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ISSN 2277 - 1921
Article type Full Length Research Article
Submission date 24 June 2012
Acceptance date 30 June 2012
Publication date 15 July 2012
Article URL http://www.crdeep.com/category/ijbas

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Evaluation of The Ethanolic Extracts Of Three Plants For Their Molluscicidal Activities Against Snails Intermediate Hosts of Schistosoma Mansoni And Fasciola

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Abstract

Schistosoma mansoni and fascioliasis are worldwide parasitic diseases infecting 83 and 17 million people respectively causing significant morbidity and mortality. The present work was carried out to evaluate the molluscicidal activities of the ethanoic extract of three plant species namely Euphorbia aphylla, Ziziphus spina-christi and Enterolobium contortisiliquum against Biomphalaria alexandrina and Lymnaea cailliaudi (natalensis), the snails intermediate hosts of Schistosoma mansoni and Fasciola respectively. The highest molluscicidal activity was recorded for Euphorbia aphylla on both snails’ species. The least activity was recorded for Enterolobium contortisiliquum which gave negative results against both snail species up to 1000 ppm. The LC₅₀ and LC₉₀ of the ethanolic extract of Euphorbia aphylla against Biomphalaria alexandrina snails were 87.6 ppm and 142.5 ppm respectively while the LC₅₀ and LC₉₀ of Euphorbia aphylla against Lymnaea cailliaudi snails were 0.66 ppm and 0.88 ppm respectively. The LC₅₀ and LC₉₀ of Ziziphus spina-christi ethanolic extract against Lymnaea cailliaudi snails were 311 ppm and 500 ppm respectively. However it showed no molluscicidal activity against Biomphalaria alexandrina snails up to 1000 ppm. The histopathological changes in hermaphrodite gland of B. alexandrina and Lymnaea cailliaudi snails 2 weeks post exposure to LC₅₀ of the ethanolic extract Euphorbia aphylla and the histopathological effect of LC₉₀ of the ethanolic extract Ziziphus spina-christi on Lymnaea cailliaudi snails were studied. Degenerative changes in hermaphroditic acini and their contents of ova and sperms were observed. The purification of active compounds present in Euphorbia aphylla and Ziziphus spina-christi plants may offers an alternative tool for the control of snails’ intermediate hosts of Schistosoma mansoni and Fasciola. Further investigations for Enterolobium contortisiliquum may increase its potential use as plant molluscicide are recommended.

Key words: Euphorbia aphylla, Ziziphus spina-christi, Enterolobium contortisiliquum, molluscicides, Biomphalaria and Lymnaea.

Introduction

Schistosoma mansoni is endemic in 54 countries. It is estimated to infect more than 83 million people worldwide (Standley et al., 2010 and Mitiku et al., 2010). In the developing world including Egypt, intestinal schistosomiasis is common, recurrent and long-lasting health problem (Sadek et al., 2008). Biomphalaria alexandrina as specific intermediate host of Schistosoma mansoni is prevalent in both Upper and Lower Egypt (WHO, 2002).

Fascioliasis is a worldwide zoonotic disease infects over 17 million people causing significant morbidity and mortality (Mas-Costa et al., 2005 and WHO, 2006). In Egypt, fascioliasis becomes hyperendemic and problematic where animal reservoir and snail vector are available (Rashed et al., 2008). Nearly 24 million Egyptians are at risk and about 800 000 suffering from fascioliosis (WHO, 1995 and Haseeb et al., 2002). Human infection causes serious hepatic pathological consequences (Soliman, 2008).

Treatment of Schistosoma and Fasciola infections remains highly problematic. Praziquantel developed drug resistance to Schistosoma strains with serious side effects while treatment of Fasciola requires high or multiple doses of drugs with frequent side effects (Ismael et al., 1999 and Abdul-Samie et al., 2010). Therefore snail control is considered essential in Schistosome and Fasciola control (Mello-Silva et al., 2006 and Jigyasu and Singh, 2010). Due to the hazardous environmental affects of niclosamide, its toxicities to non-target organisms and even man, the search for alternative safe molluscicides is still ongoing (WHO, 2002 and Abdelraezek et al., 2007). During recent years much attention has drawn for the use of molluscicides of plant origin. The use of plants with molluscicidal properties appears to be a simple, inexpensive and safe alternative (Tantawy et al., 2004, Bakry and Hamdi, 2007, Al-Daihan, 2010 and Singh and Singh, 2010).

In Egypt, several local plant species screened and proved to have molluscicidal properties against different snail species e.g. Ambrosia maritima and Conmiphora molmo) against Lymnaea cailliaudi snails (Abou Basha et al., 1994 and Allam et al., 2001), Solanum species, Guayacum officinalis, Calatropis procera and Euphorbia splendens against Biomphalaria alexandrina snails (Tantawy et al., 2000 and Bakry, 2009) and Conmiphora molmol against Biomphalaria alexandrina, Biomphalaria arabica, Bulinus truncatus and

It is now well established that in many plants the molluscicidal activity is due to the presence of saponin contents (Rawi et al., 1996, Osman et al., 2007 and Singh and Singh 2010) and alkaloid components (Melendez and Capriles, 2002, El-Ansary et al., 2001, Ahmed and Rifaa 2005, Silva et al., 2005 and Singh et al., 2010). Euphorbia aphylla (Euphorbiaceae), Ziziphus spina-christi (Rhamnaceae) and Enterolobium contortisiliquum (Fabaceae) have been described as plants rich in saponin and/or alkaloids (Hostettmann et al., 1982, Shahat et al., 2001, Mimaki et al., 2004, Anthony, 2005, Osman et al., 2007 and Siddiqui et al., 2009).

Euphorbia (Euphorbiaceae) is the largest genus of flowering plants in the Egyptian flora (El-Karemy, 2008). Over the past twenty years, they have received considerable phytochemical and biological attention (Wu et al., 2009). 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 for the treatment of intestinal parasites (Appendino and Szallasi, 1997 and Shi et al., 2008). They also possess antiamaebic (Tona et al., 2000), anti-plasmodial (Tona et al., 2004) and anti-leishmanial activity (Ahmed et al., 2006). Earlier studies indicated that the euphorbiales have molluscicidal activity (Tantawy et al., 2004, Sermsart et al., 2005, Bakry 2009 and Singh and Singh, 2010).

Ziziphus spina-christi (Rhamnaceae) is one of the plants most commonly used in Egyptian folk for treatment of different diseases (El-Rigal et al., 2006 and Nawash and Al-Horani, 2011). Different extracts of Ziziphus spina-christi showed anti-schistosomal activity (Aly and Aly, 2006 and El-Rigal et al., 2006) and anti-leishmanial activities (Tonkal et al., 2005).

Enterolobium contortisiliquum is an important species of the family Fabaceae. The essential oil of Enterolobium contortisiliquum seeds had been reported to have an antimicrobial activity (Shahat et al., 2008).

The present study aims to evaluate the molluscicidal activity of the ethanolic extracts of Euphorbia aphylla (Euphorbiaceae), Ziziphus spina-christi (Rhamnaceae) and Enterolobium contortisiliquum (Fabaceae) against Biomphalaria alexandrina and Lymnaea cailliaudi (natalensis), the snails’ intermediate hosts of Schistosoma mansoni and Fasciola species respectively, as well as their histopathological effect on hermaphrodite glands of the previously mentioned snails in a trial to apply these plant extracts as eco-friendly molluscicides.

Materials and Methods

Snail materials

Laboratory bred uninfected adult Biomphalaria alexandrina snails (6-8mm in diameter) and Lymnaea cailliaudi (natalensis) (8-10mm in shell length) from Schistosome Biological Supply Program Unit (SBSP), Theodor Bilharz Research Institute (TBRI), Giza, Egypt were used.

Plant Material

The plants used in this study were Euphorbia aphylla (Family Euphorbiaceae), Ziziphus spina-christi (Family Rhamnaceae) and Enterolobium contortisiliquum (Family Fabaceae). The plant materials were collected locally from Faculty of Agriculture, Assiut University. Plant species was kindly identified and extracted by Prof. Dr. Zedan Z. Ibrahim, Pharmacognacy Department, Faculty of Pharmacy, Assiut University. Voucher specimens of each plant were kept in the Museum of Pharmacognacy Department, Faculty of Pharmacy.

The aerial parts of Euphorbia aphylla, Ziziphus spina-christi and the mature ripe fruit of Enterolobium contortisiliquum were cleaned. Each plant was cut into small pieces and dried in shade then grounded using blender. About 250 g of air dried powdered plant material was extracted with ethanol (70%), filtered and distilled off under vacuum at temperature not exceeding 50 °C and the residues were stored in dry glass bottles according to (Bakry, 2009).

Determination of molluscicidal activity

The efficacy of the ethanolic extracts of the plants was primarily determined against the adult snails using the standered method of (WHO, 1965 and Singab et al., 2006). Thus one liter of each plant material at a concentration of 20 ppm was prepared and 10 snails were added.

The snails were maintained in the solution for 24 hours at 25°C ± 2°C. After exposure the snails were thoroughly washed and transferred to dechlorinated fresh water for another 24 hours for recovery. Three replicates were carried out and three groups of snails were maintained in dechlorinated fresh water under the same experimental conditions as negative control.

The Three plant extracts were retested by the same method using various concentrations to give mortalities between 0-100 percent Snails were considered dead if they probed and remained motionless or if the shell looked discoloured. Mortality rates were recorded at the end of recovery period.

Probit regression analysis (SPSS version 7) was used for calculating LC50 and LC95 values as well as their 95 % confidence limits according to Finney (1971).

Histopathological Study

The histopathological changes in the hermaphrodite gland of Biomphalaria alexandrina snails exposed to LC25 (58.7ppm) of the ethanol extract of Euphorbia aphylla for 2 weeks were studied. Also Lymnaea cailliaudi (natalensis) snails exposed to LC25 of Euphorbia aphylla (0.55ppm) and Ziziphus spina-christi (212 ppm) separately for 2 weeks. Three groups 10 snails each were used for tested plants. Another three groups

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were kept in dechlorinated fresh water for the same period as negative controls (Bakry, 2009). The hermaphrodite gland of treated and control snails were removed from their shells and processed for histopathological study (Harris, 1990).

**Results**

**Molluscicidal activity of Euphorbia aphylla**

The effect of various concentrations of the ethanolic extract of the aerial portion of *Euphorbia aphylla* on adult snails of *Biomphalaria alexandrina* and *Lymnaea cailliaudi (nalatensis)* snails after 24 hour exposure were evaluated (tables 1 and 2).

The LC\(_{50}\) and LC\(_{90}\) of this extract against *Biomphalaria alexandrina* snails after 24 hour exposure were 87.6 and 142.5 ppm respectively. While the LC\(_{50}\) and LC\(_{90}\) of the same extract against *Lymnaea cailliaudi (nalatensis)* snails after 24 hour exposure were 0.66 and 0.88 ppm respectively (table 4).

There was a significant difference between molluscicidal activities of the ethanolic extract of *Euphorbia aphylla* against both snails. *Lymnaea cailliaudi (nalatensis)* snails were more sensitive to *Euphorbia aphylla* extract than *Biomphalaria alexandrina* snails.

The probit mortality showed that the response of the two snail species illustrated a linear relationship with the different concentrations (dose/ppm) (fig.1, 2.).

**Molluscicidal activity of Ziziphus spina-christi**

Molluscicidal effect of the ethanolic extract of the aerial portion of *Ziziphus spina-christi* on *B. alexandrina* snails gave negative results up to 1000 ppm.

The effects of various concentrations of ethanol extract of the aerial portion of *Ziziphus spina-christi* on *Lymnaea cailliaudi (nalatensis)* snails after 24 hour exposure were evaluated (table 3). The LC\(_{50}\) and LC\(_{90}\) of the ethanolic extracts *Ziziphus spina-christi* on *L. cailliaudi* snails were 311ppm and 500 ppm respectively (table 4). The probit mortality showed that the response of *L. cailliaudi* snails illustrated a linear relationship with the concentrations (dose/ppm) (fig. 3).

**Molluscicidal activity of Enterolobium contortisilicicum**

The ethanolic extract of the mature ripe fruit of *Enterolobium contortisilicicum* gave negative results against both snail species up to1000 ppm.

<table>
<thead>
<tr>
<th>Conc. (ppm)</th>
<th>Number of tested snails</th>
<th>Number of dead snails</th>
<th>Mortality rates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>30</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>100</td>
<td>30</td>
<td>12</td>
<td>40</td>
</tr>
<tr>
<td>50</td>
<td>30</td>
<td>9</td>
<td>30</td>
</tr>
<tr>
<td>40</td>
<td>30</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>20</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 1:** Mortality rates of ethanolic extract of *Euphorbia aphylla* against *Biomphalaria alexandrina* snails.

The probit transformed responses showed a linear relationship with the concentrations (dose/ppm) (fig. 3).

**Figure 1:** Dose/probit regression line of *Euphorbia aphylla* on *Biomphalaria alexandrina* snails.
**Table 2:** Mortality rates of the ethanolic extract of *Euphorbia aphylla* against *Lymnaea cailliaudi* (*nlatensis*) snails.

<table>
<thead>
<tr>
<th>Conc. (ppm)</th>
<th>Number of tested snails</th>
<th>Number of dead snails</th>
<th>Mortality rates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>0.80</td>
<td>30</td>
<td>24</td>
<td>80</td>
</tr>
<tr>
<td>0.75</td>
<td>30</td>
<td>18</td>
<td>60</td>
</tr>
<tr>
<td>0.60</td>
<td>30</td>
<td>12</td>
<td>40</td>
</tr>
<tr>
<td>0.50</td>
<td>30</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>0.25</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Figure 2:** Dose/probit regression line of *Euphorbia aphylla* on *Lymnaea cailliaudi* (*nlatensis*) snails.

**Table 3:** Mortality rates of the ethanolic extract of *Ziziphus spina-christi* against *Lymnaea cailliaudi* (*nlatensis*) snails.

<table>
<thead>
<tr>
<th>Conc. (ppm)</th>
<th>Number of tested snails</th>
<th>Number of dead snails</th>
<th>Mortality rates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>800</td>
<td>30</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>600</td>
<td>30</td>
<td>27</td>
<td>90</td>
</tr>
<tr>
<td>400</td>
<td>30</td>
<td>24</td>
<td>80</td>
</tr>
<tr>
<td>300</td>
<td>30</td>
<td>20</td>
<td>66.66</td>
</tr>
<tr>
<td>200</td>
<td>30</td>
<td>5</td>
<td>16.66</td>
</tr>
<tr>
<td>100</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Probit Transformed Responses

![Probit Transformed Responses](image)

**Figure 3:** Dose/probit regression line of *Ziziphus spina-christi* on *Lymnaea cailliaudi*(natalensis) snails.

**Table 4:** LC$_{50}$, LC$_{90}$ and confidence limits of the ethanolic extract of tested plants on *B. alexandrina* and *Lymnaea cailliaudi*(natalensis) snails.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Molluscidal Activity (ppm)</th>
<th>Snail</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LC$_{50}$</td>
<td>(95% C.I)</td>
</tr>
<tr>
<td></td>
<td>L .l</td>
<td>U.l</td>
</tr>
<tr>
<td>Euphorbia aphylla</td>
<td>87.6</td>
<td>39.99 332.5</td>
</tr>
<tr>
<td>Euphorbia aphylla</td>
<td>0.66</td>
<td>0.62 0.7</td>
</tr>
<tr>
<td>Ziziphus spina-christi</td>
<td>311</td>
<td>163.8 465.7</td>
</tr>
</tbody>
</table>

C.I= confidence limit, L .l=Lower limit, U .l= Upper limit

**Histopathological study**

**Hermaphrodite glands of control (normal) *Biomphalaria alexandrina* and *Lymnaea cailliaudi* (natalensis) snails.**

The hermaphrodite gland of the adult *Biomphalaria alexandrina* and *Lymnaea cailliaudi* (natalensis) snails as that of any other pulmonate snail consists of number of vesicles known as acini (plate1, 3 fig. A) separated from each other by thin vascular connective tissue (plate1, 3 fig. B). Each acinus is enveloped in a sheath of squamous epithelium. In each acinus both male and female reproductive gametes are produced where mature ova are located at the periphery of the acini and bundles of male sperms are arranged in the center (plate1 fig. B, C & plate 3 fig. C).

**Histopathological Effects**

Effects of *Euphorbia aphylla* on *Biomphalaria alexandrina* snails and *Ziziphus spina-christi* on *Lymnaea cailliaudi* snails were more ore less similar. The acini lost their normal architectue and their separating connective tissues are almost damaged (plate 2, 5 fig. A). The acinar epithilum showed necrotic changes in the form of invagination (plate 2, 5 fig. B, A) and partial destruction (plate 5 fig. C). Degenerative changes were observed in most of the ova, where some of them have faint staining nuclei and others lost their nucleous (plate 2, 5 fig. C). Reduction in the number of sperms was also observed (plate 2 fig. B). Some acini appear more or less evacuated (plate 5 fig. B).

Regarding the effect of *Euphorbia aphylla* on *Lymnaea cailliaudi* snails, most of the examined sections showed sever necrotic changes where the acini completely lost their normal architectue (plate 4 fig. A), became reduced in number and shrinked (plate 4 fig. B). Marked degenerative changes of sperms and ova were also observed. Shrinkage and/or cytoplasmic evacuation were recognized in some ova. Other ova were swollen and lost their normal shape (plate 4 fig. C).
Some acini seemed to be empty and completely evacuated from both sperms and ova (plate 4 fig. B).

**Discussion**

Consideration of cost and environmental effect of synthetic molluscicides makes plants the focus of attention as an ideal source of cheap, safe and effective molluscicidal agents (WHO, 1993, 2003 and Aladesanmi, 2007).

**Molluscidal activity of Euphorbia aphylla:**

Molluscidal activity is widespread in the family Euphorbiaceae, although activity varies greatly from species to species (Al-Zanbagi, 2005 and Sharma et al., 2009). Mello-Silva et al. (2006), Bakry (2009) and Sharma et al. (2009) revealed the molluscicidal activity of different Euphorbia species with varying degrees of potency.

In the present study, the molluscicidal effect of the ethanol extract of *Euphorbia aphylla* against *B. alexandrina* snails and *Lymnaea cailliaudi* (natalensis) snails after 24 hour exposure showed considerable activity with LC$_{50}$ 87.6 ppm and LC$_{90}$ 142.5 ppm for *B. alexandrina* snails and 0.66 ppm and 0.88ppm for *Lymnaea cailliaudi* (natalensis) snails. *Lymnaea cailliaudi* (natalensis) snails were much more senstive to the extract than *B. alexandrina* snails.

In the present study, the LC$_{50}$ and LC$_{90}$ values of *Euphorbia aphylla* against *B. alexandrina* are promising in comparison with some previously studied plants. *Euphorbia schimperiana* and *Euphorbia helioscopia* caused no mortality up to 100 ppm on *Biomphalaria pfeifferi* (Al-Zanbagi 1999). The aqueous extract from *Jatropha curcas* L. (Euphorbiaceae) performed poorly against snails transmitting *Schistosoma mansoni* as 500 ppm caused 50% mortality (Rug and Ruppel, 2000).

The molluscicidal activity of the ethanol extract of *Euphorbia aphylla* is better than *Atriplex stylosa* (Chenopodiaceae), *Guayacum officinalis* (Zygophyllaceae) and *Calatropis procera* (Apocynaceae) with LC$_{50}$ ranging from 94 to 243 ppm and LC$_{90}$ ranging from 180 to 360 ppm against *B. alexandrina* snails. On the other hand this activity is lower than the activity of *E. milii* (LC$_{50}$19 ppm and LC$_{90}$ 38 ppm) (Bakry et al., 2004), *E. splendens* (LC$_{50}$ 40 ppm and LC$_{90}$ 73 ppm) (Bakry, 2009) and *Commiphora molmol* (Myrrh) oil (LC$_{50}$ and LC$_{90}$ 155 and 195 ppm) (Allam et al., 2001) against the same snail species. These differences in potency can be attributed to several factors including the locality of the plant species, time of collection of the plant sample, part used, storage conditions, method of extraction and solvents type (Brackenbury and Appleton, 1997 and Hassan et al., 2010).

The current study was extended to evaluate the molluscicidal effect of the ethanol extract of *Euphorbia aphylla* on *L. cailliaudi*. The LC$_{50}$ and LC$_{90}$ were 0.66 ppm and 0.88 ppm respectively. This activity is higher than the latex of *E. hirta* against *Lymnaea acuminata* (LC$_{50}$ 1.29 ppm) (Yadav and Singh, 2011) and *Commiphora molmol* (Myrrh) oil (LC$_{50}$ and LC$_{90}$ 50 and 85 ppm respectively) (Allam et al. 2001).

*Commiphora molmol* (Myrrh) is a plant recommended as safe molluscicides (Massoud et al., 2004 and Al-mathal and Fouad., 2006) and has been licensed for medical use in Egypt and several countries as a fasciolicidal and schistosomicidal drug with high efficacy and safety (Aly and Aly, 2006 and Abdul-Samie et al., 2010). Also this activity is much higher than that of *Meryta denhamii* (LC$_{50}$ 26.4 and LC$_{90}$ 70.8 ppm) against *Lymnaea cailliaudi* (Hassan et al., 2010).

In the present study, *Lymnaea cailliaudi* snails have been found to be more susceptible than *Biomphalaria alexandrina* snails to the molluscicidal activity of *Euphorbia aphylla* with the latter requiring high concentrations as lethal doses. This observation is in accordance with the findings of Allam et al. (2001) and Hassan et al., (2010) using *Commiphora molmol* (Myrrh) oil and *Meryta denhamii* against *Lymnaea cailliaudi* and *Biomphalaria alexandrina* snails.

The difference in susceptibility of the two snails to the lethal effect of the same extract could 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 (Bakry and Hamdi, 2007 and Osman et al., 2007).

Beside its considerable molluscicidal potency, *Euphorbia aphylla* also presents some very interesting characteristics for an ideal plant molluscicide. It is cosmopolitan and perennial plant. It is not edible to animals and easily cultivable (its multiplication is done by means of asexual reproduction which does not require frequent watering or application of pesticides or fertilizer) (Baptista et al., 1997 and Brickell and Cathey, 2008).

**Molluscidal activity of Ziziphus spina-christi:**

In the present study, the LC$_{50}$ and LC$_{90}$ of ethanol extract of *Ziziphus spina-christi* against *L. cailliaudi* after 24 hours exposure were 311 and 500 ppm respectively. This activity is much higher than that reported for the Egyptian plant, *Ambrosia maritima* (damsisssa) (LC$_{50}$ 3000 ppm) against Egyptian *Lymnaea cailliaudi* snails (Abou Basha et al., 1994).

*Ziziphus spina-christi* showed less potent molluscicidal activity than *Euphorbia aphylla* against *Lymnaea cailliaudi* which can be attributed to the differences in each plant active ingredients, their mode of action and method of penetration of the snails (Rawi et al., 1996 and Hassan et al., 2010).

In the present study, *Ziziphus spina-christi* gave no molluscicidal activity against *B. alexandrina* snails up to 1000 ppm. The possibility for the same plant extract to have molluscicidal activity against certain snail species and absence of activity against other species were recorded by Al-Zanbagi (2005) who found that *Euphorbia schimperiana* and *Euphorbia helioscopia* (Family Euphorbiaceae) have a relatively high toxicity against the snails *Bulinus wrighti* while they gave negative results against the snails *Biomphalaria pfeifferi*.

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Molluscicidal activity of Enterolobium contortisiliquum: 

Enterolobium contortisiliquum is rich in saponins (Mimaki et al., 2004), saponins have haemolytic properties and toxic effect on most cold-blooded animals including snails and are proved to have molluscicidal activity (Herlt et al., 2002, Osman et al., 2007 and Singh and Singh, 2009). However no molluscicidal activity of the ethanol extract of Enterolobium contortisiliquum mature fruits on both snail species up to 1000 ppm was detected. This could be attributed to the fact that saponins responsible for its activity are extracted in greater measures with other solvents. Supporting this explanation the results obtained by Abdel-Rahman and Hassan (2008) who found that the butanol fraction of Hedera canariensis (Family Araliaceae) has molluscicidal activity against Biomphalaria alexandrina and Lymnaea cailliaudi. While ethyl acetate extract of the same plant was inactive. On the contrary, Hassan et al. (2010) found that the butanol fraction of Meryta denhamii flowers which belongs to family Araliaceae was inactive and ethyl acetate extract was active against Biomphalaria alexandrina and Lymnaea cailliaudi.

In this study, selection of the hermaphrodite glands of B. alexandrina snails and Lymnaea cailliaudi snails based on the fact that it is a target tissues for molluscsides (Bode et al., 1996). In addition, previous studies have shown that it is strongly affected by molluscsides (El-feky et al., 2009 and Rawi et al., 2011).

The present study demonstrated histopathological changes in the hermaphrodite glands of Biomphalaria alexandrina snails exposed to the LC₂₅ of Euphorbia aphylla and Lymnaea cailliaudi snails intoxicated with Euphorbia aphylla and Ziziphus spina-christi. The histopathological changes in these snails were more or less similar showing evacuations and reduction in number of acini, marked degenerative changes of ova and decrease in numbers of sperms. However sever necrotic changes of the acini with more marked degenerative changes in ova and sperms were detected after the exposure of Lymnaea cailliaudi snails to Euphorbia aphylla extract. These findings agree with Rizk (1998) who recorded severe changes in the sperms and ova besides degeneration in the gonadal acini structure of B. alexandrina snails post exposure to sublethal concentrations of the plant Sesbania sesban. According to (El-Hommosassy and El-Sheribibi, 2011) evacuations in many gonads’ cells severely suppressed the snails’ capacity for egg-laying.

The differences in histopathological changes detected in B. alexandrina and Lymnaea cailliaudi snails using the same plant extract (Euphorbia aphylla) may be attributed to the fact that molluscsides may vary in their toxicological effects according to the species of the snails’ used (Bakry and Hamdi, 2007 and Osman et al., 2007).

Atlam (2000) found harmful effects in the hermaphrodite gland cells and gametogenic stages of B. alexandrina snails and Lymnaea cailliaudi (natalensis) snails treated with sublethal concentrations of Euphorbia peplus.

The same results obtained by Mossalem (2003) post B. alexandrina snails exposure to the LC₃₀ of the plants Dyzygotheca kerchoveana, Solanum nigrum and Panicum repens for 24 and 48 hours. Also, the histopathological effect of Guayacum officinalis, Atriplex stylosa and Euphorbia splendens methanol extract on the hermaphrodite gland of Biomphalaria alexandrina snails were studied by Bakry (2009) who obtained the same observations post two weeks of exposure to LC₂₅ of the tested plant’s extract.

Conclusion

- The use of the Ethanolic extract of Euphorbia aphylla as a molluscicide may play a vital role in controlling Schistosoma mansoni and Fasciola.

Phytochemical investigations to identify the bioactive ingredient(s) responsible for the molluscicidal potency are recommended.

- Toxicological studies on man, fauna and flora of the fresh water are needed.

- Further laboratory tests to search for the active component of Ziziphus spina-christi plant may increase its potential use as plant molluscicide.

- Further investigations using different solvents to search for other active ingredients of Enterolobium contortisiliquum may increase its potential use as plant molluscicide.

- The application of LC₂₅ of the ethanol extracts of Euphorbia aphylla, and Ziziphus spina-christi may be helpful in snail control as they resulted in evacuations in many gonads’ cells which severely suppressed the snails’ capacity for egg-laying.

Acknowledgement

The authors are grateful to Prof. Dr. Zedan Z. Ibraheim, Pharmacognacy Department, Faculty of Pharmacy, Assiut University for identifying , extracting the plant materials and for his valuable advice and continuous care to this work.

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Plate (1): Transverse sections (T.S.) in normal hermaphrodite gland of *B. alexandrina* snails (control)  
Fig. A. Showing normal acinar architecture (arrows) (X100).  
Fig. B. Showing thin vascular connective tissue (head arrow) and sperms (arrow) (X200).  
Fig. C. Showing the ova (arrows) (X400).
Plate (2): T.S. in hermaphrodite gland of *B. alexandrina* snails after exposure to LC$_{25}$ of the ethanolic extract of *Euphorbia aphylla*

Fig. A. Showing loss of normal acinar architecture and damaged separating connective tissue (arrows) (X100).
Fig. B. Showing invaginated epithelium (arrows) and reduced number of sperms (head arrow) (X200).
Fig. C. Showing partial destruction of acinar epithelium (head arrow), ova lost their nucleous and others has faint staining nucleous (arrows) (X400).
Plate (3) T.S. of hermaphrodite gland of normal *Lymneae cailliaudi (natalensis)* (control).

Fig. A. Showing normal acinar architecture (arrows) (X100).
Fig. B. Showing separating thin vascular connective tissue (arrow) (X200).
Fig. C. Showing ova (arrow) and sperms (head arrow) (X400).
Plate (4) T.S. of hermaphrodite gland of *Lymneae cailliaudi* (*natalensis*) after exposure to LC$_{25}$ of the ethanolic extract of *Euphorbia aphylla*.

Fig. A. Showing complete loss of acinar architecture (X100).
Fig. B. Showing shrinkage of acini, reduction of their number and some acini appeared to be empty (arrows) (X200)
Fig. C. Showing distortion in the shape of some ova with cytoplasmic evacuation (arrows) (X400).
Plate (5) T.S. of hermaphrodite gland of *Lymneae cailliaudi* (*natalensis*) after exposure to LC$_{25}$ of the ethanolic extract of *Ziziphus spina-christi*.

Fig. A. Showing loss of normal acinar architecture, damaged separating connective tissue and invagination of acinar epithilium (arrow) (X100).

Fig. B. Showing disruption of the acini and some of them appeared more or less evacuated (arrows) (X200).

Fig. C. Showing partial destruction of acinar epithilium (head arrow), ova lost their nucleous and others has faint staining nucleous (arrows) (X400).