HELMINTHOLOGIA, 57, 4: 335 - 343, 2020

Efficacy of Miltefosine and Artemether on infected Biomphalaria alexandrina snails with Schistosoma mansoni: immunological and histological studies

H. H. ABDEL-AZEEM¹, G. Y. OSMAN¹, M. F. EL GARHY², K. S. AL BENASY¹,3,*

¹Department of Zoology, Menoufia University, Shebeen El-koom, Egypt; ²Department of Zoology, Cairo University, Giza, Egypt;
³*College of Applied Medical Sciences, Majmaah University, Saudi Arabia, E-mail: K.albenasy@mu.edu.sa

Summary

Biomphalaria alexandrina snails have received much attention due to their great medical importance as vectors for transmitting Schistosoma mansoni infection to humans. The main objective of the present work was to assess the efficacy of miltefosin a synthetic molluscicidal drug and artemether a natural molluscicidal drug. The correlation between immunological and histological observations from light and electron microscopy of the hemocytes of B. alexandrina post treatment with both drugs was also evaluated. LC_{50} and LC_{90} values were represented by 13.80 ppm and 24.40 ppm for miltefosine and 16.88 ppm and 27.97 ppm for artemether, respectively. The results showed that the treatment of S. mansoni-infected snails and normal snails with sublethal dose of miltefosine (LC_{25}=8.20 ppm) and artemether (LC_{25}=11.04 ppm) induced morphological abnormalities and a significant reduction in hemocytes count.

Keywords: Biomphalaria alexandrina; miltefosine; artemether; hemocytes; immunological and histological studies; light and electron microscope

Introduction

Schistosomiasis represents a global health concern with over 700 million people at risk of contracting this disease (Weber et al., 2019). In Egypt eradicating this disease is a top governmental priority (Abou-El-Naga, 2018). Generally, chemotherapy is still one of the most effective methods for controlling Schistosoma infection (Bertão et al., 2012a). Since the mid-1970’s, PZQ (praziquantel derivatives) represented the anti-helminthic drug of choice. Despite its efficiency in reducing morbidity and mortality rates in Schistosoma infections (Caffrey, 2007), it is not active against early stages of schistosomes and causes species resistance (Bertão et al., 2012b). Currently, miltefosine and artemether (antimalarial drugs) have a potential application in schistosomiasis treatment (El Beshbish et al., 2018).

Miltefosine (hexadecylphosphocholine) is an alkyl phospholipid derivative that was developed as a new type of antitumor agent in the 1990s (Eibla and Unger, 1990). Miltefosine has comparative advantage over PZQ as antischistomicidal drug due to its efficacy on the differential developmental stages of S. mansoni in infected mice (Eissa et al., 2011). Miltefosine used as an schistosomicidal drug increased helminths mortality rate and induced extensive tegumental changes in the adult worms of Egyptian and Brazilian strains of S. mansoni in vitro and in vivo (Eissa et al., 2015; El-Faham et al., 2017). In addition, miltefosine presents a molluscidal activity against both Biomphalaria alexandrina and Bulinus truncatus snails (Eissa et al., 2011). Artemether is a methyl ether derivative of artemisinin, a compound extracted from the leaves of the Chinese wormwood plant (Artemisia annua) (Mossalem et al., 2013). It was first described as anti-schistosomal agent in
1980s, against juvenile worms (5-21 day-old) *S. japonicum* (Liu et al., 2012). Artemether efficiency as a schistosomicidal and molluscicidal agent has been previously shown (Al-Kazzaz et al., 2014; Madbouly et al., 2015).

The aims of this work were to compare the efficacy of miltefosine, a chemotherapeutic synthetic compound, with artemether, a natural molluscicidal through light and electronic microscopy and to elucidate their effect on normal immune-histological parameters in *S. mansoni*-infected *B. alexandrina* snails.

**Materials and Methods**

**Experimental snails**

*B. alexandrina* snails (8-10 mm in diameter) were obtained from *Schistosoma* Biological Supply Centre at the Medical Malacology Laboratory, Theodor Bilharz Research Institute, Giza, Egypt. The snails were classified as follows:

- Group (1): 50 normal control snails (unexposed snails).
- Group (2): 100 normal snails divided into two subgroups (per 50 snails) exposed to sublethal concentrations LC₉₀ of miltefosine and artemether for 2 successive week intervals in two replicates for each drug.
- Group (3): 150 snails were exposed to *Schistosoma mansoni* miracidia with a dose of 10 per snail (Liang et al., 1987). This group was further divided into 3 subgroups:
  - Group (3a): 50 infected snails
  - Group (3b): 50 infected snails treated with a sublethal concentration LC₂₅ of miltefosine for 2 successive weeks in two replicates for each drug.
  - Group (3c): 50 infected snails treated with a sublethal concentration LC₂₅ of artemether for 2 successive weeks in two replicates for each drug.

For each drug, the treatment was changed weekly with freshly prepared one to avoid the effect of storage. The snails were exposed to the tested concentrations for 24 hours, then removed from the experimental environment, washed thoroughly with dechlorinated tap water and transferred to aquaria with fresh dechlorinated tap water for the next 24 hours to recover (25 ± 2°C).

**Experimental materials**

1. The drug miltefosine (100 mg) was provided by (Sigma-Aldrich Chemie and GmbH, CA 58066-85-6, MW 407.57, Germany) and its trade name is Impavido (molar mass 407.568 g/mol, chemical formula C₂₁H₄₆NO₄P).

2. The drug artemether was obtained in the form of tablets (Kunming Pharmaceutical Cooperation, PR China) with a documented purity of 99.6%. It is sold under the trade name Riamet and Coartem among others, has a molar mass of 298.374 g/mol and the chemical formula is C₁₆H₂₆O₅. The actual concentration was calculated as the percentage of the active material in the used weight. Artemether was applied to snails as aqueous solution of tablets.

**Artemether structure**

![Artemether structure](image)

**Molluscidal properties of miltefosine and artemether drugs**

A stock solution of 1000 ppm from each drug was prepared on the basis of weight/volume using dechlorinated water. A series of concentrations was prepared for each drug according to the standard procedure recommended by WHO (1965) that allowed us to reach experimental concentrations (LC₀, LC₁₀, LC₂₅, LC₅₀ and LC₉₀). The effectiveness of each drug as a molluscide has been expressed in terms of LC₂₅ and LC₉₀ according to the procedure of Litchfield & Wilcoxon (1949). Three replicates of gradual concentrations from each stock solution were prepared. The snails were exposed to the tested concentrations for 24 hours, then removed from the experimental environment, washed thoroughly with dechlorinated tap water and transferred to aquaria with fresh dechlorinated tap water for the next 24 hours to recover (25 ± 2°C). Unexposed snails (controls) were assayed side by side with the treated groups under the same laboratory conditions in dechlorinated tap water (WHO, 1965). Dead snails were noticed and removed from the container.

**Immunological and histological study of *B. alexandrina* hemocytes**

Hemolymph samples from snails from all studied groups were collected as previously described by Michelson (1966) by removing a small portion of the shell and inserting a capillary tube into the heart. The hemolymph was collected in a vial tube (1.5 ml) and kept in ice-box. The collected hemolymph from infected snails and infected treated groups was used to estimate the total hemocytes count using a Bürker-Türk hemocytometer (while the differential haemocyte count was obtained according to a previously published method (Van der Knap et al., 1981)). While the differential haemocyte count was obtained on the light microscopy level, hemolymph samples were placed individually onto a clean glass slide. Hemocytes were fixed in 100% methanol, then stained.
with Giemsa's stain examined and counted then photographed using Agfa film according to a previously published method (Abdul-Salam & Michelson, 1980). The hemolymph samples from normal control snails (group1) and drug exposed snails (group 2) were used for transmission electron microscopy examinations (Grimaud et al., 1980).

**Statistical analysis**

The results were analysed statistically using the Statistical Package for Social Science (SPSS version 15 package software). Data were expressed as mean (M) ± standard deviation (S. D). The data were statistically analysed significantly differences between the treated and the control group using “t” test (Goldstein, 1964).

**Results**

**Molluscicidal activity of miltefosine and artemether against B. alexandrina snails**

Bioassay tests results are presented in Table 1, revealing that both compounds has a molluscicidal activity against adult snails. It was noticed that miltefosine showed a marked lethal effect against snails compared to artemether ($LC_{50}$ and $LC_{90}$ of 16.88 and 27.97 ppm, respectively). Its $LC_{50}$ and $LC_{90}$ values were 13.80 ppm and 24.40 ppm with a slope function value of 1.60. Furthermore, these results demonstrated that the sublethal concentrations ($LC_{10}$, $LC_{25}$, & $LC_{50}$) of miltefosine were lower than the corresponding values of artemether.

**Effect of the tested drugs on hemocytes of adult B. alexandrina snails**

**Total number of hemocytes of B. alexandrina snails**

The recorded data in Figure 1 denoted that the exposure of snails (groups 3b & 3c) to $LC_{25}$ of miltefosine (8.20 ppm) and artemether (11.04 ppm) for 24 hours for 2 successive weeks exhibited a significant decrease ($P < 0.001$) in hemocytes count compared with the control group (infected snails; group 3a). The reduction in hemocytes was of 65.48% and 47.62% in miltefosine and artemether treated groups, respectively.

**Effect of the tested drugs on percentage of different types of hemocytes**

As for the number of the different haemocyte types; the exposure of adult infected snails (groups 3b & 3c) to the $LC_{25}$ drugs concentrations induced a decrease in hyalinocytes percentage. Miltefosine treated–infected snails had the lowest percentage of hyalinocytes (18.40%) compared to artemether (26.60%) and control snails (group3a) (52.3%) (Figure 2). On the contrary, both drugs induced an elevation in the percentage of small round cells and granulocytes in corresponding to the infected-control group (group3a).

| Tested drug   | $LC_{10}$ Ppm | $LC_{25}$ Ppm | $LC_{50}$ Ppm | $LC_{90}$ Ppm | Slope |
|--------------|---------------|---------------|---------------|---------------|-------|
| Miltefosine  | 1.38          | 8.20          | 13.80         | 24.40         | 1.60  |
| Artemether   | 1.68          | 11.04         | 16.88         | 27.97         | 1.75  |

Table 1. Molluscicidal activity of miltefosine and artemether against adult B. alexandrina snails (24 hours exposure).
Morphological alterations induced by miltefosine and artemether observed in light and electronic microscopy

Light microscope study
Hemocytes examination of the normal control snails (group 1) by light microscopy revealed the presence of three morphologically different cell types (Plate 1 A–C): small undifferentiated cells with a spherical profile (A), large spherical granulocytes with a double membrane and a relatively large cytoplasm filled with a variable number of basophilic granules (B), and polymorphic hyalinocytes with either a large eccentric nucleus or two nuclei, suggesting atypical cell division (C).

The exposure of adult non-infected *B. alexandrina* (group 2) to LC$_{25}$ miltefosine (8.20 ppm) and artemether (11.04 ppm) for 24 hours for 2 successive weeks induced some alterations on the morphology of granulocytes and hyalinocytes, but small undifferentiated cells were not affected. In drug exposed snails noticed by light microscopy that granulocyte appeared under different sizes granules. Hyalinocytes on the other hand, had a large vacuolated cytoplasm and a shrunk nucleus (Plates 2 & 3).

Electron microscope study (TEM)
The ultrastructural examination of normal control hemocytes (group 1) showed the presence of three morphologically different cell types (Plate 4 A–C). The exposure of adult snails to sublethal concentrations (LC$_{25}$) of the examined drugs showed a different type of cells with two types of globules in the cytoplasm that appeared only in the hemolymph of treated snails (group 2) (Plate 5 A–C).

Miltefosine treatment induced the following alterations in hemocytes types of non-infected snails (group 2 a): small undifferentiated cells showed a large nucleus, an intact cell membrane, cytoplasmic extensions (pseudopodia), vacuoles, and phagolysosomes in the cytoplasm (5 A); granulocytes presented nucleus with irregular boundaries and an irregular chromatin distribution, the cytoplasm contained phagolysosomes, granules, and some cell organelles such as the mitochondria and rough endoplasmic reticulum (5 B); hyalinocytes presented a degenerated outer cell membrane, the nucleus had an irregular membrane, and the nucleus shrunk in size (5 C).

Artemether induced the following on different types of hemocytes of snails (group 2 b) (Plate 6 A–C): small undifferentiated cells had an intact cellular membrane with extended pseudopodia, the cytoplasm and the nuclei contained phagolysosomes and vacuoles (6 A); granulocytes showed pseudopodia, cytoplasmic granules,
Plate 2. The morphological effect of miltefosine LC<sub>25</sub> on different types of hemocytes in the haemolymph of treated-infected <i>B. alexandrina</i> snails. <b>A</b>): Round small non-differentiated cell, <b>B</b>): Granulocyte and <b>C</b>): Hyalinocyte (x40). S: Round small cell, G: Granulocyte, GR: Granules, N: Nucleus, (H): Hyalinocyte.

Plate 3. The morphological effect of artemether LC<sub>25</sub> on different types of hemocytes in the haemolymph of treated <i>B. alexandrina</i> snails. <b>A</b>): Round small (non-differentiated), <b>B</b>): Granulocyte and <b>C</b>): Hyalinocyte (x40). S: Round small cell, G: Granulocyte, C: Cytoplasm, GR: Granules, N: Nucleus, (H): Hyalinocyte.

Plate 4. TEM micrographs showing normal cells of <i>B. alexandrina</i> snails. <b>A</b>): Round small non-differentiated cell, <b>B</b>): Granulocyte and <b>C</b>): Hyalinocyte. CH: chromatin, CM: cell membrane, CY: Cytoplasm, N: nucleus.

Plate 5. TEM micrographs showing the effect of miltefosine LC<sub>25</sub> on hemocytes <b>A</b>): Round small (non-differentiated) cell, <b>B</b>): Granulocyte and <b>C</b>): Hyalinocyte of treated-infected <i>B. alexandrina</i> snails. CH: chromatin, CM: cell membrane, CY: Cytoplasm, GR: granules, N: nucleus, V: vacuole, PL: phagolysosome.
and vacuoles in cytoplasm (6 B); while hyalinocytes showed an intact cell membrane, degenerated nuclei, and difficult to identify organelles (6 C).

Discussion

Internal defense mechanisms of invertebrates depend upon an innate immune system including cellular and humoral components (Le Clech et al., 2016). Cell-mediated immune response resulted from the presence of various cell categories that are vital in defense and constitute the primary barrier against invading parasites and bacteria as well as accumulate different substances such as molluscsides, heavy metals and pesticides. These are mobile amoeboid cells referred to as amoebocytes or hemocytes (Bernard, 2016). Furthermore, hemocytes of the snail are used to determine the prepatency period of infection with schistosomiasis (Kamel et al. 2006). Previous experimental studies proved that the parasitic infection induced positive effect upon hemocytes count by increasing their number in the hemolymph of various species of snails (Barcante et al., 2012; Mossalem & Ibrahim, 2019; Suwanatrai et al., 2019).

In the present study, the hemocytes of S. mansoni infected snails (had higher significant increase compared to the treated -infected snail groups with the sublethal concentration (LC$_{25}$) of miltefosine and artemether as in previous studies (Mossalem et al., 2013; Ibrahim et al., 2018; Mossalem & Ibrahim, 2019). The decrease in hemocytes count in treated snail groups might be a result from tissue damage in digestive and hermaphrodite glands as they participated in tissue repair (Esmaeil, 2009). Another explanation might be the reduction in transaminase enzyme activities, sensitive tools in physiological alterations detection (Kamel et al., 2007), or perhaps, it is the result of hemocyte cells from the hemolymph migrating to connective tissues (Coehennec-laureau, 2003).

In regard to the types of hemocytes, the present study detected 3 types of cells in B. alexandrina hemolymph; hyalinocytes, small round cells and granulocytes. This finding agrees with the results of other studies (Mohamed et al., 2006; Cavalcanti et al., 2012). Some authors suggested that the hyalinocytes and the granulocytes are different cell types, while others considered that they represent different developmental phases of the same cell type (Oliveira et al., 2010). Another study revealed that granulocytes were phagocytic cells with pseudopods capable of encapsulating large particles, while the hyalinocytes were spherical smaller cells without pseudopods (Yoshino et al., 2008). Additionally, the present study showed that the percentage of hyalinocytes decreased in both miltefosine and artemether exposed groups, while the percentage of round small cells (undifferentiated cells) and granulocytes increased in exposed groups compared to the normal uninfected control group. The present data are in accordance with Bakry et al. (2012) with a study showing that methanol extracts of Azadirachta indica plant induced a significant increase in the number of granulocytes in the hemolymph of B. alexandrina snails indicating a high response of the snails against the treatment. Several studies explained the fluctuations in percentage of the types of hemocytes of aquatic snails as a result of drug treatment (Bakry et al., 2012). A study reported that granular hemocytes were the major responsive hemocytes type in B. alexandrina snails treated with a plant growth regulator (Mepiquat chloride) (Mohamed & Abdel-Gawad, 2005). Another study detected an increase in the percentage of small round undifferentiated cells, which may be due to the stimulation of the hematopoietic organ producing undifferentiated hemocytes that differentiated into granulocytes to compensate their reduction in number (El Sayed, 2006). Furthermore, a study stated that hyalinocytes are thought to be responsible primarily for wound repair requiring aggregation at injury site, thus their number decreased in the hemolymph (Barcante et al., 2012). It was reported that granulocyte cells are immunological active cells found mainly in the hemolymph of snails, instead of remaining in the damaged tissue to face external stimuli (Oliveira et al., 2010).

The ultra-structural observation of hemocytes showed that the tested drugs induced morphological alterations, such as irregular nuclear boundaries, irregular chromatin distribution, degenerated nuclei, and vacuolated cytoplasm with electron-dense phagolysosomes; this is in agreement with the results obtained by others (El Sayed et al., 2011; Ibrahim et al., 2018). Kamel et al. (2006, 2007) revealed the presence of a remarkable activation in hemocytes due to a sublethal concentration of plant treatment and mol-
luscidial compound. They attributed the degenerative changes of hemocytes cellular organelles to a direct toxic effect of these molluscicidal drugs. Further, the continuous exposure to sub-lethal concentrations of artemether caused an increase in glycogen content in hemocytes causing them to increase in size by increasing the time of exposure as reported by Mossalem et al. (2013). Despite the promising molluscicidal activity of miltefosine, studies show administration results in severe side effects, miltefosine had broad biocide activity as well as it has been shown that miltefosine is a teratogenic agent and its use in the treatment as an anti-Leishmania medication has been associated with severe side effects (Bhattacharya et al., 2007; Eissa et al., 2011). On the contrary, the present study suggests that artemether can be effectively used as a safe plant origin molluscicide in the national S. mansoni control program due to some studies, do not show evidence of harm on non-target organisms. So, further research studies are warranted to evaluate the impact of artemether as safe molluscicidal and schistosomicidal agent (Piola et al., 2010; Elmorshey et al., 2016).

Conclusion

Miltefosine and artemether have a toxic effect on B. alexandrina as they negatively affect immunological processes inducing degenerative changes and fragmentation of hemocytes. Consequently, tested drugs could be ranked as beneficial molluscicidal agents for the control program of schistosomiasis. However, artemether is cheaper to produce and safer for vector control. To further assess artemether in the control of schistosomiasis, it should be evaluated and tested for complete efficacy within the operational research bases for Schistosoma infection control.

Acknowledgment

This paper is part of Ph. D Thesis, sincere gratitude is expressed to Menuofia University, Faculty of Science, Biology Department, Egypt for the fruitful cooperation and continuous help throughout this work.

Conflict of Interest

The authors declare that there are no conflicts of interest.

References

Abdul-Salam, J.M., Michelson, E.H. (1980): Biomphalaria glabrata amoebocytes, effect of Schistosoma mansoni infection on in vitro phagocytosis. J Invertebr Pathol., 35, 241–248. DOI: 10.1016/0022-2011(80)90157-3
Abou-el-nAgA, i.F. (2016): Study of the efficacy of Nitazoxanide, Myrrh Total Oil and Mirazid in comparison with Praziquantel in experimental Schistosomiasis mansoni. M.Sc. University of Alexandria, Egypt
Al-Kazzaz, M.A.N. (2014): Influence of Atrazine and Roundup pesticides on biochemical and molecular aspects of Biomphalaria alexandrina snails. Pestic Biochem Physiol, 104: 9–18. DOI: 10.1016/j.pestbp.2012.05.012
Berrão, H.G., Ramos da Silva, A.R., Albuquerque, M., Radis-Baptista, G., de Azevedo Albuquerque, M. (2012 a): Ultrastructural analysis of miltefosine-induced surface membrane damage in adult Schistosoma mansoni BH strain worms. Parasitol. Res., 110: 2465-2473. DOI: 10.1007/s00436-011-2786-5
Bertão, H.G., Ramos da Silva, A.R., Radis-Baptista, G., de Azevedo Albuquerque, M. (2012 b): Miltefosine, an alkylphosphocholine originally developed as an antitumoral, is an effective compound against Schistosoma mansoni. Int. J. Pharm. Med. Biol. Sci., 1: 2278-5221
Bhattacharya, S.K., Sinha, P.K., Sundar, S., Thakur, C.P., Jha, T.K., Pandey, K., Das, V.R., Kumar, N., Lal, C., Verma, N., Singh, V.P., Ranjan, A., Verma, R.B., Anders, G., Sindermann, H., Ganguly, N. K. (2007): Phase 4 trial of miltefosine for the treatment of Indian visceral leishmaniasis. J. Infect. Dis., 196(4): 591-598. DOI: 10.1086/519690
Caffrey, C.R. (2007): Chemotherapy of schistosomiasis: present and future. Curr Opin Chem Biol., 11(4): 433–439. DOI: 10.1016/j.cbpa.2007.05.031
Caivalcani, M.G.S.; Mendonca, A.M.B.; Duarte, G.R.; Barbosa, C.; De Castro, C.; Alves, L.C.; Branyer, F.A. (2012): Morphological characterization of hemocytes from Biomphalaria glabrata and Biomphalaria straminea. Micron, 43: 285–291. DOI: 10.1016/j.micron.2011.09.002
Cochennec-Laureau, N., Auffret, M., Renault, T., Langlade, A. (2003): Changes in circulating and tissue-filtering hemocyte parameters of European flat oysters, Ostrea edulis, naturally infected with Bonamia ostreae. J. Invertebr. Pathol., 83 (1): 23–30. DOI: 10.1016/S0022-2011(03)00015-6
El-Banayosy, A., Unger, C. (1990). Hexadecylphosphocholine a new and selective antitumor drug. Cancer Treat Rev., 17: 233-242. DOI: 10.1016/0305-7372(90)90053-i
Eissa, M.M., El-Azzouni, M.Z., Amer, E.I., Badour, N.M. (2011): Miltefosine, a promising novel agent for schistosomiasis mansoni. Int J Parasitol., 41(2): 235-242. DOI: 10.1016/j.ijpara.2010.09.010
Eissa, M.M., El-Moslemly, R.M., Ramadan, A.A., Amer, E.I., El-Azzouni, M.Z., El-Khordagui, L.K. (2015): Miltefosine lipid nanocapsules for single dose oral treatment of schistosomiasis mansoni:
A Preclinical Study. *PLoS One*, 10: 22-29. DOI: 10.1371/journal.pone.0141788

Eissa, S.H., Rizk, E.T., Abou-Shafey, A.E., Mona, M.H., Atlum, A. (2002): Toxicological effect of *Euphorbia peplus* water suspension on haemocytes of the fresh water snails, *Biomphalaria alexandrina* and *Lanistes carinatus*. *Proc. LCBS*, 2(2): 417-447

El Beshbishi, S.N., El Bardicy, S., Tadros, M., Ayoub, M., Taman, A. (2018): Efficacy of artemisinin–naphthoquine phosphate against *Schistosoma haematobium* adult flukes: dose–effect relationship and tegumental alterations. *J. Helminthol.*, 93(4): 513-518. DOI: 10.1017/S0022149X18000421

El-Faham, M.H., Eissa, M.M., Igetei J.E., Amer, E.I., Liedell, S., El-Azzouni, M.Z., Doenhof, M.J. (2017): Treatment of *Schistosoma mansoni* with miltefosine in vitro enhances serological recognition of defined worm surface antigens. *PLoS Negl. Trop. Dis.*, 11(8): 1-26. DOI: 10.1371/journal.pntd.0005853

Elmorshedy, H., Tanner, M., Bergouist, R.N., Sharaf, S., Barakat, R. (2016): Prophylactic effect of artemether on human schistosomiasis mansoni amongst Egyptian children: A randomized controlled trial. *Acta Trop.*, 158: 52–58. DOI: 10.1016/j.actatropica.2016.02.015

El-Sayed, K.M., Mossalem, H.S. (2011): Cryptostegia grandiflora affecting compatibility of *Biomphalaria alexandrina* and *Biomphalaria galabrata* to infection with *Schistosoma mansoni* with emphasis on some hematological effects. *Aust J Basic Appl Sci*, 5(12): 2210-2217

El-Sayed, K.M. (2006): Effect of the plant *Cupressus macrocarpa* on some haematological and biochemical parameters of *B. alexandrina* snails. *J. Egypt. Soc. Parasitol.*, 36: 911-924

El-Sayed, K.M. (2007): Toxicological effect of certain plants and synbiotics on *B. alexandrina* on hemocytes of the fresh water snails, *Euphorbia peplus* (2002): Toxicological effect of *Euphorbia peplus* on ultra-structural changes in hemocytes of *Biomphalaria tenagophila*. *Proc. LCBS*, 1: 221-234

Eissa, S.H., Mona, A.H., Mossalem, H.S. (2006): The effect of *Schistosoma mansoni* infection on *Biomphalaria alexandrina* haematocytes at ultra-structural level. *Proceeding of the 4th International Conference of Biological Science* (Zoology), p. 219

Eissa, S.H., Mona, A.H., Mossalem, H.S. (2007): Toxicological effect of certain plants and synthetic molluscicides on ultra-structural changes in hemocytes of *Biomphalaria alexandrina*. *J. Exp. Biol.* (Zoo.), 3: 135-140

El-Clech, W., Anderson, T.J.C., Chevalier, F.D. (2016): Characterization of hemolymph phenoloxidase activity in two *Biomphalaria* snail species and impact of *Schistosoma mansoni* infection. *Parasit Vectors*, 9: 32-38. DOI: 10.1186/s13071-016-1319-6

LeClech, W.T., Wilcox, F. (1949): A simplified method of evaluating dose effect experiments. *J. Pharmacol. Exp. Therap.*, 96: 99-11113

Liu, R., Dong, H.F., Jiang, M.S. (2012): Artemisinin: the gifts from traditional Chinese medicine not only for malaria control but also for schistosomiasis control. *Parasitol. Res.*, 110: 2071–2074

Michelson, E.H. (1966): Specificity of hemolymph antigens in taxonomic discrimination of medically important snails. *J. Parasitol.*, 52: 466-472

Mohamed, A.Z., Abdel-Gawad, A.E. (2005): Effect of plant growth regulator (Mepiquat chloride) on circulating hemocytes and total plasma protein of *Biomphalaria alexandrina* snails. *Egypt. J. Parasitol.*, 31(1): 283-303

Mossalem, H.S., Ibrahim, A.M. (2019): The ameliorative potential of the ethanol extract of the plant *Cupressus macrocarpa* on ultra-structural and biochemical parameters of *B. alexandrina* snails. *J. Egypt. Soc. Parasitol.*, 36: 911-924

Goldstein, A. (1964): *Biostatistics: An introductory text*. Macmillan, New York, p. 51

Grimaldi, J.A., Druget, M., Peyrol, S., Chevalier, D. (1980): Collagen immunoypoting in human liver: Light and electron microscope study. *J. Hist. Chem. Cytol.*, 28: 1145-1151

Ibrahim, A.M., Ahmed, A.K., Bakry, F.A., Abdel-Gaffar, F. (2018): Hematological, physiological and genotoxicological effects of Match 5% EC insecticide on *Biomphalaria alexandrina* snails Ethylendiamine. *Ecotoxicol Environ Saf.*, 174: 1017-1022. DOI: 10.1016/j.ecoenv.2017.09.059

Kamel, E.G., Refaat, S.W., El-Daffrawy, S.M., Mohamed, A.H., Mossalem, H.S. (2006): The effect of *Schistosoma mansoni* infection on *Biomphalaria alexandrina* haematocytes at ultra-structural level. *Proceeding of the 4th International Conference of Biological Science* (Zoology), p. 219

Kamel, E.G., Refaat, S.W., El-Daffrawy, S.M., Mohamed, A.H., Mossalem, H.S. (2007): Toxicological effect of certain plants and synthetic molluscicides on ultra-structural changes in hemocytes of *Biomphalaria alexandrina*. *Egyt. J. Exp. Biol.* (Zoo.), 3: 135-140

LeClech, W., Anderson, T.J.C., Chevalier, F.D. (2016): Characterization of hemolymph phenoloxidase activity in two *Biomphalaria* snail species and impact of *Schistosoma mansoni* infection. *Parasit Vectors*, 9: 32-38. DOI: 10.1186/s13071-016-1319-6

Liang, Y.S., John, B.L., Boyd, D.A. (1987): Laboratory cultivation of schistosomate vector snails and maintenance of schistosome life cycles. In: *Proceeding of the 1st Sino-American Symposium*, 1: 34–48

Litchfield, J.T., Wilcox, F. (1949): A simplified method of evaluating dose effect experiments. *J. Pharmacol. Exp. Therap.*, 96: 99-11113

Liu, R., Dong, H.F., Jiang, M.S. (2012): Artemisinin: the gifts from traditional Chinese medicine not only for malaria control but also for schistosomiasis control. *Parasitol. Res.*, 110: 2071–2074

Michelson, E.H. (1966): Specificity of hemolymph antigens in taxonomic discrimination of medically important snails. *J. Parasitol.*, 52: 466-472

Mossalem, H.S., Ibrahim, A.M. (2019): The ameliorative potential of the ethanol extract of the plant *Ocimum basilicum* on *Biomphalaria alexandrina* snails exposed to the insecticide Bestacid. *Egypt. J. Aqu. Bio. Fisheries.*, 23(1): 161-172

Mossalem, H.S.; Abdel-Hamid, H., El-Shinnawy, N.A. (2013): Impact of artemether on some histological and histochemical parameters in *Biomphalaria alexandrina*. *Afr J Pharm Pharmacol*, 7(31): 2220-2230

Oliva, A.L.D., Levada, P.M., Zanotti-Magalhaes, E.M., Magalhaes, L.A., Ribeiro-Paes, J.T. (2010): Differences in the number of hemocytes in the snail host *Biomphalaria tenagophila*, resistant and susceptible to *Schistosoma mansoni* infection. *Genet. Mol. Cells.*, 9 (4): 2436-2445

Pioia, P., Namasum, R., Surayakaa E., Dhorda, M., Lindegard, N., Nvehanganene, D., Snouino, G., Ashley, E.A., McGready, R., Nosten, F., Guerin, P.J. (2010): Efficacy and safety of artemether–lumefantrine compared with quinine in pregnant women with uncomplicated *Plasmodium falciparum* malaria: an open-label, randomised, non-inferiority trial. *Lancet Infect Dis.*, 10 (11): 762–769. DOI: 10.1016/S1473-3099(10)70202-4

Suzannatari, K., Suzannatari, A., Donhasong, C., Arunsan, P., Thinkhamrop, K., Tapsiripap, P., Welbat, J.U., Tangkawattana, S., Sotillo, J., Tesana, S. (2019): Hemocyte subpopulation changes in *Bithynia* snails infected with *Opisthorchis viverrini* in Thailand. *bioRxiv*, 1-18. DOI: 10.1101/536292

Van der Knaap, W. P., Sminia, T., Kroese, F. G., Dikkeboom, R. (1981): Elimination of bacteria from the circulation of the pond snail *Lymnaea stagnalis*. *Dev Comp Immunol*, 5: 21
WEBER, C.J., CALVOPRA, J.H., GRAEF, K.M., CATHYRYNE, K.M., DENT, J. (2019): Platform for product-centered cross-sector partnerships for the Elimination of Schistosomiasis. *Trop. Med. Infect. Dis.*, 4(11): 1-20

WHO (1965): Molluscicide screening and evaluation. *Bull. WHO* 33: 5675-5681

YOSHINO, T.P., DINGURARD, N., KUNERT, J., HOKKE, C.H. (2008): Molecular and functional characterization of a tandem-repeat galectin from the freshwater snail *Biomphalaria glabrata*, intermediate host of the human blood fluke *Schistosoma mansoni*. *Gene*, 411: 46-58