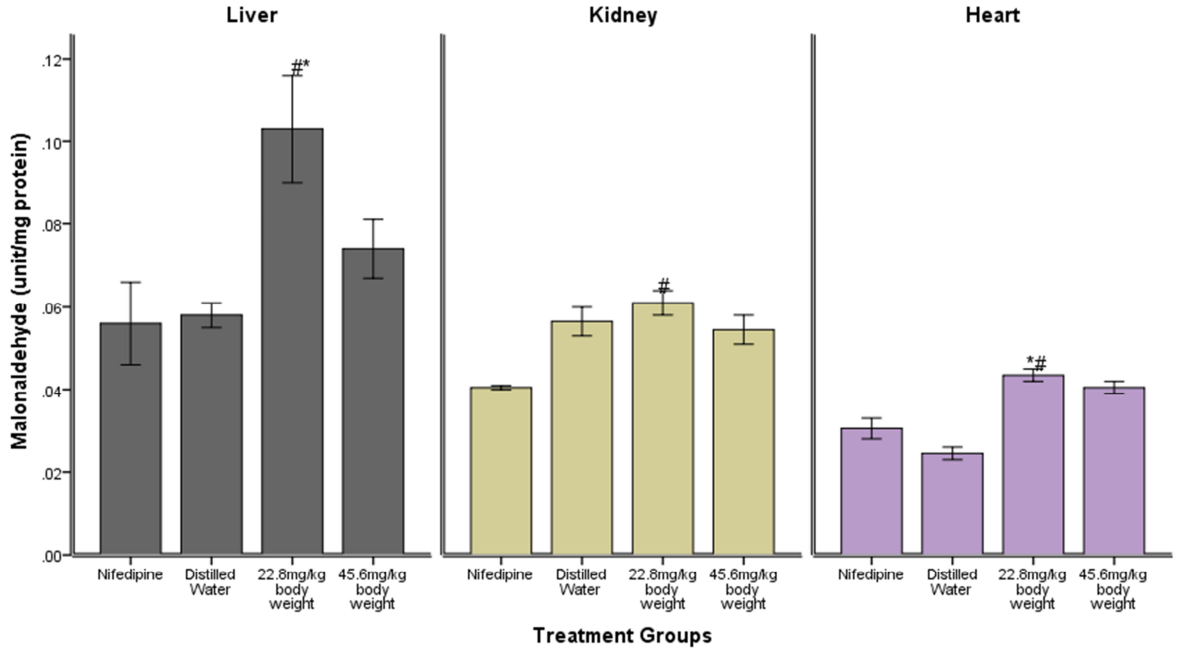

Figure 3: Graph showing levels of malondialdehyde (unit/mg protein) in the liver, kidney and heart of the control (distil water), positive control (nifedipine) and oral hemolymph (22.8 and 45.6 mg/kg body weight) administered rats.

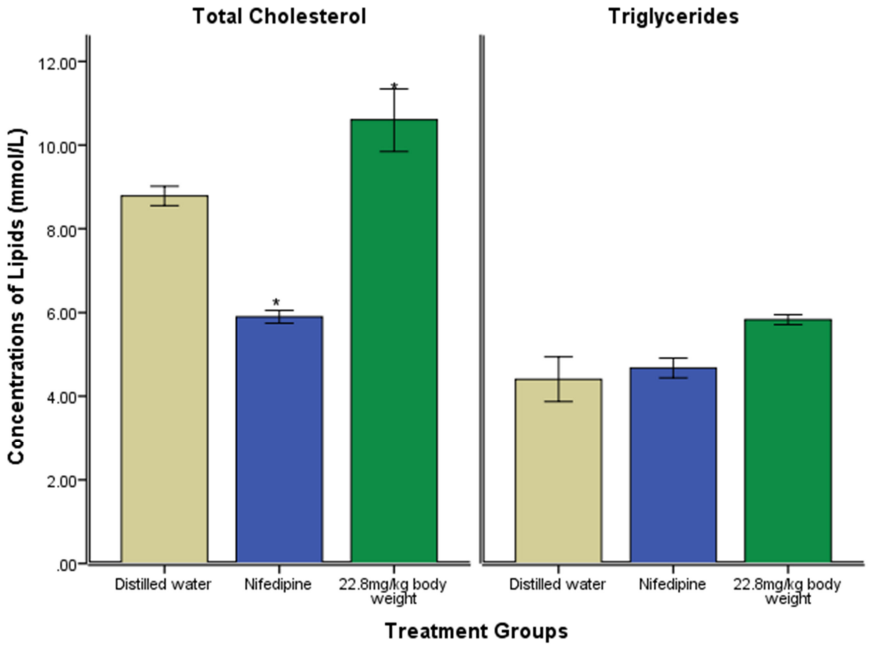

Figure 4: Graph showing levels of total cholesterol and triglycerides (mmol/L) in the control (distil water), positive control (nifedipine) and oral hemolymph (22.8 mg/kg body weight) administered rats.

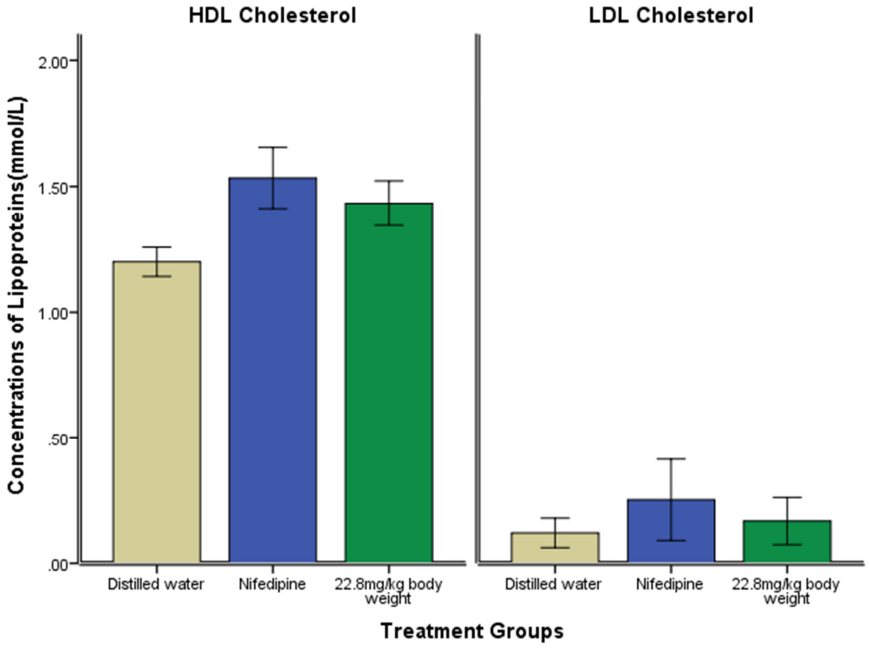

Figure 5: Graph showing levels of HDL cholesterol and LDL cholesterol (mmol/L) in the control (distil water), positive control (nifedipine) and oral hemolymph (22.8 mg/kg body weight) administered rats.

4. Discussion

The use of different types of naturally occurring matter as therapy for ailments such as hypertension, diabetes and others is a common global phenomenon. For hypertension, this includes the use of lemon juice in Turkey (Adibelli, Dilek et al. 2009), hemolymph of snails in Nigeria (Adibelli, Dilek et al. 2009, Ojekale, Agbafor et al. 2015) amongst others. The results from this study suggests a reduced level of free radicals scavengers like glutathione and superoxidase dismutase in the liver and kidney of the haemolymph treated rats (figure 1& 2) compared with the rats treated with the antihypertensive nifedipine. Hypertension is known to cause and

increase renal oxidative stress (Vaziri, Wang et al. 2000, de Faria, Silva et al. 2011), and thus concomitantly upregulate the expression of enzymic and non enzymic free radicals mop up mechanisms. An upregulation of antioxidant defence mechanisms in the presence of an external trigger factor is indicative of the biological system trying to protect itself from associated cellular damages (Peixoto, Pessoa et al. 2009), while the converse could mean an overwhelmed system. A significant increase is observed in the malonaldehyde (fig. 3) levels in the kidney, liver and heart of the hemolymph treated rats in comparison with the nifedipine treated. This suggests increased oxidative stress in the presence of the orally administered *Archachatina marginata* hemolymph via lipid peroxidation. The levels of triglycerides, cholesterol, HDL cholesterol and LDL cholesterol (fig 4 & 5) in the different group of animals treated were not significantly different from each other. Taken together, the data from this study suggests increased oxidative stress on the rats consequent upon the administration with the hemolymph.

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