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EVALUATION OF THE HAEMOLYMPH OF THE GIANT AFRICAN LAND SNAILS *Achatina achatina* AND *Archachatina marginata* FOR BACTERIA STERILITY AND INHIBITORY PROPERTIES

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ABSTRACT

An experiment was conducted to examine bacteria sterility and bacteria inhibitory properties of haemolymph of two species of Giant African Land Snails. A total number of 18 *A. achatina* and *A. marginata* snails each were used for this experiment. The shell and foot of the snails were washed with distilled water for isolation of bacteria flora and bacteria load. The experiment was a 2 x 2 x 3 factorial arrangement with 3 replicates in a completely randomized design. To isolate bacteria, a sterile inoculating loop was used to pick a portion of each dissimilar colony and transferred into another appropriate fresh sterile medium in a culture plate and streaked in a quadrant streak plate method to obtain pure cultures of the isolates. The method was aimed at trimming down the microbial load to allow for isolates to grow in pure cultures. The plates were incubated at 37 ^{0}C for 24 hours, after which the pure isolates were observed and inoculated into McCartney bottles containing nutrient agar slants. The bottles were incubated for 24 hours at 37 ^{0}C and stored in the refrigerator for further biochemical analysis. Haemolymph of the two species studied was not sterile irrespective of the type of haemolymph. To test for haemolymph inhibitory properties, Haemolymph was collected from each snail into sterile and labeled containers in the laminar flow chamber. Microorganism was seeded into the agar plate, after the agar has solidified, holes were bored into the agar using a sterilized hole borer. One ml of the haemolymph collected was poured into each well in agar plates and incubated at 37 ^{0}C for 24 hours observing for zones inhibition. Equally, there was no evidence of bacteria inhibition by the haemolymph despite the three methods of bacteria inhibition used: agar diffusion method, disk diffusion method and serial diffusion method. It could be concluded from this study that the haemolymph of the two snail species did not exhibit bacteria sterility and inhibition.

Key words: Haemolymph, bacteria, sterility, inhibitory properties, Giant African Land Snails

INTRODUCTION

Molluscs posses a natural immunity formed by anatomical and chemical protective barriers that prevents damage to the underlying tissues, body fluid losses and infections of pathogenic microorganisms and parasites. The main physical barrier is the shell and mucus, which cover the soft body of molluscs. Snails have been reported to be one of the oldest species around the globe that have survived extreme environmental conditions for more than 600 million years. This fact is an indication that snails have some special adaptive proteins with which they survive in

their environment. The integrity of the body covering is complemented by inherent agglutination ability of the haemolymph (Glinka and Jarosz, 1997; Abiona, 2010).
Haemolymph is the blood analogue found in all arthropods and most molluscs that have an open circulatory system. It is composed of water, inorganic salts mostly Na, Cl, Mg and Organic compounds mostly carbohydrates, proteins and lipids. Muscular movement by the snail during locomotion can facilitate haemolymph movement, but diverting flow from one area to another is Limited (Burton, 1965).

Wide range of research work has been conducted on the Giant African Land Snails *A. achatina* and *A. marginata* which includes considerable interest in the nutrition, growth, stocking density and housing systems of Giant African Land Snails at different body weight. Adeleke *et al.* (2002) on their part studied the effect of parent body weight on the growth rate of Giant African Land Snails (*A. marginata*) under unrestricted feeding. Ogunsanmi *et al.* (2003) reported on the haemolymph biochemical parameters. Omoyakhi (2007) evaluated the effect of aestivation on growth, body composition and reproductive performance of Giant African Land Snails *A. achatina* and *A. marginata*. There have also been reports on the environmental effects: Rosiji (2005) experimented on the effect of soil moisture level on the reproductive organs of the Giant African Land Snails *A. marginata* in different seasons. Study on the biophysical and biochemical parameters of the haemolymph of *A. marginata* was carried out by Abdussamad (2009). Abiona (2010) reported on the identification of the erythrocytes agglutinin in the haemolymph of Giant African Land Snails, *A. achatina* and *A. marginata.*
Studies on some haemolymph biochemical parameters of Giant African Land Snails, (*A. marginata*) have also been reported (Afolayan *et al.,* 1997; Akinloye and Olorode, 2000; Ogunsanmi *et al*., 2003; Ademolu and Idowu, 2005; Abdussamad, 2009 and Sodipe, 2011). The present study aim to investigate the sterility and inhibitory properties of the haemolymph of the two Giant African Land Snails against bacteria colony.

MATERIALS AND METHODS

The study was carried out at the Snail Research Unit and the Animal Physiology Laboratory of the College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta. Abeokuta lies within the rain forest vegetation zones of Western Nigeria on latitude $7^{0}10^{/}$N and $3^{0}2$/E and altitude 76 m above sea level. The climate is an average relative humidity of 82 % (60% in January and 94% from July to September) (Amujoyegbe *et al.,* 2008).
The experiment was a 2 x 2 x 3 factorial arrangement, with the following factors and levels:

Factors:

A. Type of haemolymph: Mantle cavity vs. Foot sinuses haemolymph
B. Species of snails: *A. achatina* and *A. marginata*
C. Liveweight: 150 -200g, 201 – 250g, 251 – 300g

Nutrient agar plates were prepared under aseptic condition. 18 snails from different species were classified by weight into three liveweight groups. The shell of each snail was wiped with sterile cotton wool soaked with ethanol. The snails were kept in the laminar flow chamber. The surface of each of the 48 agar plates was inoculated with the haemolymph sample and incubated for 24 hrs. at 37^{0}C for microbial growth (Duncan,

2005).

For bacterial inhibition activity preliminary trials using the boring and disk diffusion methods showed that the haemolymph did not diffuse into the agar. The dilution method was employed. Different dilutions of haemolymph were inoculated and incubated over night at 37 ^{0}C against gram-negative *E. coli* (TACC25923) and gram-positive *S. aureus* (2TACC25922).

RESULTS AND DISCUSSION

The result bacteria sterility experiment showed there was bacteria growth in the haemolymph of *A. achatina* and *A. marginata.* The different bacteria colony identified were gram positive and gram negative bacilli and cocci bacteria as well as spores (Table 1).It was observed that the haemolymph of *A. achatina* and *A. marginata* is not sterile, irrespective of the species of snails and type of haemolymph.

The result of the experiment on bacterial inhibitory effect of haemolymph of *A. achatina* and *A. marginata* using the dilution method showed that the haemolymph at $^1/_{10^{th}}$, $^3/_{10^{th}}$, $^5/_{10^{th}}$ and $^7/_{10^{th}}$ dilution ratio did not cause inhibition zones on the agar plates. Hence the effect of the haemolymph of *A. achatina* and *A. marginata* on bacteria inhibition was not established.

In the present study, it was observed that the haemolymph of *A. achatina* and *A. marginata* across the liveweight groups were not sterile. This is contrary to the sterility or purity observed in blood of other animals. This could be as a result of the open circulatory system that snails exhibit in which the haemolymph have contact with the shell cavity and foot of the snails.

The antibacterial activity of haemolymph of *A. achatina* and *A. marginata* against gram-negative *E. coli* (TACC25923) and gram – positive *S. aureus* (2TACC25922) could not be established in this study despite the fact that different methods of determining bacteria inhibition (agar diffusion, disk diffusion and dilution method) were employed. The haemolymph original concentration and at different dilutions showed no inhibitory action against gram-negative *E. coli* (TACC25923) and gram-positive *S. aureus* (2TACC25922). The result from this study does not go in line with the result obtained from the study by Abiona (2010) which revealed that substances which bind the four bacteria isolates (*Escherichia coli, Pasteurella* sp., *Salmonella* sp. and *Staphylococcus aureus*) used in the study were present in the haemolymph of Giant African Land Snails (*A. marginata* and *A. achatina*). MIC values recorded for both *Salmonella species* and *Staphylococcus aureus* is a further indication that antimicrobial proteins secreted by the snails still have capacity to control this pathogen but not as strong as in the first two species of bacteria isolate. The result of his study shows that the haemolymph possess antimicrobial properties.

CONCLUSION

From the result of this study, it could be concluded that the haemolymph of *A. achatina* and *A. marginata* is not sterile. The haemolymph of the Giant African Land Snails possesses some microbial organisms which show that the haemolymph is possibly contaminated during circulation in the open circulatory system. The haemolymph in its natural state could not inhibit bacteria growth. Further studies should be carried out on the microbial inhibition of snail haemolymph.

Table 1. The interactive effect of genera of snail, liveweight and type of haemolymph on *A. achatina* and *A. marginata* haemolymph bacteria sterility

Snail specie	Liveweight	Type of Haemolymph	Type of microorganism
A. achatina	150 – 200 g	Mantle cavity	Gram positive cocci
		Foot sinuses	Gram negative bacilli with few gram positive cocci
	201 – 250 g	Mantle cavity	Gram negative bacilli with few gram positive cocci
		Foot sinuses	Gram positive cocci
	251 – 300 g	Mantle cavity	Gram positive cocci
		Foot sinuses	Gram positive bacilli
A. marginata	150 – 200 g	Mantle cavity	Gram positive bacilli
		Foot sinuses	Gram positive bacilli with spores
	201 – 250 g	Mantle cavity	Gram positive bacilli
		Foot sinuses	Gram positive bacilli with spores
	251 – 300 g	Mantle cavity	Gram positive bacilli with spores
		Foot sinuses	Gram positive cocci

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