**In-vitro Investigation of the Effect of Bee Venom on Schistosoma mansoni Eggs**

Alaa H Saleh¹, Abeer M Badr¹, Soheir S Mahmoud², Noha A Mahana¹ and Ahmed S Abo Dena³, ⁴,*

¹Zoology Department, Faculty of Science, Cairo University, Giza 12613, Egypt; ²Department of Parasitology, Theodor Bilharz Research Institute, Warrak El-Hadar, Imbaba, P.O. Box 30, Giza, 12411, Egypt; ³Faculty of Oral and Dental Medicine, Future University in Egypt (FUE), New Cairo, Egypt; ⁴Pharmaceutical Chemistry Department, National Organization for Drug Control and Research (NODCAR), P.O. Box 29, Giza, Egypt

*Corresponding author: ahmed_said5899@yahoo.com

**ABSTRACT**

Schistosomiasis (Bilharziasis) is a fatal parasitic disease caused by parasitic worms with the genus *Schistosoma*. The release of *Schistosoma* eggs in running fresh water contributes to completing its life cycle. Therefore, finding a suitable drug having ovicidal activity towards eggs is crucial. Here, we investigate the *in-vitro* effect of bee venom (the venom of *Apis millifera*) on the eggs of *Schistosoma mansoni* (*S. mansoni*). The eggs were incubated with different concentrations of bee venom and then the percent mortality, hatchability and morphology of the eggs were observed. It was found that bee venom causes morphological alterations for *S. mansoni* eggs. In addition, there is a critical concentration (100 µg/mL) at which bee venom leads to the lowest mortality and the highest hatchability percent. Below or above this threshold, the mortality increases and the hatchability decreases. Moreover, bee venom was proven to have a lethal effect on *S. mansoni* miracidia.

**Key words:** *Schistosoma mansoni*, Bee venom, Egg mortality, Hatchability, Morphological changes

**INTRODUCTION**

Schistosomiasis is a parasitic disease caused by blood-dwelling fluke worms of the genus *Schistosoma* (Colley et al., 2014). The World Health Organization (WHO) estimates that more than 249 million people have been infected with schistosomiasis resulting in approximately 280,000 deaths annually (Gryseels et al., 2006). One of the important species is *Schistosoma mansoni* in which the female lays ~300 eggs per day.

The produced eggs are usually deposited in the liver and intestine and can cause serious complications and death. For example, eggs always cause severe inflammations that lead to hepatic granuloma formation (Hams, Aviello, & Fallon, 2013) and may result in hepatocellular carcinoma in late stages.

*S. mansoni* eggs have unique elongated and large shapes with a size of 112–175 × 45-70 µm. In addition, the eggs are characterized with prominent lateral spines near their posterior ends. The presence of an eggshell surrounding the embryo (miracidium) protects it from toxic substances. Usually, the miracidium fills the whole space inside the egg (Teixeira et al., 2007; Candido et al., 2018; LoVerde, 2019).

Bee venom (BV) is recently used as a potential drug for many diseases due to its pharmacologically active molecules such as enzymes, peptides, amines and non-peptide components. It has been used to treat various diseases such as cirrhosis, rheumatoid arthritis, Alzheimer’s disease, atherosclerosis and Parkinson’s disease (Zhang et al., 2018). Recently, the effect of BV on *S. mansoni* infected mice showed a reduction in the total worm burden, numbers of immature eggs and egg count in the hepatic tissue. However, an increase in hepatic granuloma diameter was demonstrated which might be attributed to the high dosage of BV (Mohamed et al., 2016). We report here the influence of the venom of *Apis millifera* on *S. mansoni* eggs *in vitro* in terms of mortality and hatchability percent. In addition, changes in egg morphology were investigated.

**MATERIALS AND METHODS**

**Materials**

BV powder was obtained from Vaccera, Egypt. Rosewell Park Memorial Institute 1640 (RPMI-1640) medium (Lonza, Basel, Switzerland) supplemented with 1.0 mM sodium pyruvate, 2.0 mM glutamine, 200 µg/mL penicillin, 200 µg/mL streptomycin and 10% fetal calf serum (FCS) were purchased from Sigma Aldrich (St. Louis, MO, USA).

**Preparation of the eggs**

The eggs were obtained from the intestines of Syrian hamsters infected with 50±5 cercariae. The hamsters were...
euthanized for dissection and removal of the intestine. The intestinal tissues were minced with scissors and then suspended in 1.2% NaCl solution. Thereafter, the tissues were blended in Waring blender for 30 s at low speed and then the homogenate was placed in a crude sieve with size of 420 μm. The tissues were collected and the process was repeated at medium and high speeds. The filtrate was placed in stainless steel sieves with gradual size and rinsed with 1.2% NaCl solution using a spray apparatus with. The sieves with eggs were shaken to allow all the eggs to pass through the lowest sieve. A suspension of the eggs was prepared in cold 1.2% NaCl solution and finally the mature viable eggs were counted (Tucker et al., 2013).

In-vitro treatment of the eggs

The viable mature eggs were suspended in RPMI-1640 culture medium (1000 eggs/mL) as a control group. In addition, groups of 1 mL of RPMI-1640 culture medium each containing 1000 fresh eggs were prepared with different concentrations of BV (5, 10, 25, 100, 200 and 300 μg/mL) as treated groups. Then, the eggs were distributed in 96-well microtiter plates (40 μL/well) and incubated for 4 h at 37°C in a dark place. After incubation, the eggs suspension was centrifuged and then 1 mL of deionized water was added to the pellets. The plates were incubated under illumination for 1 h and examined under an Olympus XC50 CCD camera (Olympus, Tokyo, Japan) for mortality and hatchability after a 24-h incubation period with the different concentrations of BV.

Statistical analyses

The data were tested with Statistical Package for the Social Sciences (SPSS) version 25. Significant differences were expressed at (P<0.0001) by Student’s t-test, one-way analysis of variance (ANOVA) and Duncan’s post hoc.

RESULTS

The control group showed viable immature and mature eggs with normal structures. Concerning the group treated with 300 μg/mL of BV, the miracidia inside the eggs appeared hydrolyzed. In addition, some hatched eggs, dead miracidia (outside the egg) and dead eggs were observed. Interestingly, for 100 μg/mL of BV the eggs showed morphological alterations and the miracidia began to exit from the eggs (Fig. 1).

At low BV concentrations, empty eggs were found when the eggs were incubated in 10 or 25 μg/mL. In addition, the eggs showed a variety of changes such as deformations, morphological alterations and cracks. Even at very low concentration of 5 μg/mL, the number of empty eggs was apparently high (Fig. 1).

Regarding the hatchability of the eggs, it was clearly noticed that there is a threshold value for the concentration of BV where the hatchability was maximum at 100 μg/mL of BV, while at concentrations higher or lower than this value, the hatchability was diminished reaching zero at 5 and 300 μg/mL of BV. It is worthy to mention that the hatchability percent in all groups incubated with BV were lower than that of the control by ≥ 20% (Fig. 2).

DISCUSSION

Schistosomiasis is considered one of the most important parasitic diseases that threaten millions of people. The produced eggs cause the etiology of the disease and transport the parasite (Rizk & Aly, 2015). The S. mansoni eggs were classified into immature, mature, and dead. Dead eggs include dead mature with disintegrating miracidium, retracted miracidium and dead...
immune (darkened) (Sarvel et al., 2006). In the present study, high concentrations of BV (300 and 100 µg/mL) strongly affected the miracidia either by inducing hatching or death.

In one of the previous studies, the morphological alterations in eggs were affected by tamoxifen (15 µM) in an in-vitro experiment on S. mansoni eggs (Oliveira et al., 2019). Here, an obvious morphological change in egg shapes was influenced by 10 and 25 µg/mL of BV. Moreover, the lowest concentration (5 µg/mL) of BV led to miracidium escaping.

In another in-vitro study on S. mansoni eggs, the results showed that high curcumin concentrations significantly (P<0.05 to <0.0001) affected the viability and hatchability of the eggs (Rizk & Aly, 2015). BV showed the best (lowest) hatchability percent at 5 µg/mL. This may be due to its efficacy in hindering egg hatching and causing miracidium death/disintegration.

A different in-vitro study reported that the number of S. mansoni eggs were decreased (P<0.05 to P<0.0001) by phytol, a diterpene alcohol from chlorophyll, at sub-lethal doses (25 µg/mL) (de Moraes et al., 2014). The in-vitro anti-schistosomal activity of a natural plant component called piplartine (6.3 µM), an amide isolated from Piper tuberculatum (Piperaceae) was studied and showed a reduction in the produced number of eggs by 75% (Moraes et al., 2011). Egg production was also inhibited by a natural product extracted from Eremanthus goyazensis during cultivation of S. mansoni depending on drug concentration and exposure period (Barth et al., 1997).

From the above results, it is clear that BV significantly affects S. mansoni eggs by inducing egg mortality. Thus, BV has an ovicidal effect on S. mansoni eggs.

Conclusions
In the present study, we prepared different concentrations of BV solution in order to control the eggs of S. mansoni. The morphological alterations were observed and the hatchability and mortality percent were assessed. The prepared BV concentrations showed an obvious ovicidal effect on the eggs of S. mansoni. Currently, we are working on investigating the in-vivo effect of different BV doses on S. mansoni eggs.

REFERENCES