sciendo

DOI: 10.2478/fv-2025-0011

FOLIA VETERINARIA, 69, 2: 1—5, 2025

ORIGINAL ARTICLE

ANTIOXIDANTS ACTIVITIES AND LIPID PROFILE IN THE HAEMOLYMPH OF BLACK AND WHITE-SKINNED GIANT AFRICAN LAND SNAIL (*Archachatina marginata*)

Kehinde Olutoyin Ademolu[1*], Funmilayo Abimbola Okeniyi[2], Beno Okechukwu Onunkwor[3], Foluke Adenike Akinnusi[4], Jolaade Agbetiloye[1], Adewunmi Babatunde Idowu[1]

[1]Department of Pure and Applied Zoology, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria; [2]Department of Animal Science, Landmark University, Omu-Aran, Kwara State, Nigeria; [3]Department of Biochemistry, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria; [4]Centre for Entrepreneurship Studies, Chrisland University, Abeokuta, Nigeria

OPEN ACCESS

***Correspondence:** kennyademolu@yahoo.com

Citation: Ademolu, K. O., Okeniyi, F. A., Onunkwor, B. O., Akinnusi, F. A., Agbetiloye, J., Idowu A. B., 2025: Antioxidants activities and lipid profile in the haemolymph of black and white-skinned giant African land snail (*Archachatina marginata*). Folia Veterinaria, 69, 2, 1–5.

Received: November 28, 2024

Accepted: March 6, 2025

Published: June 16, 2025

Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

Antioxidants are compounds that eliminate oxidative stress in biological systems. The activities of antioxidants in black and white-skinned snails have not been compared in literature. This study was carried out to compare the antioxidants (glutathione peroxidase – GPx, superoxide dismutase – SOD, malondialdehyde – MDA, catalase) activities in the haemolymph of the white and black-skinned snails (*Archachatina marginata*) as well as their lipids profiles. Twenty (20) giant African land snails (10 black-skinned and 10 white-skinned snails) were used in the experiment. The lipid profile of white-skinned snails was higher than that of black-skinned snails. White-skinned snails have higher SOD activities than black-skinned snails. However, MDA, catalase and GPx activities in black-skinned snails were higher than that of white-skinned snails. The black-skinned snails were more endowed to cope with environmental disturbances than their white-skinned counterparts.

Keywords: antioxidants; *Archachatina marginata*; black-skinned snails; white-skinned snails

INTRODUCTION

Snail is a terrestrial, shell-bearing animal, belonging to the phylum *Mollusca* and class *Gastropoda*. It is normally more active after dusk and when the ground is damp [1]. The haemolymph is regarded as a blood analogue found in all arthropods and most molluscs [2]. It is blue, indicating the presence of haemocyanin, a blue copper-containing pigment in the solution. It is composed of water, inorganic salts (mostly Na, Cl, K, Mg and Ca) and organic compounds (mostly carbohydrates, proteins, and lipids) [3].

Various parts (haemolymph, flesh, shell, slime) of the giant African land snail have been used in traditional medicine in Nigeria to treat tuberculosis, measles, high blood pressure, headache and cough [1]. The increased use of snail haemolymph in native medicine is likely due to its antioxidant content. Lawal et al. [4] reported the presence of antioxidant activities in the haemolymph of land snails *Achatina achatina* and *Archachatina marginata*, referring to them as natural sources of antioxidants.

Many factors influence the activity of antioxidants in land snails. Hermes and Storey [5] observed that aestivation, anoxia, and dry and hot environments increased the activities of antioxidants while Lawal et al. [4] opined that antioxidant activities in snail haemolymph have ability to protect albino rats from potential oxidative stress from liver damage by carbon tetrachloride (CCL_4) poisoning.

One ecotype of snails that has been extensively used in traditional medicine is the albino snails popularly called the white-skinned snail. This snail, although edible, is not eaten by some people because of taboos and superstitions surrounding it [6] It is believed to be dedicated as a sacrifice to gods. Ketiku and Adeleke [7] compared the nutrient composition of the flesh of the normal snails with that of the albino (white-skinned). They observed a significantly higher ($P < 0.05$) protein and minerals in the albino snail than in the normal skinned. Albinism is a hereditary deficiency of pigmentation that may involve the entire body or parts of the body. It is believed to be caused by an enzyme deficiency involving melanin metabolism during prenatal development [8]. There are two main types of albinism in snails: albino-shelled and albino-bodied but the genes responsible for them are unknown.

Literature abounds with many studies on haemolymph antioxidant activities based on species variation, body weight, age, habitat, and feeding [9, 10] while little attention is paid to the white-skinned (albino) snail. This study aims to evaluate lipids and the antioxidant composition of the haemolymph of black-skinned and white-skinned giant African land snails, *Archachatina marginata*.

MATERIALS AND METHODS

Snail Collection

Twenty giant African land snails (10 black-skinned and 10 white-skinned snails) with the average weight of 150 ± 02g were bought from a private snail farm in Abeokuta, Ogun State, Nigeria.

Haemolymph Collection

The snails were thoroughly cleaned to remove dirt from their shells and apertures. The apex of the snails was carefully cracked open, adopting the method from Akinloye and Olorode [11]. The haemolymph from the black-skinned and white-skinned snails was drained into separate and clean sample bottles.

Determination of Antioxidant Levels

Fresh haemolymph samples collected into plain sample bottles were immediately centrifuged. The supernatant (serum) was then frozen for the antioxidant analyses. The supernatants were analyzed for lipid profile (cholesterol, triglycerides, and lipoproteins) and antioxidant activities (catalase, glutathione peroxidase, superoxide dismutase, lipid peroxidation) using Brigelius-Flohé and Maiorino [12] methods. Superoxide dismutase (SOD) activity was determined spectrophotometrically at 420 nm by measuring the inhibition of autoxidation of pyrogallol, a superoxide-reacting indicator molecule (SRIM) that competes with SOD for the reaction with superoxide in alkaline medium (pyrogallol / SOD + $O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$). The specific activity of SOD was expressed as units/g haemolymph or pyrogallol 50 % oxidation auto-inhibition/min/g haemoglobin using a pyrogallol molar extinction coefficient (Σ) of $8.0 \times 10^5\ M^{-1}\ cm^{-1}$.

Catalase activity was determined spectrophotometrically at 374 nm by measuring the rate of decomposition of hydrogen peroxide ($2H_2O_2 \rightarrow 2H_2O + O_2$). The specific activity of catalase was expressed as units/g haemolymph or mmol H_2O_2 degraded/min/g haemoglobin using the H_2O_2 molar extinction coefficient (Σ) of $43.6\ M^{-1}\ cm^{-1}$.

Glutathione peroxidase (GSPx) activity was determined spectrophotometrically at 420 nm by measuring the residual GSH content during the decomposition of hydrogen peroxide using GSH as co-factor ($H_2O_2 + 2GSH \rightarrow 2H_2O + GSSG$). GSPx specific activity was expressed as units/g haemolymph or nmol of residual GSH/min/g haemoglobin using a GSH molar extinction coefficient (Σ) of 9.6 0.017 $mM^{-1}\ cm^{-1}$.

Statistical Analysis

Data collected from the experiments were analyzed using the Student T-test. Significant difference was set to $P < 0.05$.

RESULTS

The lipid profile of the haemolymph of black- and white-skinned snails is presented in Table 1. The haemolymph of the white-skinned snails had significantly ($P < 0.05$) higher total cholesterol, triglycerides, and High-Density Lipoprotein than the black-skinned snails. However, the haemolymph of black-skinned snails recorded significantly higher Very Low-Density Lipoprotein than their white-skinned counterparts.

Table 1: Lipid Profile of Black and White-Skinned Snails Haemolymph (Mean± SD)

Lipids	Black-skinned snail	White-skinned snail
Total cholesterol (mg/dl)	15.10 ± 0.05^{b}	17.30 ± 0.08^{a}
Triglycerides (mg/dl)	18.30 ± 0.10^{b}	20.20 ± 0.04^{a}
VLDL (mg/dl)	10.33 ± 5.77^{a}	4.04 ± 0.04^{b}
HDL (mg/dl)	7.55 ± 0.07^{a}	8.65 ± 0.06^{a}

Values in rows with different superscripts are statistically different ($p < 0.05$)

The black-skinned snails had significantly higher antioxidant activities in the haemolymph than the white-skinned snails (except for the SOD), however, there was no significant difference in the activities of GPx (Table 2).

Table 2. Antioxidant Activities in the Haemolymph of Black and White-Skinned Snails (Mean± SD)

Antioxidants	Black-skinned snail	White-skinned snail
SOD (U/L)	1.52 ± 0.03^{b}	2.01 ± 0.01^{a}
MDA (U/L)	2.34 ± 0.05^{a}	1.59 ± 0.04^{b}
Catalase (U/L)	2.11 ± 0.06^{a}	1.95 ± 0.03^{b}
GPx (U/L)	1.24 ± 0.02^{a}	1.03 ± 0.02^{a}

Values in rows with different superscripts are statistically different ($p < 0.05$)

DISCUSSION

White-skinned snails had been reported to exhibit slower growth and less activity than the black-skinned ones [3]. This reduced activity recorded by the white-skinned snails might be responsible for accumulating lipid derivatives in their haemolymph. Lipids are energy sources that supply more energy than carbohydrates to both snail embryos and adults [14, 15], but when not utilized, they accumulate in the tissues. Ketiku and Adeleke [7] similarly reported higher protein and mineral concentrations in the flesh of white-skinned snails. The absence of melanin pigment in white-skinned snails makes them sensitive to light and heat, thus withdrawing most times, especially during the day [13].

Black-skinned snails are usually active and carry out mating and feeding activities during the last hours of the day (18:00- 24:00 GMT) [2]. They also perform some of these tasks immediately after the rain during the day when the temperature is low and relative humidity is high [1]. These activities expose black-skinned snails to more reactive oxidative stress (ROS) than white-skinned snails that are usually restricted in their habitat [16].

Higher activity rates displayed by black-skinned snails either in the wild or in captivity expose them to more oxidative stress than the white-skinned snails, hence requiring more antioxidant defenses. The land snail, *Otala lacteal,* exhibits an increase in activities of foot muscle superoxide dismutase, catalase, and glutathione S-transferase [17] in preparation for dealing with oxidative stress during the arousal process from aestivation. Other factors like habitat, age, and feed have significant roles on the antioxidant activities of snails. For instance, pollutants (CCL_4 and AG-NP) reduced the antioxidant activities in *Archachatina marginata* and *Biomphalaria alexandrina,* respectively [4, 7]. Water snail, *Cornu aspersum aspersum* fed with different protein sources showed significant changes in their antioxidant activities and chemical composition [9].

Furthermore, due to the higher metabolic activity of black-skinned snails, they consume more oxygen than their white-skinned counterparts, which can cause ROS. The increased rate of oxygen consumption in the desert snail, *Oreohelix* spp., led to a higher rate of ROS generation which also elicits increased antioxidant defense mechanisms to protect the tissues [17].

CONCLUSION

In conclusion, black-skinned snails had more antioxidant activities and less lipid concentrations in the haemolymph than the white-skinned snails in order to pro-

tect their tissues from damage caused by ROS warranted by their higher activities.

Data Availability Statement

The raw data of this article will be made available by the authors, without undue reservation.

Ethical Statement

The study did not require any ethical approval.

Conflict of Interest

No conflict of interest.

Funding

This study was financially supported by the authors

Generative AI Statement

The authors declare that no Gen AI was used in the creation of this manuscript.

Authors' Contributions

Conceptualization: Ademolu, K.O. and Onunkwor, B.O.

Manuscript writing: Ademolu, K.O. and Okeniyi, F.A.

Sampling and Methodology: Agbetiloye, J., Ademolu, K.O., Akinnusi, F.A,

Statistical Analysis: Idowu, A.B. and Onunkwor, B.O.

REFERENCES

1. **Akinnusi, O., 1997:** A practical approach to backyard snail farming. *Nig. J. Ani. Prod.*, 25, 2, 193–197.
2. **Abdussamad, M. A., Osinowo, O. A., Smith, O. F., Onadeko, S. A., 2010:** Some haemolymph biophysical parameters in the giant African land snail *Archachatina marginata* during a six-week aestivation period. *Glob. Vet.*, 4, 4, 400–408.
3. **Ademolu, K. O., Jayeola, O. A., Idowu, A. B., Elemide, I. O., 2011a:** Circadian variation in locomotor and feeding periods of two land snail species. *Arch. Zootec.,* 60, 232, 1323–1326.
4. **Lawal, B. K., Shittu, O. C., Ossai, P. N., Abubakar, A. M., Ibrahim, A., 2015:** Evaluation of antioxidant activity of Giant African Snail (*Archachatina marginata*) haemolymph in CCl4-induced hepatotoxixity in albino rats. *J. Pharm. Res. Int.*, 6, 3, 141–154. DOI: 10.9734/BJPR/2015/15887
5. **Hermes-Lima, M., Storey, K. B., 1995:** Antioxidant defenses and metabolic depression in a pulmonate land snail. *Am. J. Physiol*., 268, 6, 2, R1386–R1393. DOI:10.1152/ajpregu.1995.268.6.R1386
6. **Amusan, J. A., Omidiji, M. O., 1998:** Edible land snails. A technical guide to snail farming in the Tropics, Verity Printers, Ibadan, 1, 16.
7. **Ketiku, A. O., Adeleke, A. O., 1998:** Nutrient composition of black fleshed and white fleshed African giant land snails (*Archachatina marginata*). *W. Afri J. Biol. Appl. Chem.*, 44, 21–23.
8. **Allegretti, S. M., Catvalho, J. F., Magalhess, L. A., Zanotti-malgahaes, E. M., 2009:** Behavior of albino and melanic variant of *Biomphalaria glabrata* (Mollusca: Planorbidae) following infection by *Schistoma mansoni*. *Braz. J. Biol.,* 69, 8, 11.
9. **Gogas, A., Laliotis, G. P., Ladoukakis, O., Trachana, V., 2021:** Chemical composition and antioxidant profile of snails (*Cornu aspersum aspersum*) fed diets with different protein sources under intensive rearing conditions. *J. Anim. Feed Sci*., 30, 4, 391–398. DOI: 10.22358/jafs/143107/2021
10. **Khaled, M. Z., Yun-Hai, G., Shan, L. V., Yi, Z., Xiao-Nong, Z., 2022:** Molluscicidal and antioxidant activities of silver nanoparticles on the multi-species of snail intermediate hosts of schistosomiasis. *PLoS Negl. Trop. Dis.*, 16, 10, e0010667. DOI: 10.1371/journal.pntd.0010667
11. **Akinloye, O. A., Olorode, O., 2000:** Effect of different feeding condition on performance, haemolymph biochemical and mineral value of Giant African Snail (*Archachatina marginata*). *J. Agri. Envt.*, 1, 1, 143–147.
12. **Brigelius-Flohé, R., Maiorino, M., 2013:** Glutathione peroxidases. *Biochim. Biophys. Acta*, 1830, 5, 3289–3303. DOI: 10.1016/j.bbagen.2012.11.020
13. **Ademolu, K. O., Jayeola, O. A., Dedeke, G. A., Idowu, A. B., 2011b:** Comparative analysis of the growth performance and haemolymph biochemical properties of normal and albino giant land snail (*Archachatina marginata*). *Ethi. J. Environ. Stu. Mgt.,* 4, 2, 101–106.
14. **Odiete, W. O., 1999:** Basic Animal Physiology. Diversified Resources Ltd. Lagos. 257 pp.

15. **Egbebi, J. M., Ademolu, K. O., Idowu, A. B., Aladesida, A. A., Ojo, V., 2023:** Biochemical composition of eggshell and egg yolk of giant African land snail (*Archachatina marginata*) during gestation period. *Arch. Zoote.*, 72, 280, 284–287.

16. **Okon, B., Ibom, L. A., 2012:** Snail breeding and snailery management. Fresh Dew Productions, Calabar, Nigeria, 1–70.

17. **Rees, B. B., Hand, S. C., 1990:** Heat dissipation, gas exchange and acid-base status in the land snail *Oreohelix* during short-term estivation. *J. Exp. Biol.,* 152, 1, 77–92. DOI: 10.1242/jeb.152.1.77