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## **Archachatina marginata Haemolymph Potentiates Hypoglycemic Effect by Mimicking Insulin in Streptozotocin-Induced Diabetic Rat**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author OMO designed the study. Author SKA managed the analyses of the study and performed the statistical analysis. Author ATO wrote the protocol, first and final drafts of the manuscript. Author OOA managed the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aims:** This study investigates the hypoglycemic potential of *Archachatina marginata* haemolymph and its mechanism in streptozotocin-induced diabetic rats.

**Study Design:** One factor experimental design.

**Place and Duration of Study:** Department of Chemical Sciences, Joseph Ayo Babalola University, Ikeji Arakeji, Osun State, Nigeria between January to June, 2015.

**Methodology:** The rats were set into four groups (n=5). Group 1 - Non-diabetic control (NDC), Group 2 - Diabetic Control (DC), Group 3 - Diabetic rats with 1 mL administration of snail

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haemolymph (DSS1), Group 4 - Diabetic rats with 2 mL snail haemolymph administration (DSS2). The blood glucose concentration of each rat was taken and thereafter, they were weighed and then administered with 55 mg/kg STZ in citrate buffer (pH 4.5) peritoneally non-diabetic control group (NDC). Rats with blood glucose level of 200 mg/dL and above were considered diabetic. Haemolymph of 1 mL and 2 mL were administered to rats in DSS1 and DSS2 respectively while DC received 2 mL distilled water for 14 consecutive days. Physiological oranges and blood samples were harvested for various analysis.

**Results:** The *A. marginata* haemolymph significantly improved the plasma concentration of insulin and reduced plasma glucose level in a dose dependent manner  $P=.05$ .

**Conclusion:** The *A. marginata* haemolymph has potential to exact hypoglycemic effect and improve the insulin concentration in streptozotocin induced diabetic rat.

**Keywords:** *Archachatina marginata*; haemolymph; streptozocin diabetes rats.

## 1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease characterized by persistent hyperglycemia resulting from insulin deficiency or insulin resistance or both. It is estimated that in year 2000 there were approximately 150 million individuals with the disease and that this number is likely to double by year 2025 [1]. Type 2 diabetes is the fourth or fifth leading cause of death in most developed countries and there is growing evidence that it has reached epidemic proportions in many developing and newly industrialised countries [2]. High blood sugar can produce long-term complications such as cardiovascular and renal disorders, retinopathy, and poor blood flow [3]. Its development can be prevented or delayed in people with impaired glucose tolerance by implementing lifestyle changes or the use of therapeutic agents [3]. Despite the variety of therapies currently available, achieving desirable glycemic target has become a burden to the patients leading to sourcing for complementary and alternative medicine [4]. Recently, orthodox sources have continued to be valuable therapeutic agents especially in treating non communicable diseases such as snail's hemolymph which is used in folk medicine [4,5]. Haemolymph is the blood analogue found in all arthropods and most mollusks which have an open circulatory system. It is composed of water, inorganic salts [6,7] and organic compounds (mostly carbohydrates, proteins and lipids). In the traditional ethno medicine of South West Nigeria, the African Giant Snail is included as part of recipes for the treatment of various ailments and diseases. It is believed that various preparations containing snails are useful in restoring fertility, virility and to cure smallpox [8]. Also, it is used as relieve in labour pains, blood loss in pregnancy and during delivery.



**Fig. 1. Live *Archachatina marginata***

The meat of *A. marginata* according to scientific finding contains low level of sodium, fat and cholesterol but high in proteins, iron and calcium hence it's claimed usefulness in the treatment of Atherosclerosis, Anaemia, Hypertension and Obesity [9]. Also, it has been reported that protein is relatively higher than other metabolites in the haemolymph of *A. marginata* [10,11,12] with heamocyanin a respiratory pigment as the major protein [10]. In continuation to source for a safe therapeutic agent in treating diabetes, this study investigates the hypoglycemic potential of *A. marginata* haemolymph and its mechanism in streptozotocin -induced diabetic rats.

## 2. MATERIALS AND METHODS

Reagent kits for glucose were obtained from Randox laboratory, USA. Streptozotocin (STZ) was obtained from Sigma-Aldrich (Germany). Other chemicals used were of analytical grade and obtained from FLUKA, BDH (Germany) and other standard commercial suppliers.

## 2.1 Animals

Healthy female albino rats were acquired from the animal holding unit of the Institute for Advanced Medical Research and Training (IMARAT), University College Hospital, University of Ibadan. Animals were maintained with food and water ad libitum and under a 12-h light/12-h dark cycle. African Giant Snails were bought from Ipetu-Ijesa Market and taken to the Department of Animal Science, Joseph Ayo Babalola University, Ikeji Arakeji for authentication. The "principle of laboratory animal care" (National Institute of Health-NIH publication No. 85- 23) guidelines and procedures were followed in this study (NIH publication revised, 1985). The ethical committee of the department of chemical sciences, Joseph Ayo Babalola University, Ikeji Arakeji approved the research work.

### 2.1.1 Induction of diabetes through the administration of streptozotocin to experimental animal

The rats were set into four groups (n=5) for Streptozotocin administration.

**Group 1:** Non-diabetic control (NDC)

**Group 2:** Diabetic Control i.e. induced rats without haemolymph administration (DC)

**Group 3:** Diabetic rats with 1 mL snail haemolymph administered (DSS1)

**Group 4:** Diabetic rats with 2 mL snail haemolymph administered (DSS2)

After the initial two weeks period of acclimatization, the animals were fasted overnight but allowed access to water ad libitum. The blood glucose concentration of each rat was taken and thereafter, they were weighed and then administered with 55 mg/kg STZ in citrate buffer (pH 4.5) peritoneally [13] with the exception of the non-diabetic control group (NDC) that received only citrate buffer.

The rats were returned to their respective cages and fed glucose laden water to guide against the immediate hypoglycaemic effect of STZ administration. After three days the blood glucose level of the rats were again checked to ascertain successful diabetes inducement with the presence of hyperglycaemia. Rats with blood glucose level of 200 mg/dl and above were considered diabetic and used as diabetic animals in subsequent studies.

## 2.2 Preparation of *A. Marginata* Snail Extract

The whole snails were washed with copious amount of tap water and then rinsed with distilled water. The apex of the snails were opened by method adopted from Akinloye and Olorode [14] and the haemolymph collected into a clean beaker. It was stored at 4°C.

## 2.3 Treatment with *A. Marginata* Snail Haemolymph

The Snail haemolymph was administered orally using the gastro-enteral cannula. 1 mL of haemolymph was administered to rats in DSS1 while 2 mL was administered to those in DSS2. The diabetic control, DC, received 2 mL distilled water. Administration was done once a day in the morning for fourteen (14) consecutive days.

## 2.4 Blood Sample Collection

At the end of the fourteenth day of administration the animals were fasted overnight i.e. no food but they were given access to water. The animals were weighed and sacrificed by cervical dislocation before slicing their carotid artery with blade and the flowing blood was collected into appropriately labeled fluoride oxalate bottle for blood glucose and plain bottle for other analysis. The blood in the plain bottle was allowed to stand until effective clotting and clot retraction was observed. After appreciable retraction, the blood samples were spun at 3,000 rpm for 15 minutes using the centrifuge. The top supernatant i.e. the serum was carefully transferred to appropriately labeled plain tubes with the aid of a Pasteur pipette. Samples were immediately stored at 4°C till analysis.

### 2.4.1 Organ- body weight ratio

Immediately after sacrifice, vital organs such as liver, heart, spleen, lung and kidneys were quickly excised into normal saline to remove blood, blotted with clean laboratory tissue, freed of fat and then weighed. The organ to body weight ratio was determined by dividing the weight of each organ with the final body weight of each rat.

### 2.4.2 Determination of blood glucose

The glucose was determined after enzymatic oxidation in the presence of glucose oxidase.

The formed hydrogen peroxide reacts, under catalysis of peroxidase, with phenol and 4-aminophenazone to form red-violet quinoneimine dye as indicator [15].

#### **2.4.3 Determination of insulin**

The serum insulin quantitative determination is based on a solid phase enzyme – linked immunosorbent assay as described by Burtis and Edward [16].

#### **2.4.4 Estimation of serum $\alpha$ -amylase activity**

Amylase activity assayed using enzymatic method of Klein et al. [16] where amylase hydrolyses starch at pH 6.9 to form a product which couple with iodine to give a coloured complex whose intensity is directly proportional to the concentration of amylase in the plasma when read at 590 nm.

### **2.5 Statistical Analysis**

All data were subjected to one way analysis of variance. The mean, standard error of mean (SEM) and percentage were used for statistical analysis. The statistical significance between the control and each treated groups were determine using student t-test. The level of significance was set at  $P=.05$ .

## **3. RESULTS AND DISCUSSION**

### **3.1 Effects of Snail Haemolymph on Body Weight of STZ Induced Diabetic Rats**

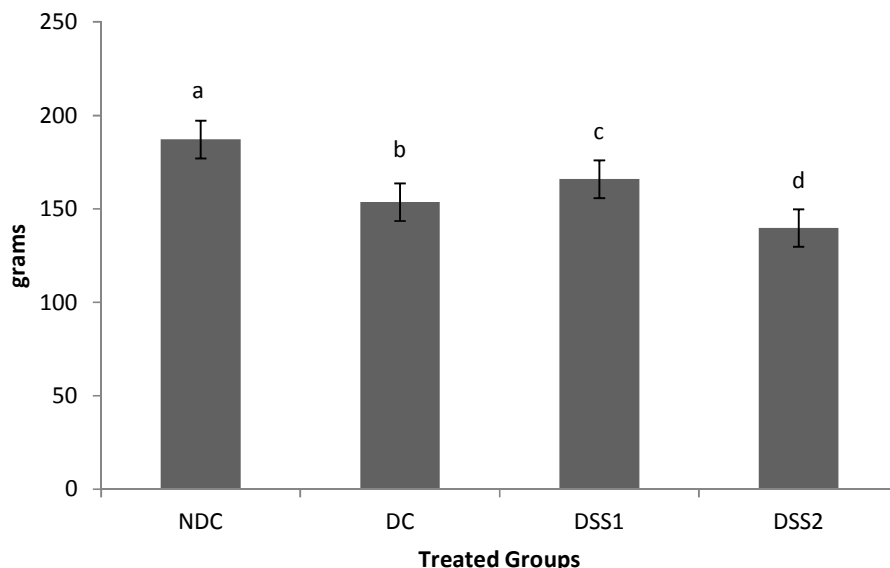
There was a significant reduction ( $P=.05$ ) in weight of all the induced groups when compared with the non-diabetic control group (Fig. 2). After treatment with snail haemolymph, no significant improvement observed.

### **3.2 Effects of Snail Haemolymph on Body Organ Ratio of STZ –Induced Diabetic Rats**

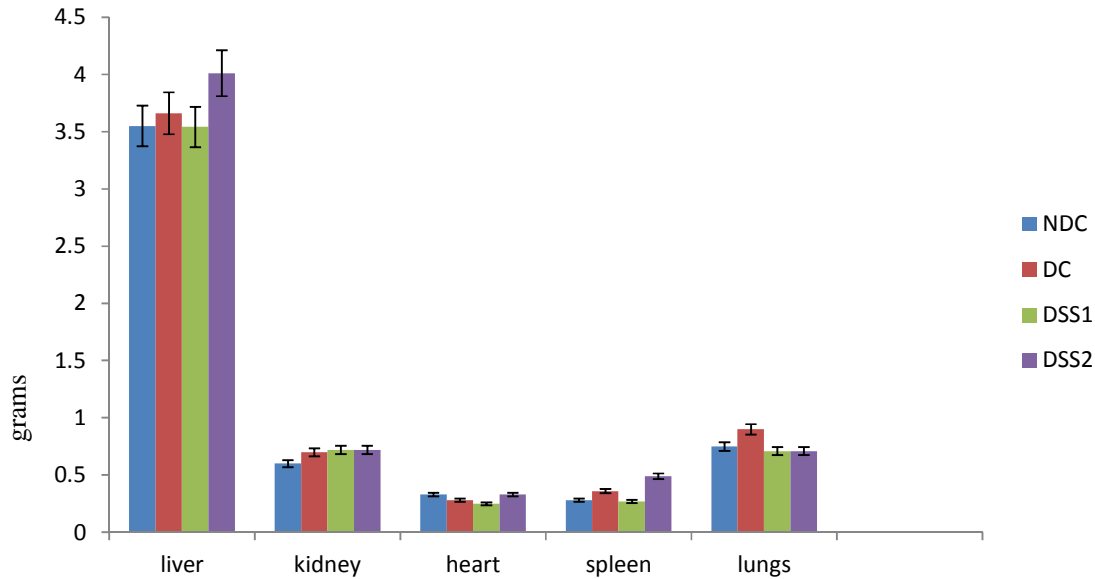
There was no significant change in major organs weight of all the induced groups when compared with the non diabetic control group (Fig. 3).

### **3.3 Effects of Snail Haemolymph on Blood Glucose Concentration in STZ-Induced Diabetic Rats**

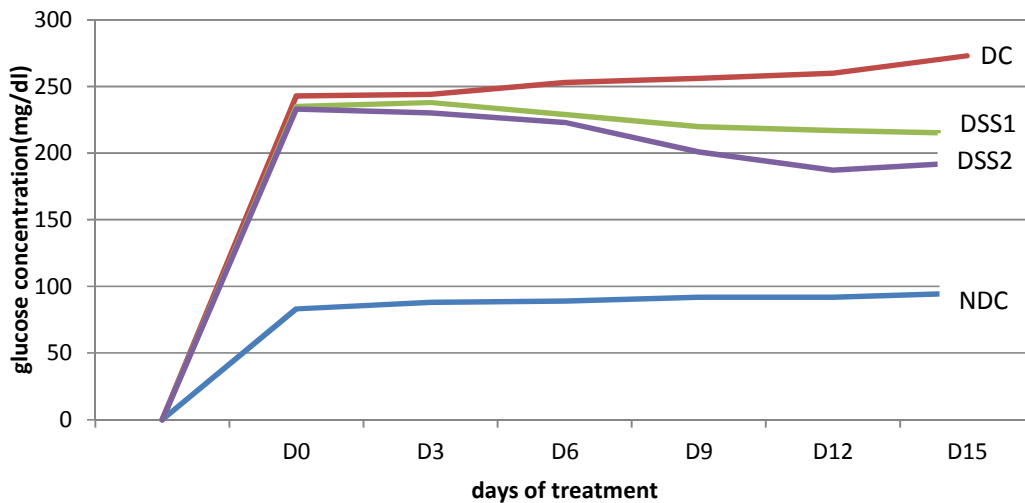
The results are presented as Means  $\pm$  SEM of five independent determinations. The determinations were done at 3 days interval commencing from the day treatment also commenced (D0). There was a significantly increase in blood glucose level ( $P=.05$ ) in all the induced groups DSS1 and DSS2 when compared with the NDC throughout the period of



**Fig. 2. Effects of snail haemolymph on body weight of STZ induced diabetic rats. Each bar is expressed as Mean  $\pm$  SEM of level of measurements of the body weights of rats in gram in each treatment group, (n=5). Bar carrying different alphabetical superscripts have significant differences ( $P=.05$ )**



**Fig. 3. Effects of snail haemolymph on body organ ratio of STZ – induced diabetic rats Each bar is expressed as Mean  $\pm$  SEM of level of measurements of the body organs weights of rats in gram in each group (n=5)**



**Fig. 4. Effects of snail haemolymph on blood glucose concentration in STZ-induced diabetic rats. The results are presented as Means  $\pm$  SEM of five independent determinations**

15 days within the six different set of samples. On D6, DSS2 was found to be significantly reduced when compared with DC. On D9, there were significant reduction ( $P=.05$ ) in glucose level when DC was compared with DSS1 and when DC was compared with DSS2. On D12, there were significant reduction ( $P=.05$ ) in blood glucose level when DC was compared DSS1/DSS2 and DSS1 with DSS2. Also on D15, there were significant reduction ( $P=.05$ ) when DC

was compared with DSS2 but with no significant change when compared with DSS1 (Fig. 4).

### 3.4 Effects of Snail Haemolymph on Insulin Concentration in STZ-Induced Diabetic Rats

There was a significant reduction in blood Insulin concentration when NDC was compared with each of the other groups ( $P=.05$ ). Also, There

was significant decrease in insulin concentration when DC was compared with DSS1/DSS2, (P=.05). There was an increase in insulin concentration when African giant snail haemolymph was increased from 1 mL to 2 mL (DSS1 and DSS2) (p<0.05) (Fig. 5).

### 3.5 Effects of Snail Haemolymph on Serum $\alpha$ -Amylase of STZ-Induced Diabetic Rats

$\alpha$  – Amylase activities significantly increased when NDC was compared with each of the other groups (P=.05). Comparing DC with DSS1/DSS2 showed a significant increased activities  $\alpha$  – amylase (P=.05). Also, there was a significant decrease in  $\alpha$  – amylase activity when comparing DSS1 with DSS2 (P=.05) (Fig. 6).

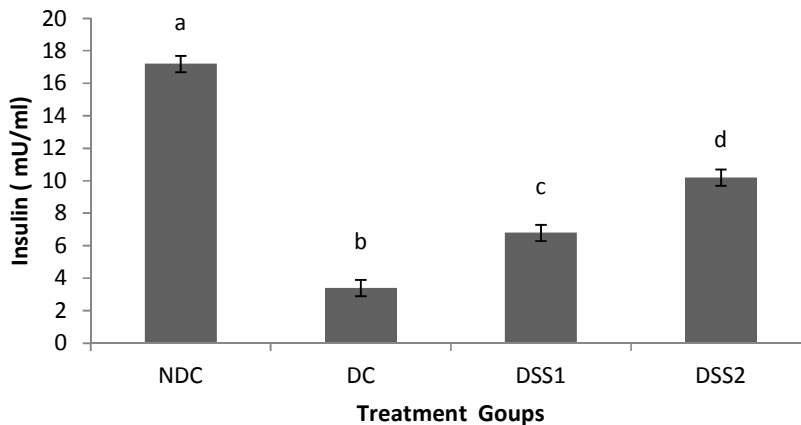
Rats injected with streptozotocin (STZ), provide model of insulin-dependent diabetes mellitus with hyperglycaemia, loss of significant weight and partial deficiency in insulin activity were all presented by the Wistar rats in this study after induction prior to treatment with snail (*A. marginata*) haemolymph. This is consistent with earlier reports [17,18,19].

Diabetes as a metabolic disease at the onset is usually associated with loss of weight due to unavailability of glucose for metabolism in the tissue and increased utilization of alternative source of energy. This is observed in the significant reduction in weight of all the induced groups when compared with the non-diabetic control group. After treatment with snail

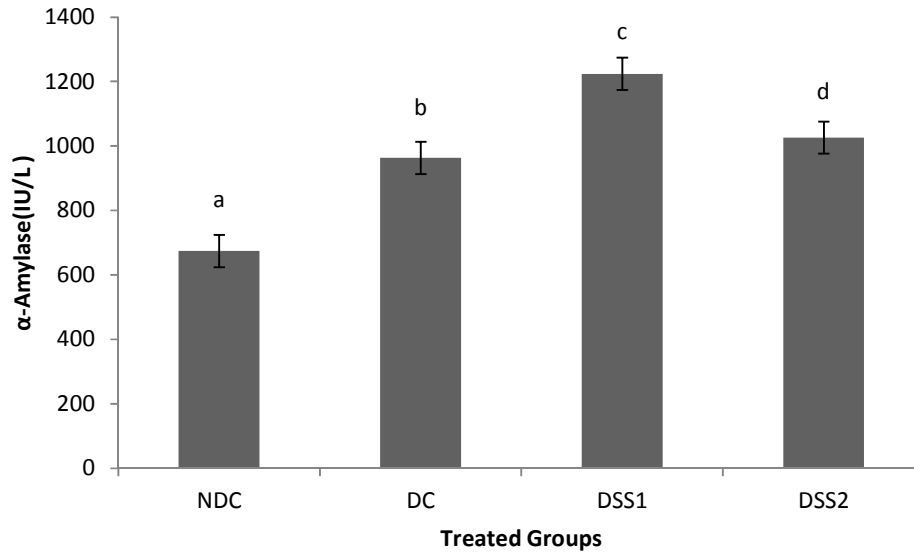
haemolymph there was no significant improvement observed. Also, there was no significant difference in major organs weights.

Diabetes is an emergency with worldwide financial burden on millions sufferers [20]. Its complications are severe and therefore needed cheap and available medication. Snail haemolymph in this study shows potential of being of high value in reducing blood glucose level at 2 mL regimen which could be attributed to the nutritive values associated with haemolymph [21]. Streptozotocin induces selective necrosis of beta cells of the pancreas that produces insulin. This degenerative process led to the observed significant decrease in the insulin concentration and the attendant DM. Snail haemolymph of 2 mL regimen significantly improved the insulin concentration over the 1 mL regimen. This is also reflected in the gradual and consistent reduction in the blood glucose concentration as opposed to the consistent increase in the diabetic control group.

Plasma  $\alpha$ - amylase consist of two forms, of salivary gland and pancreatic origin respectively. Because the salivary iso-enzyme is the principal form in plasma, total enzyme activity is not significantly lowered when the pancreatic secretory cell mass is reduced by chronic pancreatic disease. However, in acute pancreatitis, total plasma amylase activity is usually significantly increased due to release from damaged cells [22]. Streptozotocin damage of pancreatic beta cells, released the excess alpha amylase observed.



**Fig. 5. Effects of snail haemolymph on insulin concentration in STZ-induced diabetic rats. Each bar is expressed as Mean  $\pm$  SEM of concentration of blood insulin, (n=5). Bar carrying different alphabetical superscripts have significant differences (P=.05)**



**Fig. 6. Effects of snail haemolymph on serum  $\alpha$ -amylase of STZ-induced diabetic rats. Each bar is expressed as Mean  $\pm$  SEM of concentration of blood  $\alpha$ - amylase, (n=5). Bars with different alphabets superscript are significantly different (P=.05)**

#### 4. CONCLUSION

*A. marginata* haemolymph has potential to exact hypoglycemic effect and improve the insulin concentration in streptozotocin induced diabetic rat.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

The “principle of laboratory animal care” (National Institute of Health-NIH publication no. 85- 23) guidelines and procedures were followed in this study (NIH publication revised, 1985). The ethical committee of the Department of Chemical Sciences, Joseph Ayo Babalola University, Ikeji Arakeji approved the research work.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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