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Ultrastructure and histopathological effects of some plant extracts on digestive gland of *Biomphalaria alexandrina* and *Bulinus truncatus*

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KEYWORDS

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Abstract The present investigation is concerning with studying the histopathological effects of three Egyptian wild plant-extracts, as botanic toxic agents; namely *Euphorbia splendens* (Euphorbiaceae), *Ziziphus spina-Christi* (Rhamnaceae) and *Ambrosia maritima* (Asteraceae) on the digestive gland of freshwater snails *Biomphalaria alexandrina* and *Bulinus truncatus* (uninfected and infected with *Schistosoma mansoni* and *Schistosoma haematobium*). In addition, ultrastructural studies carried out using the resulted effective plant against non-infected *B. alexandrina* and *B. truncatus* snails. According to the present results, *E. splendens*-plant was the most effective plant on the target two snails was *E. splendens* followed by *Z. spina-chriti* and the least molluscicidal activity was for *A. maritima*. Moreover, the susceptibility of *B. alexandrina* to the present target plants is higher than those of *B. truncatus*. The histopathological alterations due to the present three plants on the digestive gland of target snails included, cytoplasmic vacuolization and fragmentation and accumulation of the toxic agents of the target plants inside cytoplasm of digestive and excretory cells.

Concerning histopathological effects of the present plants on digestive gland of infected-target snails, they showed numerous vacuoles in digestive and excretory cells. On the other hand, ultrastructural examinations due to the most effective plant "*E. splendens*" revealed cytoplasmic fragmentation in excretory cells and accumulation of the toxic agents of the plants inside digestive cells. Finally, it was recommended that the application of LC_{90} of *E. splendens*-extract in a trial to open new areas of application of extract of this plant as eco-friend molluscicide.

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Introduction

Schistosomiasis remains as one of the world's most prevalent diseases (King and Dangerfield-Cha, 2008). Approximately tenth of the world population are living with the risk of infection (WHO, 2010). In Egypt, the disease is not only a prime health problem, but it affects millions of farmers at the early age diminishing their productivity and exerting a serious

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socioeconomic problem (El-Baz et al., 2003). Today, molluscicide is regarded as an important aggressive strategy in the control of the snail hosts of these diseases (Giovanelli et al., 2001; Mello-Silva et al., 2006). The use of plants with molluscicidal properties appears to be a simple, inexpensive and safe alternative (Singh and Singh, 2010; Al-Daihan, 2010). In Egypt, several local plant species screened and proved to have molluscicidal properties against different snail species, from which, *Ambrosia maritima* (Abou Basha et al., 1994), S*olanum*-species (Tantawy et al., 2000) *Euphorbia splendens* (El-Karemy, 2008; Bakry, 2009, Singh and Singh, 2010; Hassan et al., 2011). According to Mwine (2011) a good number of *Euphorbia*-species are actually potent as medicinal plants and their extracts have been isolated and patented as modern drugs. Recent studies indicated that the euphorbiales have molluscicidal activity (Tantawy et al., 2004; Sermsart et al., 2005, Mello-Silva et al., 2006, Bakry 2009; Singh and Singh, 2010; Hassan et al., 2011).

Alkaloids and saponins are reported among active compounds of *Euphorbia*-species (Siddiqui et al., 2009).

Ziziphus spina-christi is one of the plants that used in Egypt for treatment of different diseases (Mello-Silva et al., 2006; Nawash and Al-Horani, 2011). The phytochemical composition of *Ziziphus spina-cristi* reported the presence of four saponin glycosides and alkaloids as molluscicides (Shahat et al., 2008; Anthony, 2005).

Belot et al. (1993) declared that *Ambrosia maritima* plant is toxic to snail intermediate hosts of schistosomiasis. (Belot et al., 1993). Abdelgaleil (2010) isolated the sesquiterpenes; neoambrosin, damsinic acid, damsin, ambrosin and hymenin from *A. maritima* that have molluscicidal activities.

Histologically, Bakry (2009) studied changes following exposure to three plants; *Euphorbia splendens*, *Guayacum officinalis* and *Atriplex stylosa* on the digestive gland of *B. alexandrina*-snails that caused a great damage, included vaculations, disappearance of secretory cells from the digestive tubules as well as connective tissue between shrinked acini was damaged.

It is now well established that in many plants, the molluscicidal activity is due to the presence of saponins and alkaloid components (Singh and Singh, 2010). Based on these facts and since *E. splendens*, *A. maritima* and *Z. spina*-christi have been described as plants rich in saponins and/or alkaloids.

Thereby, the present study is aimed to evaluate the molluscicidal activity of the present target plants against digestive gland of *B. alexandrina* and *B. truncatus*-snails that are the intermediate hosts of *Schistosoma mansoni* and *S. haematobium*, respectively in a trial to open new areas of application of extracts of these plants as eco-friend molluscicides.

Materials and methods

Tested snails

The non-infected and infected fully grown adult snails of *B. alexandrina* and *B. truncatus*, with *Schistosoma mansoni* and *S. haematobium*, were established from laboratory colonies provided from Theodor Bilharz Institue Imbaba, Giza, Egypt, through July 2011. Large stock colonies of these snails were reared under the laboratory conditions in glass aquaria filled with aerated de-chlorinated tap water. The snails were fed on lettuce leaves.

Tested plants

The three native plants; *E. splendens*, *Z. spina-Christi* and *A. maritime*; that obtained from a folk market at Cairo city "as dried leaves", had been identified by Department of Botany, Faculty of Science, Ain Shams University.

Preparation of plant extracts

The preparation of the present plants had been achieved according to Bakry (2009). The target plants were overdried in an electric oven, adjusted at 50 °C for three days. Then, ethanol (70%) and plant-leaves had homogenated, filtered and evaporated in adjusted electric oven at 70 °C to obtain gum-shaped substance. The obtained residues were dissolved in distilled water, at ratio 1:5 by volume. Finally, the obtained plant extracts were used to prepare all aqueous concentrations to determine LC_{90} of each plant extract.

Molluscicidal evaluation of different plant extracts

All bioassay experiments were carried out under laboratory controlled conditions according to methods of Abbott (1925) and Finney (1971).

To determine LC_{50} and LC_{90} of the target plants, four concentrations (100 snails/concentration) (150, 300, 450, and 600 ppm) of the plants were prepared for 48 h (Abbott, 1925).

Regression mortality lines for tested concentrations and the corresponding mortalities were established on log dose-mortality sheets. LC_{90} values for the three tested plant extracts had calculated according to Finney' method (1952).

Histopathological technique

Routine histological technique was achieved where healthy normal (untreated) snails had used as control. The shell of the snails were carefully broken under a binocular dissecting microscope and soft-body parts were removed and then digestive gland as well as foot of infected target snails separated and placed in the fixing fluid (Carnoy's fixative that composed of 60% methanol, 30% chloroform, and 10% glacial acetic acid, time of fixation for at least 24 h). The fixed samples of snail tissues were dehydrated in ascending series of ethanol and cleared in methyl benzoate for 6–8 h, followed by washing in two changes of benzene, each for two minutes, followed by infiltration with paraplast-wax.

Preparation of samples

Samples of snails were divided into two groups; the first control snail-group and the second group of snails that exposed to LC_{90} of the target plants for 48 h.

Preparation of stained-slides

The paraplast sections (6 μ-thickness) had de-paraplastinized, hydrated to water and stained with Haematoxylin–Eosin solutions (Haematoxylin for 15 min and 1% eosin for 30 s). Stained sections were dehydrated, cleared and mounted in DPX. The stained slides had inspected microscopically (using Carl Zeiss-microscope, Germany). The series of foot-sections of infected target snails had inspected to reveal sporocysts.

Transmission electron microscope (TEM) technique

The present technique had been achieved according to (Reynolds, 1963). This technique included: anesthetizing the target snails with 30% ethyl alcohol, dissection to obtain the digestive gland and cutting it into small pieces, fixing with 2.5% paraformaldehyde-3% glutaraldehyde (pH 6.7) and post-fixed with (1%) phosphate buffered OsO_4 for one hour "for the first five minutes, fixation was carried out at 23 °C after which the specimens were placed in water bath at 4 °C". Then, rinsing of specimens in 0.2 M phosphate buffer (pH 7.3), dehydration in ethyl alcohol and embedding in Epon 812 mixture. Thick sections "5 μm" for light microscopy and thin sections for transmission electron microscopy were prepared using both glass and diamond knives on LKB Nova ultra-microtome. The specimens were stained with freshly lead citrate and uranyl acetate. Bright-field and NIC photomicrographs were taken with Olympus BHS microscope. Transmission electron micrographs were taken using TEM (JEM 100CX II transmission electron miscoscope operated at 80 kV).

Results

According to the present results, *E. splendens*-plant was the most effective plant on the target snails followed by *Z. spina-chriti* and the least activity was for *A. maritima*. The LC_{90} of *E. splendens* against *B. truncatus* were 42.871 and 51.12 ppm against *B. alexandrina*, *Z. spina-chriti* against *B. truncatus* were 82.2 and 107.694 ppm against *B. alexandrina* and *A. maritime* against *B. truncatus* is 384.33 and 455.2 ppm for *B. alexandrina*) and these data indicate that *E. splendens*-plant was the most effective plant on the target snails (Table 1).

The digestive gland of the non-treated "control" of the present snails composed of acini that lined by two types of cells; digestive and excretory cells (Figs. 1 and 5). On the other hand, the feet of the infected target snails have obvious sporocycts (Figs. 9 and 10).

The histopathological alterations due to the present plants on the target non-infected snails included cellular swelling and vacuolation in excretory cells and cytoplasmic fragmentation and accumulation of the toxic agents of the target plants inside both digestive and secretory cells of the two target snails (Figs. 2–4 and 6–8). On the other hand, histopathological effects on digestive gland of infected-target snails with *Schistosoma mansoni* and *S. haematobium* showed obvious vacuolation in digestive and excretory cells (Figs. 11 and 12).

Fig. 13 displaying the ultrastructural examination of part of digestive gland-acinus of non-treated "control" of *B. Alexandrina*. It shows adjacent digestive cells with cytoplasmic density and elongated excretory cells and well developed lysosome that composed of tubulo-vesicular endosomes. Concerning the cytological effects of the effective plant "*E. splendens*", Figs. 14 and 15 revealed vacuolation in secretory cells and accumulation of the toxic agents of the target plants inside the digestive

Table 1 LC_{90}, slope function and X^2 data for the three target plants *E. splendens*, *Z. spina-christi* and *A. maritima* against the snails *B. alexandrina* and *Bulinus truncates*.

Plant extract	LC_{90}		Slope	X^2
	B. alexandrina	*B. truncatus*		
E. splendens	51.120	42.871	1.52	2.41
Z. spina-christi	107.694	82.200	1.21	0.33
A. maritima	455.200	384.330	1.41	1.49

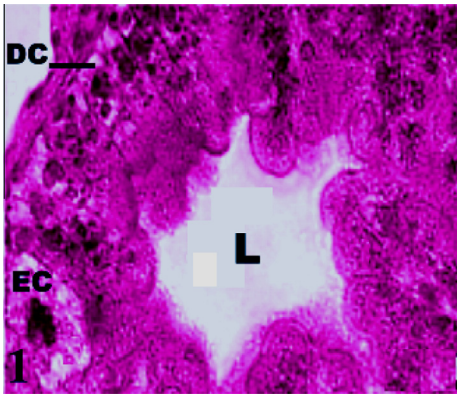

Figure 1 Illustrating part of digestive gland of untreated *B. alexandrina* snail (X = 500). [L, Lumen; DC, digestive cell; EC, excretory cell.]

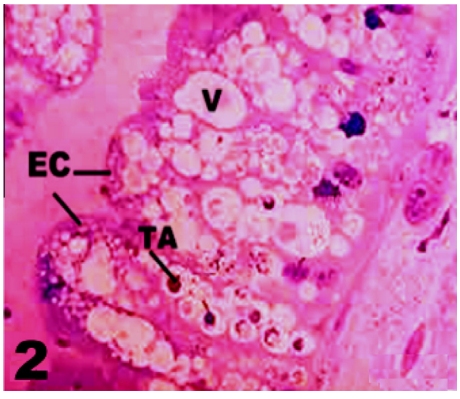

Figure 2 Displaying part of digestive gland of treated snail *B. alexandrina* with LC_{90} of *A. maritime*-extract (X = 1250). [TA, toxic agent; V, vacuole; EC, excretory cell.]

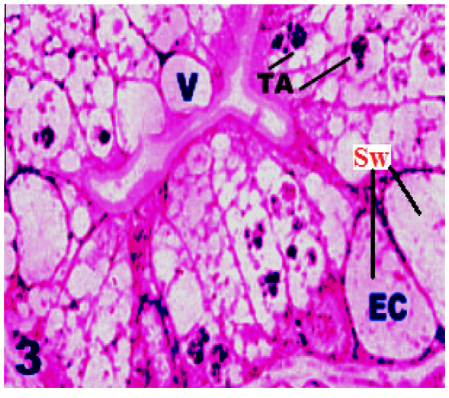

Figure 3 Revealing part of digestive gland of treated snail *B. alexandrina* with LC_{90} of *Z. spina-christi*-extract (X = 1250). [TA, toxic agent; V, vacuole; EC, excretory cell; Sw, swelling.]

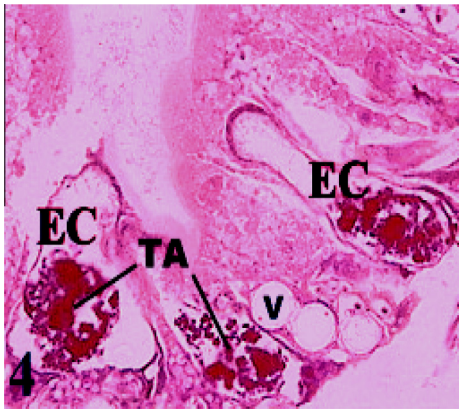

Figure 4 Showing part of digestive gland of treated snail *B. alexandrina* with LC_{90} of *E. splendens*-extract ($X = 1250$). [TA, toxic agent; V, vacuole; EC, excretory cell.]

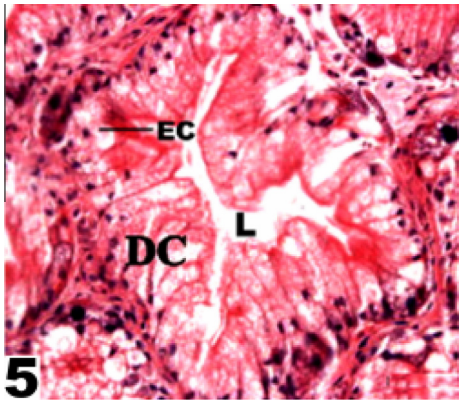

Figure 5 Illustrating complete acinus of digestive gland of untreated *B. truncatus* snail ($X = 500$). [L, lumen; DC, digestive cell; EC, excretory cell.]

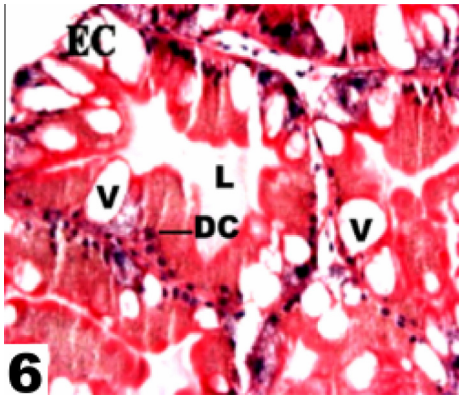

Figure 6 Displaying part of digestive gland of treated snail *B. truncatus* with LC_{90} of *A. maritime*-extract ($X = 500$). [DC, digestive cell; EC, excretory cell; V, vacuole.]

cell beside scattered cytoplasmic vacuoles and excess excretory granules or residual bodies in the excretory cells.

Figure 7 Revealing part of digestive gland of treated snail *B. truncatus* with LC_{90} of *Z. spina-christi*-extract ($X = 1250$). [TA, toxic agent; V, vacuole; EC, excretory cell; Sw, swelling.]

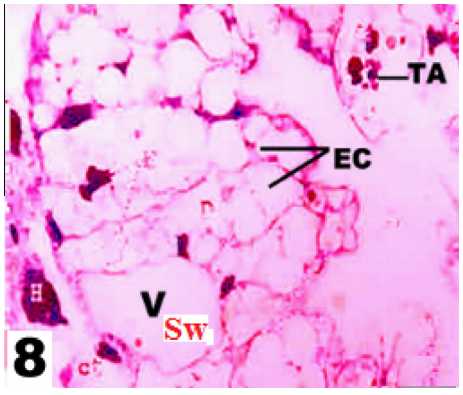

Figure 8 Showing part of digestive gland of treated snail *B. truncatus* with LC_{90} of *E. splendens*-extract ($X = 1250$). [TA, toxic agent; V, vacuole; EC, excretory cell; Sw, swelling.]

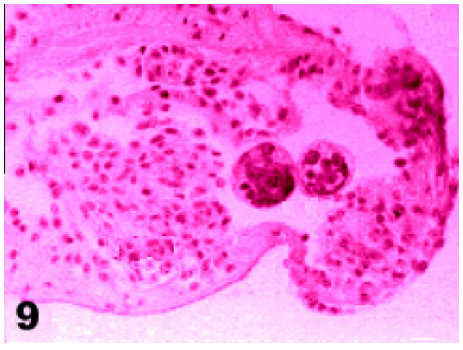

Figure 9 Histological section of sporocysts in foot-tissue of *B. truncatus* ($X = 200$).

Discussion

Schistosomiasis is worldwide parasitic diseases infecting 207 and 17 million people respectively causing significant morbidity and mortality (WHO, 2010).

The use of *Euphorbia splendens* plays vital role in controlling schistosomiasis Bakry (2009). The plant is commonly

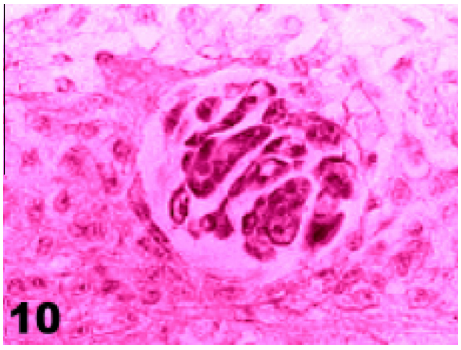

Figure 10 Histological section of sporocyst in foot-tissue of *B. alexandria* ($X = 500$).

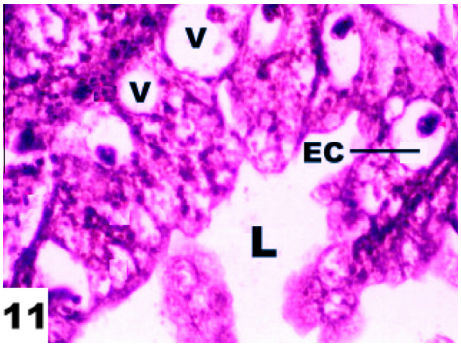

Figure 11 Histological section of part of digestive gland of infected *B. alexandrina* snail ($X = 1250$). [L, lumen; V, vacuole; EC, excretory cell.]

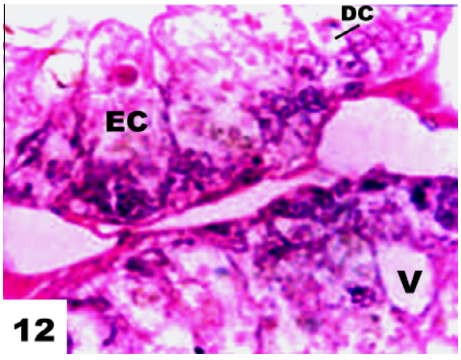

Figure 12 Histological section of part of digestive gland of infected *B. truncatus* snail ($X = 1250$). [DC, digestive cell; V, vacuole; EC, excretory cell.]

available, easy to collect and prepare for use. Therefore, it is the most suitable for biological application which offers a potentially simple, readily available and inexpensive molluscicidal agent of plant origin. In future, more attention should be paid to the mechanism of action of *Euphorbia splendens* on molluscs and application techniques for its use as plant molluscicides in rural communities. In this respect, the present study demonstrated that the extract of *Euphorbia splendens* possesses the highest molluscicidal activity. These results are in harmony with Mello-Silva et al. (2006) and Bakry (2009)

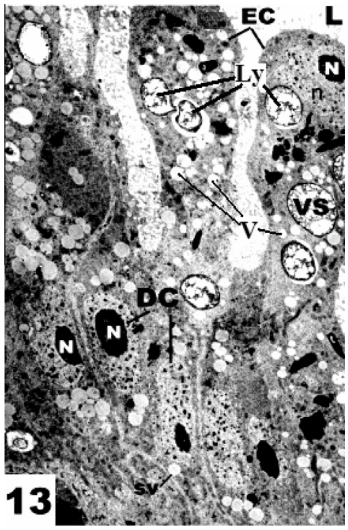

Figure 13 Transmission Electron Microscopy (TEM) of part of digestive-acinus of non-treated *B. alexandrina* (DC, digestive cell; EC, excretory cell; N, nucleus; V, vacuole; Ly, lysosome; L, lumen; VS, vesicle).

Figure 14 TEM of digestive-acinus, including digestive and excretory cells, of non-infected and treated *B. alexandrina* with LC_{90} of *E. splendens* (DC, digestive cell; EC, excretory cell; TA, toxic agent; V, vacuole; mv, brush border microvilli).

who revealed the molluscicidal activity of different *Euphorbia*-species with varying degrees of potency.

The recent study of Hassan et al. (2011) demonstrated that *Ziziphus spina-christi* has less potent molluscicidal activity than *Euphorbia aphylla* against is up to 1000 ppm on *B. alexandrina*. However, the present LC_{90} of *Euphorbia splendens* was comparatively lower "107.694 ppm". Abou Basha et al. (1994)

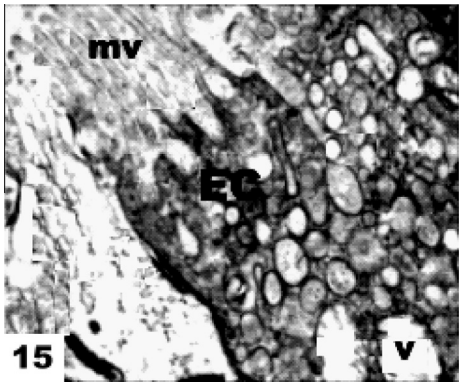

Figure 15 TEM of part of digestive-acinus, including excretory cells, of non-infected and treated *B. truncatus* with LC_{90} of *E. splendens* (EC, excretory cell; V, vacuole; mv, brush border microvilli).

declared that the LC_{90} of *Ziziphus spina-christi* against *B. truncatus* were 500 ppm although the present result scored LC_{90} equal 82.2 ppm on the same species.

A. maritima have been used for the control of schistosome intermediate hosts at 150–300 ppm (Belot et al., 1993). The present LC_{90} of the same plant "455.2 ppm" is nearly equal with these authors on another species of *Biomphalaria* "*B. pfeifferi*".

The reason of the wide variety in susceptibility between present results and other authors may be attributed to the natural resistance of different snail's genera and that the molluscicides may vary in their toxicological effects according to the species of the snails' used.

The present histopathological alterations due to the target plants on the two snails, especially the most effective *E. splendens*-plant, included vacuolation, cytoplasmic fragmentation and accumulation of the toxic agents in the cytoplasm of digestive and excretory cells. On the other hand, histopathological effects on digestive gland of infected-snails with *Schistosoma mansoni* and *S. haematobium* showed obvious vacuolation in digestive and excretory cells. In this respect, Bakry (2009) studied changes following exposure to three plants; *E. splendens*, *Guayacum officinalis* and *Atriplex stylosa* on the digestive gland of *B. alexandrina* and he found that *E. splendens* was the effective plant. In addition, he achieved phytochemical investigations to identify the bioactive ingredient responsible for the molluscicidal potency of *E. splendens*.

In addition, Mello-Silva et al. (2006) had been cytologically studied the molluscicidal effects of *E. splendens*-extract on *Biomphalaria glabrata* and found that lethal dose for 24 h of *E. splendens* var. *hislopii* is the most promising because it meets the recommendations of the World Health Organization (WHO) and their results reinforced the present result on the same plant. The results also showed a sharp reduction in the reserves of glycogen in the digestive gland.

Then, Hamed et al. (2007) studied the ultrastructural changes induced by two carbamate molluscicides on the digestive gland of the snail *Eobania vermiculata* and found that severe cytoplasmic vacuolization, disruption and reduction of microvilli and excess excretory granules or residual bodies in the excretory cells.

It was recommended that the application of LD_{90} of *E. splendens*-extract in a trial to open new areas of application of extract of these plant as eco-friend molluscicide.

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