POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 35(4): 471–479, Y. 2020

EFFECT OF SHELL INJURY ON HAEMOCYTE CONCENTRATION AND SHELL REGROWTH IN GIANT AFRICAN LAND SNAILS (*Archachatina marginata*)

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Key words: shell injury, haemocyte, shell growth, Land snail, *Archachatina marginata.*

Abstract

The effect of shell injury on growth and haemocyte concentration in *Archachatina marginata* was evaluated in this study. Thirty two snails between 130–180 g were randomly divided into four treatments with eight replicate each. The four treatments included: T1 (control), T2 (1 cm shell damage), T3 (2 cm shell damage) and T4 (3 cm shell damage). Haemolymph was collected on weekly basis for four weeks. Parameters monitored were total haemocyte count and shell growth. Results showed that shell injury had significant effects ($P < 0.001$) on total haemocyte count and shell growth. It can be concluded from this study that shell injury has an influence on the immune response of the animal and that is irrespective of the level of shell damage used in this study. This humoral response is put in place to defend the animal body against any opportunistic infection that may gain entrance into the system of this animal.

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Introduction

Land snails generally have a shell, which protects them from physical damage, predators and dehydration (ADEMOLU 2015, ADAMOWICZ and BOLACZEK 2003). Similarly, the shell houses the animal especially during unfavorable conditions. The shells are twisted into spiral levels known as whorls. The whorls are largest at the base and each one gets progressively smaller as it gets to the tip, known as the apex. The snail shell has a large opening called aperture (ADAMOWICZ and BOLACZEK 2003). Due to the current trend of intensive rearing of snails to meet up with demand, there is need for cage culture or semi-intensive rearing of this animal. During intensive rearing, snails at times try to escape from their rearing vicinity and thus fall off from some height and as such break their shell. This occurrence puts snails at a great danger depending on the site of injury. It could also lead to haemolymph loss, which may result in death of this animal if such injury is much. In most occasions, the damage to the shell calls for the process of healing which requires regrowth of the damaged part and this may be energy demanding and costly (JONATHAN 1990). Studies have also shown that the wound healing process requires the activity of macrophages which promote angiogenesis and collagen formation (LEIBOVICH and ROSS 1975, POLVERINI et al. 1977, HUNT et al. 1984, KOVACS and DIPIETRO 1994). For invertebrates like molluscs, shell formation is known to be a complex process, which involves deposition of both organic and inorganic materials (WILBUR 1983). The shell formation process comprises shell mineralization known to occur in succession of compartments (CRENSHAW 1972, SALEUDDIN and PETIT 1983). The first to be reckoned with is the mantle cavity, which secretes the molecules that form the shell, followed by the periostracum (with mostly organic layers) and the extrapallial cavity, into which the outer fold epithelium secretes a calcifying mixture of proteins, glycoproteins and calcium carbonate ($CaCO_3$) (MUTVEI 1987, FENG et al. 2000, MARIN and LUQUET 2004, DALBECK et al. 2006, MARIE et al. 2011, MARIN et al. 2012). The longitudinal section of a shell is made up of a multilayer of calcium carbonate in two or more concentric layers, which are usually covered by an external layer (SALEUDDIN and PETIT 1983). Below the periostracum is an inner nacreous layer, followed by inner primastic (MARIE et al. 2011).

During rearing of snails under intensive system, damages in shell do occur due to climbing of housing facility by this animal and such may lead to economic losses due to mortality. Therefore, is very important to understand the influence of this damage on the immune status of this animal within a specific period of time and to monitor the recovery period depen-

ding on the level of damage. The aim of this study is to evaluate the effect of shell injury on haemocyte concentration and shell regrowth in Giant African Land snails (*Archachatina marginata*).

Materials and Methods

Experimental Site

The research was carried out at the Snail Research Unit of the College of Animal Science and Livestock Production (COLANIM), Federal University of Agriculture, Abeokuta, Ogun State. Abeokuta lies between the rain forest vegetation zone of Western Nigeria on latitude 7º10'N, longitude 3º2'E and altitude 76 m above sea level. The climate is humid with a mean annual rainfall of 1.037 mm, an average temperature of 34.7ºC and an imminent average humidity of 82% throughout the year (*Google Earth* 2017).

Materials and Methods

A total of thirty – two snails (*Archachatina marginata)* between 130–180 g was purchased from a local market. The snails were kept in plastic cages (30 cm by 40 cm by 24 cm). Feeding trough, watering trough, sensitive scale, plier, Eppendorf tube, syringe and needle (5 ml), ruler, vernier caliper and concentrate feed were used during this study. Marker and masking tape were also used for proper identification.

Snails and Their Management

The plastic cages along with the plastic feeders and drinkers were cleaned before the arrival of the snails and the commencement of the experiment. Feed and water were also provided *ad libitum* throughout the period of the experiment. Four weeks was set aside for the acclimatization of the snails before the commencement of the experiment. The experiment lasted for six weeks.

Experimental Esign

Thirty-two snails used for this experiment were randomly assigned into four different treatments with 8 replicates for each treatment. The treatments were: Treatment 1: No shell damage (control); Treatment 2: 1 cm shell damage; Treatment 3: 2 cm shell damage and Treatment 4: 3 cm shell damage.

All snails in both groups were treated equally in terms of feeding and drinking water provision. The composition of feed used is given in Table 1.

Table 1

Composition of experimental diets [g/100 g]

Ingredients	Quantity [g]
Maize	50
Wheat offal	27.5
Groundnut cake	12.25
Soy bean meal	4
Bone meal	3
Oyster shell	3
Salt	0.25
Total	100

Shell Damage/Injury

The snails were cleaned with damp foam in order to remove the dirt on them. The snails were weighed on a sensitive scale before the damage of the shells. The snails in each treatment (1, 2, 3, 4) were brought out of the cages, a ruler was placed on the tip of the shell a and white board marker was used to mark out the part to be damaged as 0 cm, 1 cm, 2 cm and 3 cm. After marking out, a plier was used to cut out the part as marked to be damaged. Shell growth was measured weekly for six weeks using a Venire caliper (Fig. 1).

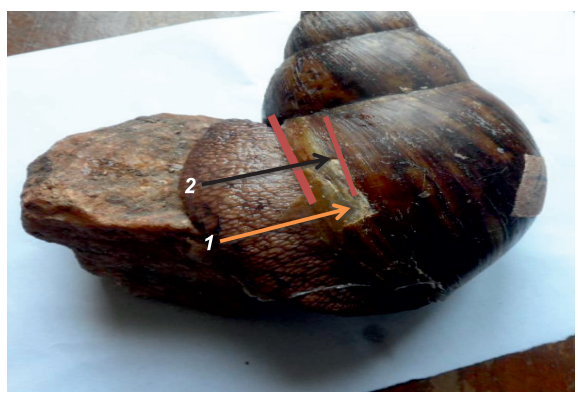

Fig. 1. Portion of shell damage and regrowth there after: *1* – fresh shell growth; *2* – length of damage

Collection of Haemolymph

Haemolymph was collected from the anterior portion of the head region after full extension of the foot muscle with the aid of syringe and needle. Haemolymph was collected from the control group and other treat-

ment levels (1 cm, 2 cm and 3 cm) immediately after shell damage and stored in eppendof tubes for haemocyte count. Haemolymph collection was also carried out on a weekly basis.

Total Haemocyte Count

Haemolymph from eight snails per treatment was selected from the four groups of snails (control, 1, 2, 3 cm). A dilution of 1 : 19 was made with the aid of 5% eosin solution, which was loaded into an improved haemocytometer. Haemocyte found in the four squares were counted. Thereafter, numbers of cells counted were multiplied by a conversion factor (50,000) to obtain the total haemocyte count.

Statistical Analysis

The data generated from this experiment were subjected to a least square analysis of variance using the SYSTAT Statistical package (SYSTAT 1992) in randomized complete block design (RCBD). Significant treatment means were separated using the Duncan multiple range test (GOMEZ and GOMEZ 1984). The model used for this experiment is stated below.

$$Yijk = \mu + Ti + Wj + (TW)ij + \sum ijk$$

where:
Yijk – dependent variable
μ – population mean
Ti – *ith* effect of level of shell damage (i = 1, 2, 3, 4)
Wj – *jth* effect of week of haemolymph collection (j = 1, 2, 3, 4)
TWij – interaction between level of shell damage and week of haemolymph collection
∑ijk – random error

Results and Discussion

The result of analysis of variance showing the effect of shell injury on haemocyte count in Giant African Land snails is summarized in Table 2.

Table 2
Analysis of variance (ANOVA) showing the effect of shell injury on the haemocyte count in Giant African Land snails (*Archachatina marginata*)

Source	Degree of freedom	Mean square
Treatment	3	330945.650***
Week	3	2425.117NS
Error	73	399960543

$P < 0.001$***

Different levels of shell damage had a significant effect on the haemocyte count ($P < 0.001$), while the effect of week on the haemocyte count during the shell damage was not significant ($P > 0.05$).

The significant effect seen in the haemocyte count is a result of anti-inflammatory responses which are very common during injury in many animal models. Allograft inflammatory factor-1 (AIF-1) which is an interferon inducible calcium-binding cytokine has been associated with inflammatory response in molluscs (LI et al. 2013). Studies have also shown that macrophages which facilitate wound healing, angiogenesis and collagen formation are found at the site of injury (LEIBOVICH and ROSS 1975, POLVERINI et al. 1977, HUNT et al. 1984, KOVACS and DIPIETRO 1994). Inflammatory response is vital to body injury, wound repair and immune response (OTTAVIAN et al. 2010). In mollusc, especially in snails, haemocytes are the analogue of various types of immune cells found in vertebrates and as such, they are known to be released whenever there are challenges in the system of this animal. Table 3 shows the least square means of the effect of shell damage on the haemocyte count in Giant African Land snails.

Table 3
Least square means showing the effect of shell injury on the haemocyte count in Giant African Land snails (*Archachatina marginata*)

Parameter	Least square means [10^6/mm^3]	S.E.M (±)
Control (undamaged shell)	345.200^a	44.719
1 cm shell damage	107.000^b	44.719
2 cm shell damage	109.400^b	44.719
3 cm shell damage	114.500^b	44.719

Means within the same column having different superscript differ significantly ($P < 0.001$)

The control group had the highest number of means compared to other levels, which were not significantly different from each other. This observation is an indication that damages of the shell at any magnitude compromises the immune status of this animal which is largely represented by total haemocyte population.

Haemocytes are known to be the chief immunoeffector cells which perform diverse immunological activities such as phagocytosis, encapsulation and cytotoxicity (RAY et al. 2013). If damages to the shell could affect the population of these cells, then it means that any other challenge at this moment of injury may be very dangerous to the survival of the animal. JONATHAN (1990) reported that experimentally shell-damaged snails had higher rate of mortality than did uninjured snails. Also, RAY et al. (2013) reported that exposure of two species of snails (*B. bengalensis* and *L. marginalis*) to cypermethrin and fenvalerate led to haemocyte density shift

and morphological damage. All these reports are testifying to the fact that both physical and chemical damage could compromise the population of haemocytes which are known to be responsible for immune activities in the system of this animal.

Table 4 shows least square means showing the effect of shell injury on the weekly haemocyte count in Giant African Land snails (*A. marginata*). Results show that haemocyte count was not significantly different ($P > 0.05$) across the three weeks of collection.

Table 4

Least square means showing the effect of shell injury on the weekly haemocyte count in Giant African Land snail (*Archachatina marginata*)

Week	Least square means [$\cdot 10^6/mm^3$]	S.E.M (±)
0	149.400	44.719
1	172.400	44.719
2	152.600	44.719
3	149.700	44.719

The implication of this observation is that a quick adjustment within the system of the animal had taken place thus nullifying the effect of damage within the three weeks of the study. Least square means showing the effect of different levels of shell damage on growth after damage is shown in Table 5.

Table 5

Least square means showing the effect of different levels of shell growth after injury

Parameter	Least square – means	S.E.M (±)
Control (no shell damage)	0.175^{c}	0.057
1 cm shell damage	0.241^{bc}	0.057
2 cm shell damage	0.347^{ab}	0.057
3 cm shell damage	0.444^{a}	0.057

Means within the same column having different superscript differ significantly ($P < 0.001$)

It is obvious that snails with 3 cm shell damage had the highest regrowth of 0.444 cm, followed by 1 cm and 2 cm shell damage which are not significantly different from each other (0.241 vs 0.347 cm) while the control had the least growth (0.175 cm). Figure 2 shows the freshly secreted shell after shell damage. The observation made in this study may be as a result of calcium and phosphorous mobilization from the body of the animal to compensate for the losses that occur during shell damage procedure. According to JONATHAN (1990), this process of shell repair is highly energy demanding. It was also reported that experimentally damaged shells grew significantly more new shell than the undamaged ones (JONATHAN 1990).

Fig. 2. Freshly secreted shell after shell damage

This assertion is in line with the observation made in this study. Mollusc shell formation has been reported to be complex and involves deposition of calcium carbonate ($CaCO_3$) which is known to be an inorganic material mixed with organic material (HARE 1963, WILBUR 1983).

Conclusion

This study has shown that shell injury has significant effect on haemocyte concentration. Irrespective of the level of shell damage used in this study, total haemocyte count was reduced compared to the control group. This observation is an evidence of immunosuppression and this call for adequate care during this period of shell injury. If adequate care is not taken during this period of injury, opportunistic infections may kill the animal as haemocytes play a crucial role in the immune defense of this animal. The implication of this study is that snail farmers should maintain a hygienic environment with adequate care during any eventuality of shell damage under intensive method of production.

Accepted for print 4.05.2020

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