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Assessment of the antioxidant capacity of *Lanistes carinatus* tissue extract and its immune-boosting influence on *Biomphalaria alexandrina* against infection with *Schistosoma mansoni*

Rasha M. Gad El-Karim[1], Mohamed R. Habib[1], Mosad A. Ghareeb[2], Rasha E.M. Ali [1*]

[1] Medical Malacology Department, [2] Medicinal Chemistry Department, Theodor Bilharz Research Institute, Corniche El Nile St., Warrak El-Haddar, Imbaba, Giza, 12411, Egypt.

[*]Corresponding Author: roshaezzat@yahoo.com

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ABSTRACT

Schistosomiasis remains a public health problem in many developing countries including Egypt. The disease control initiatives stress on control of snail intermediate hosts side by side with treatment of infected human cases. Blocking the parasite transformation within the snails will interrupt schistosomiasis transmission. Thus, the present study assessed the antioxidant efficacy of *Lanistes carinatus* tissue extracts and its role as immunostimulant for *Biomphalaria alexandrina* against infection with *Schistosoma mansoni*. *L. carinatus* soft tissues were extracted with methanol and the resulting crude extract was further fractioned with petroleum ether, methylene chloride, and ethyl acetate (EtOAc). The total antioxidant capacity (TAC) and total phenolic content (TPC) of all extracts were determined. Furthermore, the effect of the highest antioxidant extract on hemocytes activity (phagocytosis), antioxidant parameters, and infection rate of *B. alexandrina* was assessed. The results showed that, EtOAc extract had the highest antioxidant capacity and total phenolic contents. EtOAc extract led to activation of phagocytic process with a significant increase in the phagocytic indexes of hemocytes after 1, 2, and 4 h of exposure. In addition, it increased the levels of total antioxidant capacity, superoxide dismutase, reduced glutathione, and glutathione-s-transferase in *B. alexandrina* compared to controls. The infection rate of snails was significantly reduced due to EtOAc extract exposure. These results collectively suggest that EtOAc extract from *L. carinatus* has an antioxidant capacity and immuostimulatory effect for *B. alexandrina* infectied with *S. mansoni*.

INTRODUCTION

Schistosomiasis is a neglected tropical disease that represents a public health challenge in many tropical and subtropical countries **(WHO, 2020)**. The disease is caused by trematode parasites belonging to the genus *Schistosoma* of which there are several species that infect human **(Colley *et al*. 2014)**. All these schistosome species depend on

specific snails that support their transmission into human **(Bayne 2009)**. In the case of *Schistosoma mansoni*, the snails that act as intermediate hosts are of the genus *Biomphalaria* **(Morgan *et al.* 2001)**. *Biomphalaria alexandrina* is the species responsible for *S. mansoni* transmission in Egypt **(Mohamed *et al.* 2012; Abou-El-Naga 2013).** An integrated control measure for schistosomiasis includes an extensive mass drug administration (MDA) to infected population and the use of molluscicides to kill the snail intermediate hosts **(Sokolow *et al.* 2016; Jia *et al.* 2019)**. Alternative methods to control the disease can be mediated through blocking the intramolluscan transformation of the parasite. In this respect, some medicinal plant extracts or compounds isolated from plants with antioxidant activities have been used to support the immune system of *Biomphalaria* in its fight against schistosomes' larvae **(Mossalem *et al.* 2018; Mansour *et al.* 2021).**

Overproduction and accumulation of reactive species within the human body lead to a phenomenon recognized as oxidative stress that initiates several health disorders like cancer, cardiovascular, and inflammation. The destructive effects of such phenomenon can be diminished via utilizing naturally occurring antioxidant agents as free radical scavengers **(Ghareeb *et al.* 2018; Sobeh *et al.* 2018).** Additionally, naturally occurring antioxidants play an important function in the balance of endogenous antioxidants production of free radicals such as hydroxyl radical, anion radical super-oxide, and hydrogen peroxide are highly reactive forms of reactive oxygen species (ROS) **(Gayathri *et al.* 2017a)**. From a pharmaceutical point of view, marine and freshwater mollusks are considered possible candidates for various bioactive compounds with antibacterial, anti-inflammatory, antineoplastic, and antioxidant properties. Extracts from molluscs possess many molecules such as peptides, sterols, sesquiterpenes, terpenes, polypropionates, nitrogen compounds, macrolides, prostaglandins, and alkaloids, which are known to have pharmacological properties **(Lawal *et al.* 2015)**.

A variety of marine were shown to have potent antioxidant activity in preceding studies, extracts from *Patella rustica* and *Littorina littorea*, for example, have been able to scavenge the radical diphenylpicrylhydrazyl (DPPH) **(Khalil *et al.* 2019)**. The antioxidant activity of the giant African snail's (*Achachatina maginata*) hemolymph was verified, and for that it can be used as an accessible source of natural antioxidants with potential application to reduce oxidative stress induced liver damage. In previous studies, methanol extract from freshwater molluscs such as *Pila virens* snail had antioxidant property. Also, crude methanol and chloroform extracts from the freshwater apple snail, *Pomacea maculata* was reported to have a high antioxidant activity **(Nagash *et al.* 2010; Khalil *et al.* 2020)**.

Molluscs have an efficient mechanism of internal defense consisting of cellular and humoral defense factors **(Boehmler *et al.* 1996; Le Clec'h *et al.* 2016)**. The main mediator of cellular defense reactions in molluscs is circulating hemocytes. Several roles

have been assigned to gastropod hemocytes, including an essential role in protective reactions such as phagocytosis **(Adema *et al*. 1992)**.

Detoxification and getting rid of microorganisms engulfed by hemocytes or larval stages of a pathogen is accomplished by a sudden discharge of ROS within the hemocytes, which is referred to as an oxidative burst **(Hahn *et al*. 2001)**. Since antioxidants are essential to oppose the activities of *in vivo* generated ROS and reactive nitrogen species (RNS) that may cause serious damages to DNA, lipids, proteins, and other biomolecules, as aresult of the insufficiency of endogenous antioxidant defenses (superoxide dismutase "SOD", hydrogen peroxide-removing enzymes, and metal binding proteins) to fully prevent damage, exogenous antioxidants especially of natural origins are used to balance the system **(Kurutas 2015)**.

Based on these studies, it is expected that freshwater molluscs' extracts can be a novel candidate for compounds with antioxidant properties instead of being labeled as pests. Hence, this study aims to evaluate the antioxidant activity and total phenolic content of freshwater *snail, L. carinatus* methanol extract and its fractions and their influence on *B. alexandrina* snails' immune system particularly regarding the dynamics of infection with *S. mansoni*.

MATERIALS AND METHODS

Sample collection, extraction and fractionation

Live specimens of *Lanistes carinatus* were randomly collected by hand picking from the west bank of the Nile of Sohag Governorate (26°33′N 31°42′E). The collected fresh molluscs were transported to Medical Malacology Department and identified according to the standard published keys **(Brown 1994; Lotfy and Lotfy 2015**). The soft bodies of snails were removed by breaking the shell, the flesh samples were then snap-frozen using liquid nitrogen to stop the snail metabolic activity and kept at –80ºC until extraction.

Frozen snail tissues were mixed on a vortex mixer until the tissue is well homogenized within the extraction mixture, then extracted with methanol at room temperature with stirring daily followed by filtration and re-extraction for 4 times. Filtration of the extract was performed using Whatman filter paper No. 1 and concentrated using rotatory evaporator (Buchi, Switzerland) at 40±2°C. The crude extract was then collected and stored at room temperature in a dark place for further investigations. The methanolic crude extract (MeOH) was washed several times with petroleum ether (60–80°C) to get rid of the fat content, then sequentially fractionated with different organic solvents in the following sequence: petroleum ether, methylene chloride (CH_2Cl_2), and ethyl acetate (EtOAc).

Determination of total antioxidant capacity (TAC)

This assay was carried out depending on phosphomolybdenum method using ascorbic acid as standard and based on the reduction of molybdenum "Mo" (VI) to Mo (V) by the sample contents and subsequent formation of a green colored [phosphate=Mo (V)] complex at acidic medium with a highest absorption at 695 nm. In this method, 0.5 ml of each sample (500 µg/ml) in methanol was mixed in dried vials with 5 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The reaction mixtures were plugged and incubated in a thermal block at 95℃ for 90 min. followed with measuring the absorbance at 695 nm against a blank. All experiments were carried out in triplicate. The antioxidant activity of the sample was expressed as the number of ascorbic acid equivalent (AAE) **(Prieto *et al*. 1999; Ghareeb *et al*. 2016)**.

Determination of total phenolic content (TPC)

The total phenolic content of the crude extract and its fractions was estimated using Folin - Ciocalteu's reagent according to **Prior *et al*. (2005)** using gallic acid as a standard. This process is based on a molybdotungstate reagent oxidation/reduction reaction. The reaction of electron transfer contributes to blue color formation, which can be quantified at 765 nm by spectrophotometry. The reaction mixture consisted of 100 µl of extract (500 µg/ml), 500 µl of the Folin-Ciocalteu's reagent and 1.5 ml of sodium carbonate (20%). After shaking, the mixture was allowed to stand for 2 h. The absorbance was measured at 765 nm using spectrophotometer (UV-VS spectrophotometer, Milton Roy 601, Co, USA). The total phenolic content was expressed as mg gallic acid equivalent (GAE) per g extract.

In vitro phagocytosis assay

B. alexandrina snails were exposed to 500 µg/ml of ethyl acetate fraction of the extract for different time intervals (1, 2, 4, 6, and 24 h, respectively). Freshly collected hemolymph from 3-5 snails was pooled from both control and treated snails and challenged with common baker's yeast cells (*Saccharomyces cerevisiae*) as target for phagocytosis. Approximately, 0.1 g of freshly obtained yeast was dissolved in 10 ml PBS (pH 7.4) at 40°C in water bath for 5 min in glass test tube. The tube was transferred directly into ice for 5 min to stop the reaction. Centrifugation at 2000 g for 3 min and 3 times wash with cold PBS. The washed yeast cells were resuspended in 10 ml PBS. The last suspension was diluted in PBS to reach the ratio of 10000 cell/ml for use.

The monolayer technique described by **Abdul-Salam and Michelson (1980)** was used to estimate the percentage of phagocytic index of *B. alexandrina* hemocytes. Approximately, 100 µl of pooled hemolymph was superimposed with an equal volume of yeast suspension on a clean glass slide. Three slides were prepared for each experimental group. Slides with experimental mixture were incubated in a humid chamber for 60 min.

The phagocytic reaction was stopped using absolute methanol after washing with PBS (pH 7.4). The fixed monolayers were stained with Giemsa stain for 15 min, rinsed with tap water, air dried and mounted in DPX. The monolayers were microscopically examined using microscope with digital camera (Carl-Ziess, Germany). The hemocyte was considered positive if it engulfed 1 or more yeast cells. The phagocytic index was calculated as a proportion of positive hemocytes from the original 100 cells counted.

Determination of antioxidant enzymes

B. alexandrina snails were treated with 500 µg/ml of ethyl acetate fraction of the extract for different time intervals (1, 2, 4, 6, and 24 h, respectively). The soft parts of snails were dissected out from their shells after gentle crushing, and then the tissues were weighed and homogenized in phosphate buffer at a ratio of 1:10 W/V using a glass homogenizer for 5 minutes. The homogenates were centrifuged for 15 minutes at 1,700 g for 15 min at 4°C and the fresh supernatant was then used to estimate the total antioxidant capacity by the reaction of antioxidants in the sample with a defined amount of exogenously provide hydrogen peroxide (H_2O_2), the antioxidants in the sample eliminate a certain amount of the provided hydrogen peroxide. The residual H_2O_2 is determined colorimetrically by an enzymatic reaction which involves the conversion of 3,5,dichloro-2-hydroxy benzensulphonate to a colored product using a colorimetric kit (Biodiagnostic Company, Dokki, Giza, Egypt; Cat. No. GR 2513) based on the method of **Koracevic *et al*. (2001)**.

SOD activity was performed by using an assay kit (Biodiagnostic Company, Dokki, Giza, Egypt; Cat. No. SD 25 21) which relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye according to Nishikimi *et al*. (1972). Also, the concentration of glutathione reduced (GSH) was assessed using a colorimetric GSH kit (Biodiagnostic Company, Dokki, Giza, Egypt; Cat. No. GR 2511) based on **Beutler *et al*. (1963).** Glutathione-s-transferase (GST) total activity was measured following the method of **Habig *et al*. (1974)** using an assay kit (Biodiagnostic Company, Dokki, Giza, Egypt; Cat. No. CA 2519).

Survival rate at first shedding and infection rate of B. alexandrina snails with S. mansoni miracidia

Adult snails (6-8 mm in shell diameter) were pretreated with 500 µg/ml of ethyl acetate fraction of the extract for 1.0 hr then exposed individually to 8-10 *S. mansoni* miracidia per snail in multidish plates filled with 2 ml dechlorinated tap water for 24 h as described by **Anderson *et al*. (1982)**. After 21 days post-miracidial exposure, surviving snails were individually examined for cercarial shedding in multidish plates after exposure to light (desk lamp) using 2 ml dechlorinated water for each snail/well, positive snails were removed, marked and transferred to clean aquaria with dechlorinated water and maintained in the dark under laboratory conditions. This examination was carried out once weekly to avoid exhausting of snails.

Statistical analysis

Calculations were performed using the Statistical Package of Social Science (SPSS) software (version 20.1, Chicago, IL, USA). Data are presented as mean values ± standard deviation or standard error of the mean. The limit for statistical significance was set at $p<0.05$ (i.e. a confidence level of 95%).

RESULTS

Total antioxidant capacity (TAC) and total phenolic content (TPC) values

As shown in Table (1), TAC of *L. carinatus* tissue extract and its fractions was measured via phosphomolybdenum method using ascorbic acid as standard. TAC of crude extract and its fractions came in the following order: EtOAc > CH_2Cl_2 > MeOH > residue > petroleum ether, recording 804±3.92, 503.35±1.15, 471.54±2.85, 309.91±3.33, and 218.83±5.09 mg AAE/g extract, respectively.

TPC of *L. carinatus* tissue extract and its fractions was consistent with TAC results, where EtOAc fraction had the highest phenolic content being 276.0±3.23 mg GAE/g extract, while the petroleum ether fraction showed the lowest phenolic content (72.83±4.02 mg GAE/g extract**).**

Table 1. Total antioxidant capacity (TAC) and total phenolic content (TPC) values of the methanolic extract of snail's tissues as well as its derived fractions

Sample	Total antioxidant capacity (mg AAE/g extract)[1,2]	Total phenolic content (mg GAE/g extract)[3]
MeOH	471.54±2.85	95.49±3.11
Petroleum ether	218.83±5.09	72.83±4.02
CH_2Cl_2	503.35±1.15	135.21±2.95
EtOAc	804±3.92	276.0±3.23
Residue	309.91±3.33	112.56±1.85

[1]Results are (means±S.D.) (n = 3). [2]AAE (ascorbic acid equivalent). [3]GAE (gallic acid equivalent)

In vitro phagocytosis assay

Exposure of *B. alexandrina* snails to EtOAc fraction of *L. carinatus* tissue extract led to activation of phagocytic process with a significant increase in the phagocytic indices of hemocytes after 1, 2, and 4 h recording 64.3±0.69%, 61±0.88%, and 58.3±1.17%, respectively compared with 47±1.2% of control snails, as illustrated in Figure (1).

In addition to the significant increase in phagocytic indices as a result of treatment with EtOAc fraction of *L. carinatus* tissue extract, a qualitative improvement was also noticed as shown in Figure (2). Under normal conditions hemocytes adhere commonly to the outer membrane of one yeast cell (Fig. 2I, a & b) and tend to form pseudopodia (Fig. 2I, c). After treatment with the extract, it was noticed that the extracellular trapping and endocytosis of multiple yeast cells either through pseudopodia or surface adherence increased obviously (Fig. 2II, d-f, III, j-i, IV, j-m).

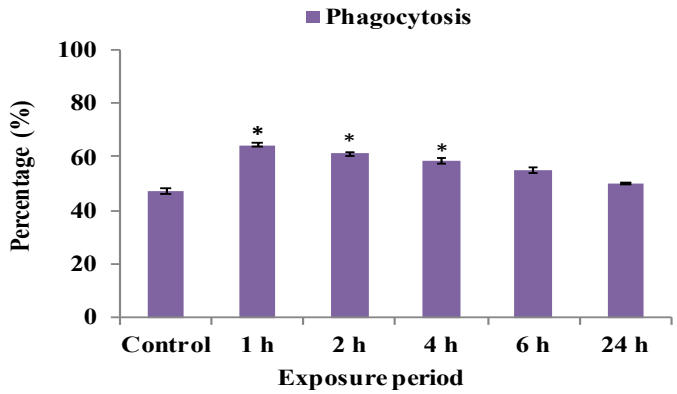

Fig. 1. Phagocytic indices of *Biomphalaria alexandrina* snails' hemocytes challenged with *Saccharomyces cerevisiae* after exposure to ethyl acetate fraction of *Lanistes carinatus* tissue extract. * significance compared to control at P<0.05

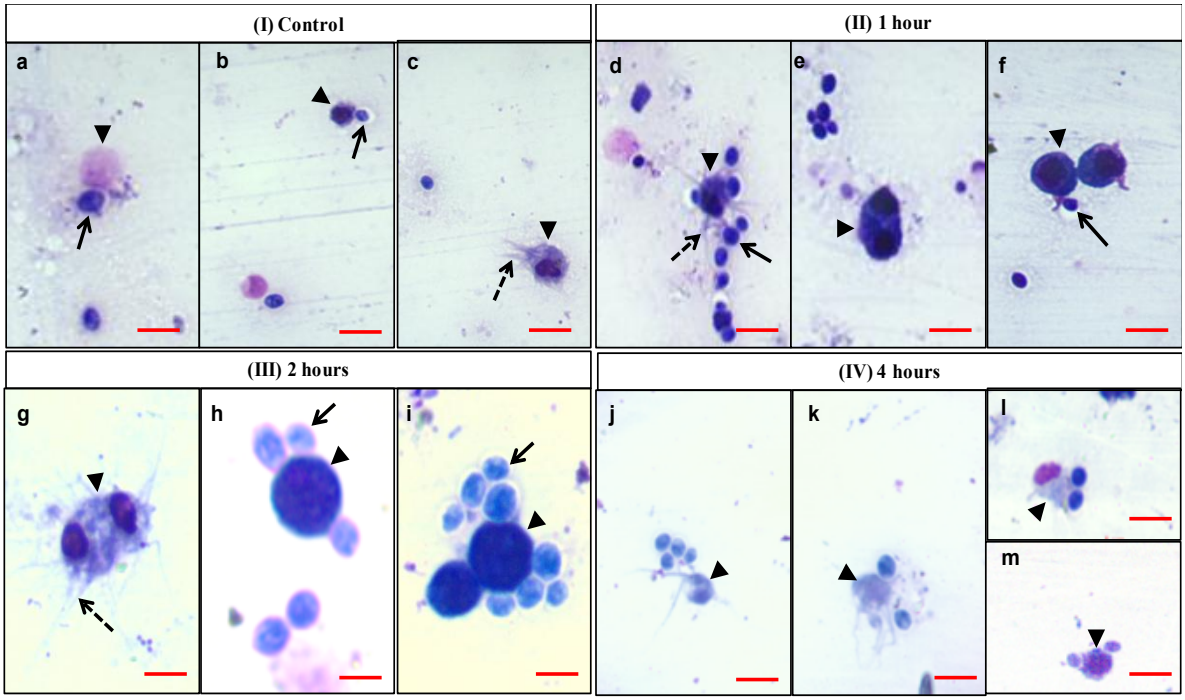

Fig. 2. Examples of *Biomphalaria alexandrina* circulating hemocytes undergoing *in vitro* phagocytosis of *Saccharomyces cerevisiae* cells after exposure to ethyl acetate fraction of *Lanistes carinatus* tissue extract. (I) control samples showing in (A & B) hemocytes (arrowheads) adhering to the yeast cells (arrows). Notice in (C) the formation of pseudopodia (dashed arrow). (II) after 1 hour of exposure showing in (D & F) an extracellular trapping of yeast cells (arrows) by pseudopodia (dashed arrow). In (E) there is an activated hemocyte undergoing mitosis (arrowhead). (III) after 2 hours of exposure showing a hemocyte (arrowhead) undergoing mitosis and the formation of pseudopodia (dashed arrow). In (H & I) multiple yeast cells (arrow) are adhering to the outer membrane of hemocytes (arrowhead). (IV) after 4 hours of exposure showing the adherence of hemocytes (arrowheads) and an undergoing endocytosis of yeast cells (J-M). Scale bar = 5 µm.

Oxidative stress and antioxidant parameters

Data presented in Figure (3) revealed that exposure of *B. alexandrina* snails to EtOAc fraction of *L. carinatus* tissue extract led to a significant elevation in the total antioxidant capacity after 1.0 hour and 2 h recording 0.71±0.04 and 0.83±0.05 mM/L, respectively compared to 0.49±0.03 mM/L of the control group. The level of total antioxidant capacity nearly restored to normal values after 24 hours post exposure (Fig. 3a).

SOD exhibited a pattern of increased activity during the 24 hours period following exposure to the extract. The highest significant ($p<0.001$) activity was noticed 2 hours post exposure being 202.69±1.12 U/g tissue versus 92.09±0.77 U/g tissue for the control snails (Fig. 3b). Concerning the concentration of GSH after exposure to the tissue extract, results in Figure (3c) showed an obvious increment during 24 hours interval. After 4 hours from exposure GSH level reached its peak recording 78.07±0.63 mg/g tissue while the control group has a concentration of 45.57±0.90 mg/g tissue. Then GSH concentration exhibited a temporary stability between 6 and 24 hours at about 1.5 fold value of the control concentration (Fig. 3c).

Exposure of *B. alexandrina* snails to EtOAc fraction of *L. carinatus* tissue extract affected the total activity of GST by raising it significantly after 1 and 2 h being 2.20±0.09 and 2.03±0.08 U/g tissue, respectively compared to 1.56±0.04 U/g tissue for the control snails (Fig. 3d).

Survival rate at first shedding and infection rate of B. alexandrina snails with S. mansoni miracidia

It is obvious from results in Figure (4) that pretreatment of *B. alexandrina* snails with EtOAc fraction of *L. carinatus* tissue extract prior to infection with *Schistosoma mansoni* miracidia did not affect the survival rate of snails at first cercarial shedding, while the infection rate was significantly reduced at $p< 0.001$ showing 48% of reduction from the control infected snails.

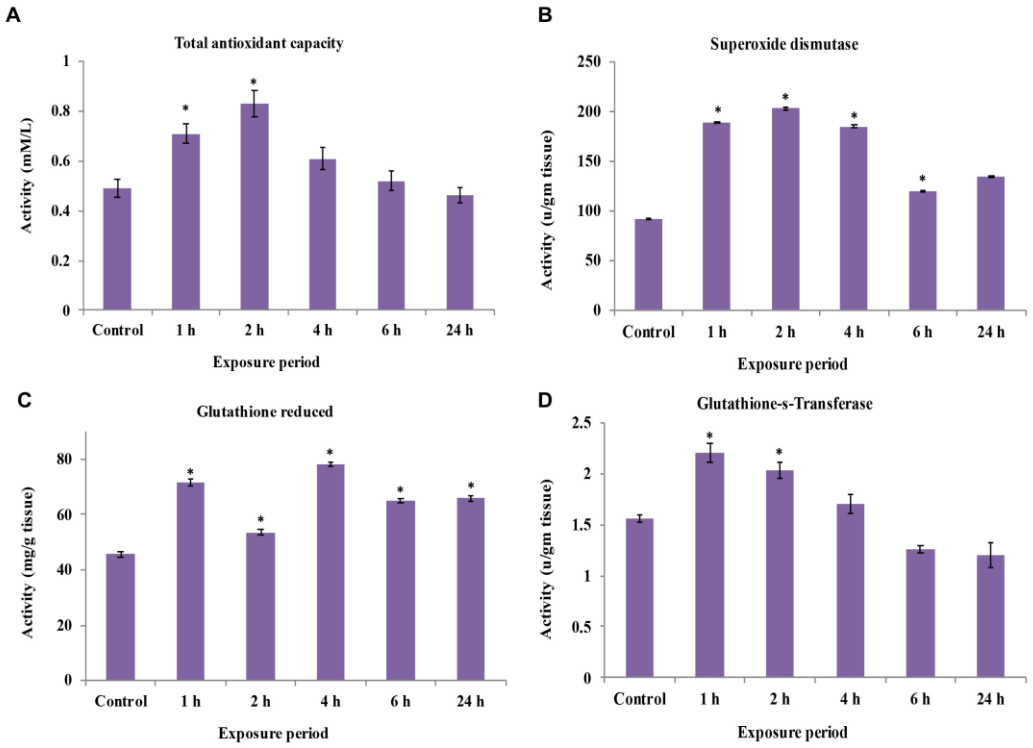

Fig. 3. Antioxidant parameters in the tissue homogenate of *Biomphalaria alexandrina* snails following exposure to EtOAc fraction of *Lanistes carinatus* tissue extract. A) Total antioxidant capacity, B) Superoxide dismutase, C) Glutathione reduced and D) Glutathione-s-Tansferase. Data presented as Mean ± Standard Error. * Significance compared to control at P<0.05

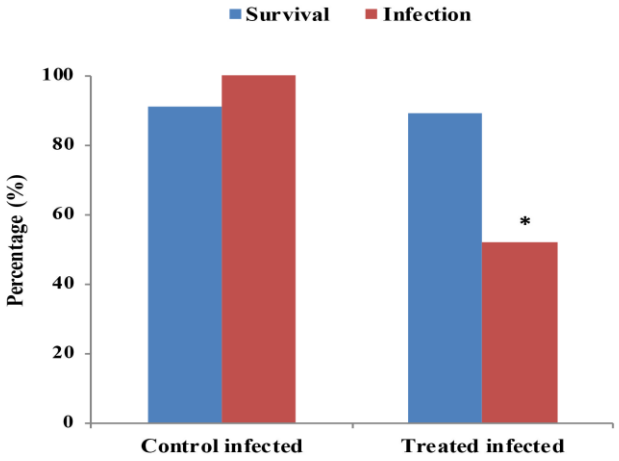

Fig. 4. Survival rate at first shedding and infection rate of *Biomphalaria alexandrina* snails infected with *Schistosoma mansoni* miracidia and exposed to EtOAc fraction of *Lanistes carinatus* tissue extract for 1 hour pre-miracidial exposure. * significance compared to control at P<0.05.

DISCUSSION

Success of schistosomiasis control depends largely on controlling the intermediate host snails, while most of the mechanisms used to achieve this goal depend on using natural or synthetic compounds as molluscicides, other initiatives aim to target the parasite development at the intramolluscan stage to block schistosomiasis transmission **(Mossalem *et al*. 2018)**. In this respect, using natural compounds to enhance the snail's immunity against the parasite seems an efficient approach. In the present study, we tested the antioxidant properties of tissue extracts of the freshwater snail, *L. carinatus* and used it improve the immune interaction of *B. alexandrina* to *S. mansoni* infection.

The results showed that, the crude methanol extract of *L. carinatus* tissues and its fractions have an adequate TAC. Methanolic tissue extract of the ampullariid snail, *Pila ampullacea* showed its tissue extracts showed a high free radical scavenging activity **(Gayathri *et al*. 2017a).** Different fractions of the methanol extract showed variable total antioxidant activities that can be supported by the fact that molluscan extracts are usually miscellaneous mixtures of bioactive molecules mainly proteins, peptides and sterols **(Ebada *et al*. 2008).**

Several studies indicated that molluscs are a very good source of biomedically important products and have developed very effective mechanisms that are part of their innate immunity **(Chakrabort and Joy 2020).** The methanolic extract of *Pila virens* proved also to be a reservoir of bioactive elements and may be recommended as a pharmaceutical **(Gayathri *et al*. 2017b)**.

In the present study, a proportional correlation between the total antioxidant capacity and total phenolic content was observed, where EtOAc fraction had the highest antioxidant activity and maximum phenolic content as well. Phenolic compounds are known to have various biological activities including antioxidant, antigenotoxic, and anti-inflammatory **(Ghareeb *et al*. 2018, 2019)**. Also, a positive relationship between phenolic content and antioxidant activities of plants has been reported by **Pereira *et al*. (2015)**.

Snail hemocytes have an effective innate immune reaction and have the ability to recognize, adhere to, surround and finally eliminate invading parasites and foreign subjects in a key defense-related function known as phagocytosis **(Humbert and Coustau 2001; Oliveira *et al*. 2010)**. Treatment of *B. alexandrina* with EtOAc fraction of *L. carinatus* tissue extract led to a noticeable increase in the phagocytic indexes of hemocytes with a qualitative improvement in the process through extracellular trapping and endocytosis of multiple yeast cells especially within few hours post treatment. This finding agrees with **Bender at al. (2005)** who noticed that *B. glabrata* hemocytes have the ability to discover the invading parasites within minutes and can cause significant damage within few hours due to adhesion and phagocytosis. Similar effects have been

established following viral, bacterial, and fungal infections, suggesting a broadly effective immune response in invertebrates **(Chambers and Schneider 2012)**. The upgrading of phagocytosis process after treatment with the extract may be related to the high antioxidant capacity and total phenolic content. **Lawal *et al*. (2015)** found that giant African snail, *Achachatina maginata*, extract can improve the immune ability and inhibit the acute liver toxicity induced by CCL_4 in rats due to high antioxidant capacity of the hemolymph extract.

The present data showed also that, treatment of *B. alexandrina* snails with EtOAc fraction of *L. carinatus* tissue extract led to an increase in the total antioxidant activity levels in *B. alexandrina* during the first 2 hours post treatment. Like other invertebrates, snails have immune functions complemented by an array of killing mechanisms, which include release of toxic reactive oxygen species (ROS) **(Moné *et al*. 2011)**. Infection with *S. mansoni* was shown to cause oxidative stress within *B. alexandrina* with an increased levels of lipid peroxidation and a reduction in the internal antioxidant system of infected snails **(Mossalem *et al*. 2018; Habib *et al.* 2020)**. Increase in production of free radicals stimulates and increases antioxidant activities to cope with increased oxidative stress and protect the cells from damage **(Monaghan *et al*. 2009)**. Indeed, an increase in SOD and GST levels within *B. alexandrina* were observed in the few hours following treatment with the extract. SOD is the first line of defense against oxidative stress is provided **(Johnson and Giulivi 2005)**. Moreover, GSTs are known to have double function in conjugation of electrophilic compounds on one hand, and in the defense against oxidative damage on the other hand **(Van der Oost *et al*. 2003)**. GSH exhibited high activity in the first 24 hours period post treatment and this may be due to the role played by GSH in protecting cells against lethal consequences of toxic compounds **(Hayes and McLellan 1999)**.

From the analysis of the previous results, it is clear that treatment of *B. alexandrina* with EtOAc fraction of *L. carinatus* tissue extract improved the general immune responses of snails which in turn led to a significant reduction in the infection rate of *B. alexandrina* snails with *S. mansoni* miracidia to the half of control values which is considered a targeted result of the current study. Numerous studies tested the effect of immunostimulation on *B. alexandrina* snails and similar results were obtained. For example, **El Sayed *et al*. (2017)** reported that treatment of *B. alexandrina* snails with sodium alginates enhanced their immune ability to overcome infection with *S. mansoni* miracidia. In the same context, treatment of *B. alexandrina* snails with β-glucan prior to infection with *S. mansoni* miracidia led to retarded infection dynamics and depressed production of cercariae accompanied with decrease in the final infection rates **(Soliman *et al*. 2017).** Moreover, antioxidants from plants sources were also effective in stimulating the immunity of *B. alexandrina* and reduced their infection rate with *S. mansoni*. For example, a significant reduction in the infection rate of *B. alexandrina* following treatment with EtOAc extract of *Eucalyptus camaldulensis* was reported by

Mossalem *et al*. (2018). Moreover, treatment of *B. alexandrina* with the antioxidant compound, methyl gallate reduced the snail's infection rate and increased the hemocytes number in snail's hemolymph **(Mansour *et al*. 2021).** The antioxidant activity of these natural extracts is due to its high ratios of phenolic contents that modulated the oxidative stress of infection in snails. Indeed, EtOAc extract of *E. camaldulensis* decreased the level of lipid peroxidation and increased catalase and glutathione reduced activities in the hemolymph and tissues of the treated *B. alexandrina* compared to untreated snails **(Mossalem *et al*. 2018)**.

CONCLUSION

The results obtained, collectively, confirm the high antioxidant properties of EtOAc fraction of *L. carinatus* tissue extract and its ability to stimulate the immunity of *B. alexandrina* and reduce the snail's infection rate with *S. mansoni*. Thus, it can be used as a safe, alternative method to control schistosomiasis. Further studies are needed to characterize the chemical constituents in this extract responsible for antioxidant and immunostimulant effects.

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