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Molluscicidal impacts of *Anagallis arvensis* aqueous extract on biological, hormonal, histological and molecular aspects of *Biomphalaria alexandrina* snails

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ABSTRACT

Controlling of *Biomphalaria alexandrina* snails by plant molluscicides is the cornerstone in treating schistosomiasis in Egypt. The objective of this study is, to evaluate the molluscicidal activity of the aqueous leaves extract of *Anagallis arvensis* against *B. alexandrina* snails. The present results showed that this aqueous extract was lethal for *B. alexandrina* snails at (LC_{50} 37.9 mg/l; LC_{90} 48.3 mg/l), and caused reduction in survival; reproductive rates and hormonal activity (testosterone (T) and 17β-estradiol (E)) of these snails. Histopathological changes occurred in the hermaphrodite glands of snails exposed to the sub lethal concentrations of this aqueous extract are detected, where, there were degeneration in both eggs and sperms and there were losses of connective tissues between acini. The present investigation revealed that this plant had a genotoxic effect especially with its concentration (LC_{10} and LC_{25}), where, the length of olive tail moment was significantly increased than control group. These observations prove the potent molluscicidal activity of aqueous leaves extract of *A. arvensis* against the intermediate hosts of *Schistosoma mansoni* and provide natural biodegradable resources for snails' molluscicidal agents.

1. Introduction

Schistosomiasis is a widespread neglected tropical parasitic disease transmitted by snails (WHO, 2017). Freshwater snails of *Biomphalaria* genus are the intermediate hosts of *Schistosoma mansoni* in Egypt (Ibrahim and Abdalla, 2017) and several strategies have been used to control snail populations through breaking the life cycle (El-Ghany and El-Ghany, 2017). Manufactured molluscicides is an imperative part in the incorporated schistosomiasis control programs (Abdel-Ghaffar et al., 2016), but because they have high cost and being poisonous to creatures of land and water (WHO, 2014), have stimulated interest to find suitable plant molluscicides (Elsareh et al., 2016).

Plant molluscicides are promising choices that may grow the scope of molluscicides accessible for controlling of *B. alexandrina* snails (Kiros et al., 2014), as these plants are cheaper, safer and having a high level of degradability (Salawu and Odaibo, 2011). *A. arvensis* is the name of Scarlet Pimpernel, with about 20–25 species of flowering plants in the family *Myrsinaceae* (Khoshkholgh-Pahlaviani et al., 2013). It is used in European traditional medicine for dermatological purposes (López et al., 2011). This plant is toxic to ruminants (Pande et al., 2016) at high doses and during long-term consumption (López et al., 2011). The bio active constituents of this plant have antibacterial, antifungal, antitumor, antidiabetic and hepatoprotective activities (Bruneton, 2001) and were found to be less toxic through the toxicity prediction tool. Also, *A. arvensis* has no toxicity on humans as it is used to treat diseases like Gout, Leprosy, Epilepsy and Urinary infection (Pande et al., 2016).

In Aquaculture, studies aimed to find a plant-based remedy for Saprolegniasis (Caruana et al., 2012), which is a pathogen causing “water mold” threatens the fresh-water breeding facilities by infecting fish eggs and fry and is caused by *Saprolegnia parasitica*. In recent study by Wirth and Stadtlander (2016), they tested the alcoholic and aqueous extracts of 29 plants that had antimicrobial properties *in vitro* against *S. parasitica*, they found that aqueous extracts were ineffective and that the concentration of 5000 ppm 70% ethanol extracts of Scarlet Pimpernel (*A. arvensis*) and rosemary (*Rosmarinus officinalis*) showed fungistatic effects in the first 24 h.

A. arvensis is considered one of the most promising molluscicidal plants and its saponin fraction has been reported to express high molluscicidal activity against schistosome intermediate snails (Abdel-Gawad et al., 2000; El-Sayed et al., 1990). The molluscicidal and cercaricidal properties of this plant are found in all its parts and the use of water suspension of its powder is an inexpensive means of controlling

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both snails and schistosome free larval stages (Elkhyat and Gawish, 2006). (Ibrahim et al., 1994) stated that the LC_{90} of the dry powder of three local plants namely *Anagallis arvensis*, *Agave lophantha* and *Bassia muruicata* tested in the laboratory against *B. alexandrina* snails were 50,100 and 165 ppm, respectively.

In two field trials carried out in Sharkia Governorate, Egypt (El-Emam et al., 1996), used relatively high concentrations of dry powder of *A. arvensis* to control vector snails of schistosomiasis and fascioliasis and used the concentrations of 125 and 100 ppm to induce death of *B. alexandrina* snails.

Also (Mosta-Fa et al., 2005), carried out semi-field trials under stimulated natural conditions to evaluate different modes of exposure to *A. arvensis* and *Calendula micrantha* as plant molluscicides and chemical molluscicides like baylucide and they found that the pre exposure to sub lethal concentrations of *A. arvensis* (55 ppm) increases the snail mortality in all experiments of bayluscide and *A. arvensis* than the other plant.

The biological response of an organism to xenobiotics starts with toxicant induced changes at the cellular and biochemical levels, leading to changes in the structure and function of the cells, tissues, physiology and behavior of the organism and these changes may affect the integrity of the population and ecosystem (Parvez and Raisuddin, 2005).

The endocrine system regulates hormone-dependent physiologic functions necessary for survival of the organism and the species (Hontela, 1998). An endocrine disruptor is an exogenous substance that affects the function of the endocrine system and causes deleterious effects in an organism, or its progeny (WHO, 2002). Some substances either of natural or man-made can cause endocrine disruption both *in vitro* and *in vivo* (Schug et al., 2011). The degree of this disruption depends on some parameters including reproductive stage dependent changes in steroid action, and whether the steroid action is genomic or non-genomic (Thomas, 2000).

The endocrine disruptions in terms of steroid levels (testosterone (T) and 17b-estradiol (E)) can be studied in *B. alexandrina* snail (Omran and Salama, 2016), because their hormonal system is comparable to that of vertebrates (Janer and Porte, 2007; Oehlmann et al., 2007). Certain compounds act as endocrine disrupters either by binding to the hormone receptors or modulating it, or by modulating endogenous hormone levels through interfering with biochemical processes associated with the production, availability, or metabolism of hormones (Oetken et al., 2004). Testosterone and estrogen hormones have an important role in the development of gonads in *B. alexandrina* (Omran, 2012). These snails can be used as a bio indicator for endocrine disrupters in terms of steroid levels (testosterone (T) and 17b-estradiol (E)), after exposure to sub lethal concentrations of any molluscicides (Omran and Salama, 2016). DNA damage produced by the androgens, testosterone (TES) and β-oestradiol in keratinocyte cell line can be examined by the Comet assay (Gopalan et al., 2006).

Comet assay is a sensitive method for direct visualization of DNA damage on the level of a single cell (Azqueta et al., 2009). Some recent studies link DNA strand breaks in aquatic animals to effects on the immune system, reproduction, growth, and population dynamics (Lee and Steinert, 2003; Shaldoum et al., 2016). So, in the current study, molluscicidal effects of *A. arvensis* plants were determined to study how it affects the biological system of *B. alexandrina* snails through evaluating its effects on biological, hormonal, histological and molecular parameters of these snails.

2. Materials and methods

2.1. Experimental animals (snails)

B. alexandrina snails (8–10 mm) provided from Medical Malacology Laboratory, Theodor Bilharz Research Institute (TBRI), Giza, Egypt were used. Snails were kept in plastic aquaria (16 × 23 × 9 cm). The aquaria were provided with dechlorinated aerated tap water (10 snails/L) and covered with glass plates. Oven dried lettuce leaves and blue green algae (*Nostoc muscorum*) were used for feeding and water in the aquaria was changed weekly. Pieces of polyethylene sheets were put into the aquaria to collect egg masses.

2.2. Plant materials

A. arvensis (Family *Myrsinaceae*) plants were collected from the shores of water courses in Aboelkhawy, El-beheira Governorate, Egypt during the spring of 2016. It was identified by Prof. Dr. Shadia Mohamed El- Dafrawy, Theodor Bilharz Research Institute, Giza, Egypt. The plants were shade dried, finely powdered using an electrical grinder. The dry powder of the experimental plants was weighed and added directly to filtered water in individual beakers.

2.3. Molluscicidal screening

To calculate LC_{50} and LC_{90}, series of concentrations were prepared on the basis of weight/volume as water extract of leaves powder (50, 40, 30, 20, 10 mg/l) and ten *B. alexandrina* snails (8–10 mm) were placed in beakers for each concentration (Litchfield and Wilcoxon, 1949). Another snail group of the same size was dipped in dechlorinated water only as control. Three replicates were used, each of 10 snails, for each concentration. The exposure period was 24 h, then, the snails were removed from the experimental test solution, and washed thoroughly with dechlorinated tap water and transferred to containers with fresh dechlorinated tap water for another 24 h of recovery, and then, the percentages of observed mortalities were recorded. LC_0 is the concentration of a toxicant, below which no measurable effects take place (Warren, 1900), and is estimated as 1/10 LC_{50} value (WHO, 1965).

2.3.1. Effect on snails' egg-laying capacity of adult snails

B. alexandrina snails (8–10 mm) were exposed for 24 h/day for 2 weeks to the concentrations LC_0, LC_{10}, and LC_{25} of the herbicide. Three replicates, each of 10 snails/L, were prepared for each concentration, another group considered as control group was maintained in dechlorinated water, the following parameters were weekly recorded: Lx (the survival rate as a proportion of the correct one), Mx (the number of eggs/snail/week) and R_0 (the reproductive rate which is the summation of LxMx during the experimental period) (El-Gindy et al., 1965).

2.3.2. Biochemical assays for steroid hormones

Ten snails (8–10 mm) were subjected to each sub lethal concentrations (LC_0, LC_{10} and LC_{25}) of the tested plant for 24 h (exposure), followed by another 24 h of recovery for two weeks. Three replicates of each concentration were prepared. Unexposed snails (control) were assayed side by side with the experimented groups. Snail's shell was gently crushed between two glass slides and digestive gland was dissected out and pooled in 1 ml Eppendorf tube. The tissues of snails from each group were weighed and then homogenized in ice cold, twice-distilled water (1 g tissue/5 ml water) using a glass Dounce homogenizer. The homogenates were centrifuged at 3000 rpm for 10 min at 4 °C and the supernatants were stored at −80 C until used. Hormone concentrations (testosterone (T) and 17b-estradiol (E)) were assayed according to the manufacture instructions of T EIA kit (Enzo Life Science, Michigan, USA, ADI-900-065) and E EIA kit (Cayman Chemical Company, Michigan, USA, item no. 582251).

2.3.3. Histological study

Adult snails (8–10 mm) were exposed to the aqueous extract of plant leaves at (LC_0, LC_{10} or LC_{25}) for 24 hours/week for 2 successive weeks (exposure), then, the snails were removed from the experimental test solution, and washed thoroughly with dechlorinated tap water, and transferred to containers with fresh dechlorinated tap water for another 24 h of recovery, and this was done for two weeks. Changes in histology