RESEARCH ARTICLE

El-Sayed Taha Rizk

TOXICITY OF COMMIPHORA MOLMOL (MYRRH) WATER SUSPENSION TO SOME NON-TARGET AQUATIC ORGANISMS AND ITS MOLLUSCICIDAL AND BIOLOGICAL ACTIVITIES ON BULINUS TRUNCATUS AND BIOMPHALARIA ALEXANDRINA (MOLLUSCA, GASTROPODA)

ABSTRACT

The molluscicidal activity of Commiphora molmol (myrrh) water suspension showed that Bulinus truncatus snails were found to be about 3 times more susceptible than Biomphalaria alexandrina snails. The 24-hour LC50 values recorded were 46.4 and 145.8 ppm, respectively. There was a significant negative correlation between LC values and exposure periods. The present study is yet the first attempt to investigate the toxicity of C. molmol aqueous suspension (in vitro) against non-target aquatic organisms coexisting with vector snails in their habitat. The obtained results revealed that the plant water suspension was more toxic at the molluscicidal levels to the crustaceans Daphnia sp, Cypris sp, Cyclops sp, Cardina nilotica and the fish Gambuzia affinis at a concentration of 50 ppm (in average) after 48 hr of exposure. Culex larvae were more tolerant to the plant suspension than other organisms. Chronic effects of sublethal concentrations of C. molmol water suspension on some biological parameters of B. truncatus snails showed a significant decrease in food consumption and egg production of snails. At the sublethal concentration of LC50, no hatching was observed after 17 days of exposure. After 24 hour of exposure to C. molmol water suspension 70% mortality of newly hatched snails (3-days old) was observed at 50 ppm. In addition, biochemical investigation showed significant differences as compared to the control regarding the total protein content, and the activity of transaminases (AST, ALT) and Lactate dehydrogenase (LD) except a mild decrease in glycogen concentration and alkaline phosphatase activity in digestive gland tissue of snails. Ultrastructural investigation of exposed snails to C. molmol water suspension showed some alterations in the digestive gland of snails (i.e. marked vaculation, increased lysosomal activity, swollen mitochondria and consumption of stored lipid and glycogen). Based on the toxicity of C. molmol to the aquatic ecosystem at the examined sublethal levels, it is not recommended as a herbal molluscicide.

Key words: Commiphora molmol (myrrh), water suspension, vector snails, molluscicidal activity, Toxicity, non-target aquatic organisms.

El-Sayed Taha Rizk
Zoology Department.
Faculty of Science,
Tanta University.
Tanta, Egypt.

INTRODUCTION

Various strategies including health education, mass diagnosis and chemotherapy and snail control have been recommended for management of schistosomiasis and prevention of its transmission or at least reduction of its prevalence among people at risk (Croft et al., 2003). Mollusciding has taken considerable concern for the control of disease vector snails. Such snail control by means of synthetic molluscicides, though effective to eliminate the intermediate host populations, their prohibitive cost, potential environment damaging effects and occurrence of resistance have limited their widespread use today. Other methods including environmental modifications and biological control have limited applicability. Plant molluscicides are gaining increased attention, as they seem to be less expensive and have low toxicity to non-target organisms (including humans) than synthetic molluscicides (WHO, 2000).

Recently, Gum Myrrh is an oleo-gum resin obtained from the stem of the plant Commiphora molmol (Burseraceae), one of medicinal plants that has been tested as schistosomicide and molluscicide (Massoud et al., 2000). They indicated its oil extract possesses a higher molluscicidal potency than the oleo-resin which against the vector snails Biomphalaria alexandrina, Bulinus truncatus and Lymnaea natalensis (Massoud & Habib, 2003 and Al-Mathal & Fouad, 2006). C. molmol. It also showed a molluscicidal effect on B. connollyi at 80 ppm after 72 hr exposure (Shoukry et al., 2006). Until now, no studies were so far carried out on the toxicity of Commiphora molmol water suspension against...
the most common aquatic non-target species, which co-exist with the snails in the same habitat.

However, the ideal plant molluscicide must have high specificity against the target molluscan species and its toxicity should be at acceptable levels before it can be recommended for further development (WHO, 2002). Therefore, in order to diminish the ecological damage, a considerable interest should be imposed in determining the effect of molluscicides on aquatic fauna, which constitute an important link in the ecological food chain (Andrews et al., 1983, Al-Sharkawi & Rizk, 1996). Virtually, before field application, laboratory toxicity data are normally used to predict the potential impact of the molluscicides on aquatic ecosystem, to assess their potential hazard and to establish their safe values to the aquatic taxa (Anton & Ariz, 1989).

Only few attempts were carried out on the effect of the oil extract of Commiphora molmol on the ovicidal activity of snails (Allam et al., 2001, Al-Mathal & Fouad, 2006).

Nowadays a great attention is paid to evaluate the toxic action of plant molluscicides on the snail’s tissues and their structure and function (Triebskorn, 1991, Brackenbury and Abd-El-Megeed, 1999, Eissa Tiwari, 2004). The digestive gland in gastropods is primarily involved in intracellular and extracellular digestive processes. It also plays an important role in the internal defense mechanism (Ottaviani, 1990), in detoxification processes (Marigomez et al., 1986) and in the storage of glycogen (Rebecchi et al., 1996).

In the context of the information provided above, the present study aimed to determine the molluscicidal effects of Commiphora molmol water suspension against the snail vectors of schistosomiasis in Egypt, (Biomphalaria alexandrina and Bulinus truncatus). Chronic toxicity of sublethal concentrations of plant water suspension on some biological parameters of Bulinus truncates was studied (e.g. food consumption, egg production, hatchability of eggs, effect on offspring of snails and its toxicity on some biochemical activities, histological study and ultrastructural observations of the digestive gland. In addition, assessment of the plant toxicity to the most common aquatic non-target species which co-exist with the snails in their habitat.

**MATERIALS AND METHODS**

**1- Snails:**

*Bulinus truncatus* snails were obtained from the Schistosome Biological Supply Centre (SBSC) at Theodor Bilharz Research Institute (TBI) Imbaba, Egypt. They were 6-7 mm in height. *Biomphalaria alexandrina* snails were collected from irrigation canals in El-Gharbia Province, which had not been previously treated with any molluscicides, and then were carried to the laboratory where they were successively examined for natural trematode infection. Non-infected specimens of both snail species were acclimatized to laboratory conditions for at least 2 weeks before experimentation. Snails were maintained in plastic aquaria in dechlorinated tap water, of pH 7.4 and under constant temperature of 27 ± 1°C. The snails were fed fresh Lettuce leaves *ad libitum* and the water of the aquaria was renewed twice a week.

**2- Non target aquatic organisms:**

The aquatic samples which co-exist in the snail habitat were collected from the same irrigation canals including *Culex* larvae, and the crustacean, *Caridina nilotica* that were collected with nets of Nylon cloth. Surface water minute crustaceans as, *Daphnia* sp, *Cyclops* sp and *Cypris* sp were filtered through a zooplankton net of 55 µm mesh size. These samples were acclimatized to laboratory conditions in 2 liter culture dishes at a temperature of 27 ± 1°C, with a photoperiod of 16 h light and allowed to produce two to three broods over 9 days to establish stock cultures. Gambuzia affinis fish were trapped and let to acclimatize in the laboratory for 7 days before testing, in 1 L glass aquaria provided with suitable aeration and kept at a water temperature of 27 ± 1°C. Fish were fed a commercial food fish.

**3- Plant material:**

*Commiphora molmol* (family: Burseraceae) is one of medical plants collected from north-east of Africa and south of Arabia. Gum myrrh is an Oleo-gum resin; obtained from the stem of *C. molmol*. Myrrh was collected from the local market in Tanta city as a reddish brown hard mass.

For preparation of *C. molmol* (Oleo-gum resin) water suspension, 1 gm of the mass material was crushed and suspended overnight in dechlorinated tap water at room temperature in 1 liter, then warmed until 60°C with continuous shaking to get a final concentration of 1000 ppm. A series of concentrations were then prepared from the stock suspension.

**4- Biological bioassays:**

**4.1 Determination of the molluscicidal activity of *C. molmol* water extracts against *Bulinus truncatus* and *Biomphalaria alexandrina* snails.**

The provisional plan of the WHO (1965) for testing the molluscicides was followed. The density of the snails per liter was 10 snails. During the exposure period, experimental container of snails and control snails were kept at room temperature (27± 1°C) and light/dark cycle. After exposure periods of 24, 48 and 72 hrs, the snail mortalities were determined after a recovery periods of 24 hour. At the same time, control snails were placed in containers
of the same volume of dechlorinated tap water to ascertain normal mortality of snails under the present experimental conditions. Dead and live snails were counted and the mortality percentages were calculated. The lethal concentration values LC50, LC90 and the slope of the concentration values were calculated, using the method of probit analysis according to Finney (1978).

4.2 Determination of the toxicity of C. molmol water suspensions to some non-target freshwater species:

The lethal concentrations of the plant water suspension on non-target aquatic organisms coexisting with the snails in their habitat were determined. Three replicates of 250-ml glass beakers containing 30 animals/100 ml of test concentrations and control were used for small size organisms i.e. Daphnia sp, Cyclops sp, Cypris sp, Caridina nilotica and Culex larvae. For each tested concentration, mortalities were recorded after 24 and 48 hr. For larger organisms (Gambuzia affinis) fish, all bioassays were done on 20 animals/10 L of dechlorinated tap water provided with constant aeration at 27 ± 1°C. Three replicates of each concentration and of the control were used. The percentage of mortalities in both the exposed and the control aquaria was calculated.

5. Chronic effects of sublethal concentrations of C. molmol water suspension on some biological parameters of B. truncatus snails.

5.1. Food Consumption:

About 40 snails were placed in 4 liter glass aquaria and exposed to half- of sublethal dose (1/2 LC50) of C. molmol water suspension. The control aquaria had the same number of snails and volume of dechlorinated tap water. This experiment was run for 5 weeks. Tested suspensions and water were changed every 2 days, and to each of them, 1 gm of dry lettuce leaves was added. The faeces of exposed and control snails were removed and dried in an incubator, then weighed to determine the amount of food consumed by a snail each week.

5.2. Egg production (Fecundity):

About 40 adult healthy snails were exposed to sub-lethal concentrations (of 1/10, 1/4 and 1/2 LC50) of C. molmol emulsion (4.6, 11.6 and 23.2 ppm, respectively) in 4 litter dechlorinated tap water. The exposure period extended for 6 weeks and each concentration was renewed every 2 days. The total number of egg masses and dead snails were collected daily during the experimental periods. The mean number ± SD of eggs / snail / week was calculated to each concentration of plant suspension and the control groups.

5.3. Hatching of egg masses:

The egg masses deposited by control snails were collected separately in petri-dishes then exposed to different concentrations of water suspensions of plant extract. Every two days the plant suspension was changed by freshly prepared one. After one week, the control egg masses started in hatching. Every 2 days after 1st week, the hatching of egg masses was determined at each concentration and the control group and the mean of hatchability rate was calculated and computed by t-test.

5.4. Effect on offspring:

Newly hatching snails (3-days old) from untreated control snails were separated in Petri-dishes then exposed to different concentration of plant water suspension (range of 10 – 70 ppm). After 24, 48 and 72 hr of exposure, dead offspring were counted then the mean mortality percentage of offspring at each concentration and control group was calculated.

5.5. Effect of C. molmol water suspension on some biochemical activities of B. truncatus digestive gland tissue:

About 40 adult healthy snails in 4 liters dechlorinated tap water were exposed to sub-lethal concentrations of 1/2 LC50 of C. molmol emulsion. The exposure period of the experiment was 5 weeks and the concentration was renewed every 2 days. Snails were first blotted with filter paper and then dried. The shells were crushed between 2 slides and the digestive gland was freely dissected. The fresh tissue samples were weighed and homogenized in tissue homogenizer with phosphate buffer solution (1:5 w/v ratios) at pH 7.4. Homogenate was centrifuged at 500 rpm for 15 min at 5°C, the sediment was removed and the supernatant used as tissue extract.

Total protein and glycogen contents as well as alkaline phosphatase, aspartate and alanine aminotransferases (AST and ALT) enzyme activities and lactate dehydrogenase (LDH) in digestive gland tissue were estimated by colorimetric method-end point determination, using biological kits purchased from Diamond Diagnostic Co., Egypt.

5.6. Chronic effects of C. molmol water suspensions on the histological structure of the digestive gland of snails:

Adult healthy snails of B. truncatus were exposed to sub-lethal concentration of 1/2 LC50 of C. molmol water suspension (23.2 ppm). The exposure period of the experiment was 5 weeks and plant suspension was changed every two days. For histological investigation, the soft parts of tested snails were separated from the shells in both control and treated specimens. The digestive gland was quickly separated then fixed in Bouin’s fluid for 72 hours, washed and dehydrated through ascending grades of ethyl alcohol. The specimens were embedded in paraffin wax at 58°C for 3 hours and sectioned at 5-6 μm.
Finally, sections were stained with haematoxylin and eosin, and then the slides were prepared for microscopical examination.

For TEM exposed the digestive gland was separated as above, fixed in 0.1M phosphate buffer formaldehyde / glutaraldehyde at pH 7.4 and room temperature for four hours, and then rinsed twice in 0.1M phosphate buffer (15 minutes), rinsed in 1% tannic acid overnight. The tissues were washed by buffer for 30min, post fixed in 2% osmiumtetroxide with the same buffer at 0-4°C for two hours and washed twice again in buffer. The tissues were dehydrated in ascending ethanol series and finally embedded in Araldite of Epon. Polymerized tissue blocks were sectioned by Ultramicrotome with glass knives (Joel) on a porter-Blom Mt2B (Reichert). Ultrathin sections were mounted on uncoated copper grids, the tissues were then double stained with aqueous uranyl acetate for 30 min and lead citrate for 3 min. Grids were examined and photographed in Joel TEM at an accelerating voltage of 60 KV at 100 CX TEM.

6. Statistical analysis of the data:

For the data obtained, the significance of the difference between means was determined using Students t-test. The regression analysis was done using the least-squared method (Finney, 1978).

RESULTS

1- Determination of the molluscicidal activity of *Commiphora molmol* aqueous suspension against *Bulinus truncatus* and *B. alexandrina* snails.

Laboratory evaluation was carried out to assess the molluscicidal activity of *Commiphora molmol* aqueous suspension against *Biomphalaria alexandrina* and *B. truncatus*. The molluscicidal activity of the plant suspension showed that *B. truncatus* snails were about 3 times more susceptible than *B. alexandrina* snails. The 24-hour LC50 and LC90 values recorded were 46.4 and 145.8 ppm, respectively. There was a significant negative correlation between LC values and exposure periods, the LC50 value of plant suspension was decreased from 46.4 ppm (24 hr) to 21.8 ppm (72 hr) against *B. truncatus* and from 145.8 ppm (24 hr) to 21.8 ppm (72 hr) for *B. alexandrina* (Table 1).

<table>
<thead>
<tr>
<th>Exposure periods/ hours</th>
<th>Lethal conc. LCP (ppm)</th>
<th>B. alexandrina</th>
<th>B. truncatus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LC50</td>
<td>LC90</td>
<td>r²</td>
</tr>
<tr>
<td>24 hr</td>
<td>145.8</td>
<td>185.4</td>
<td>0.98</td>
</tr>
<tr>
<td>48 hr</td>
<td>130.4</td>
<td>164.8</td>
<td>0.95</td>
</tr>
<tr>
<td>72 hr</td>
<td>105.9</td>
<td>141.3</td>
<td>0.84</td>
</tr>
</tbody>
</table>

* At a water temperature of 27±1°C, pH = 7.4±1 and number of 40 snails tested in each concentration / 4 liter / 3 replicates. ** r² = Correlation coefficient.

Table (1): Lethal concentration values (ppm) of *C. molmol* water suspension against *B. truncatus* and *B. alexandrina* snails under laboratory condition*.

2- Determination of the toxicity of *C. molmol* water suspensions on some non-target organisms:

The toxicity of *C. molmol* water suspension on the non-target organisms coexisting with the snails in their habitat was determined (Figure 1- a, b, c, d, e, f). The results revealed that *Daphnia* sp is more susceptible to the plant water suspension than other crustaceans (*Cardina nilotica*, *Cypris* sp and *Cyclops* sp) 100% mortality after 48 hr exposure was obtained at concentrations of 20, 40, 50 and 50 ppm, respectively. The plant suspension was moderately toxic to the fish *G. affinis*, which all were killed (100 % mortality) at a concentration of 50 ppm after 48 hr exposure. *Culex* larvae were more tolerant to the plant suspension than other organisms. At a concentration of 90 ppm, 50% mortality was obtained after 48 hr exposure.

3- Chronic effects of sublethal concentrations of *C. molmol* water suspension on some biological parameters of *B. truncatus* snails.

3.1. Food Consumption:

The results of the test performed during the exposure period (day 8-40) are shown in Figure 2. Food consumption (the amount of food consumed by snail) was significantly decreased by exposure of snails to a concentration of 23.2 ppm (1/2 LC50) (P ≤ 0.05) after the 2nd and 3rd weeks. At the 4th week, the snails’ faeces were completely missing when the feeding stopped.

3.2. Egg production (Fecundity):

Egg production of *B. truncatus* was not affected by sublethal concentrations after the 1st week of exposure. Control snails laid significantly higher number of eggs (p ≤ 0.05) than these treated with 1/10 LC50 & 1/4 LC50 compared to control was 80% & 87.1%, respectively after the 5th week of exposure. *B. truncatus* snails stopped egg laying at 25 days after exposure to 1/2 LC50 (23.2 ppm). The results suggest that the egg laying capacity of *B. truncatus* snails did not depend only on plant suspension concentrations but was also time dependent. Meanwhile, the snails exposed to 1/2 LC50 caused decreased egg laying from 40.1 eggs / egg mass / snail / week at 1st week, to 6.8 eggs / egg mass / snail / week after 2nd week of exposure to *C. molmol* water suspension (Fig. 3).

3.3. Egg hatchability:

The obtained results (Fig. 5) show that *C. molmol* water suspension caused significant inhibition of egg hatchability at sublethal concentration or delayed hatching eggs at low concentrations. Exposure of egg masses to 1/2 LC50 (23.2 ppm) water suspension led to
The obtained results showed that 50% mortality for newly hatched snails (3-days old) was observed at 20 ppm after 48 hr of exposure. While after 24 hr of exposure to C. molmol water suspension 70% mortality was observed at 50 ppm (Fig. 5). The mortality rate of newly hatched snails was concentration and time- dependent. The mortality rate of treated newly hatched snails with 50 ppm caused 82% and 100% after 48 and 72 hr of exposure, respectively.

4. Biochemical assays:

- Effect on total protein content:

  The level of total protein in digestive gland tissue for exposed snails to plant water suspension was significantly decreased than control snails (P ≤ 0.05) where the recorded values were 3.9 ± 0.81 gm / 100ml and 7.55 ± 0.59 gm/100ml, respectively (Table 2).

- Effect on glycogen content:

  Exposure of snails to C. molmol water suspension caused insignificant decrease in glycogen concentration in the digestive gland of snails reaching 29.8% (Table 2).

- Effect on transaminases (AST and ALT) activity:

  The obtained data (Table 2) showed that plant suspension caused an increase in the activity of aspartate and alanine aminotransferases (AST and ALT). Their activities showed...
concentrations of

Figure (3): Effect of sublethal concentrations of C. molmol water suspension on egg production of B. truncatus snails under laboratory conditions\(^1\) for five weeks exposure.

\(^1\) At a water temperature of 27 ± 1 °C, pH 7.4 ± 1, concentration of molluscicide water extract= 1/2 LC\(_{50}\) (23.2 ppm) and no. of 40 snails tested in each concentration / 4 liter. * (Significant at P ≤ 0.05). (0) means snails stopped egg production.

![Figure 3](image3.png)

Figure (4): The effect of sublethal concentrations\(^a\) of C. molmol water extract on the egg hatchability rate in vitro of B. truncatus snails at standard laboratory conditions\(^b\).

\(^a\) concentrations of C. molmol water suspension 1/10, 1/4, 1/2 LC\(_{50}\) and LC\(_{50}\), and no. of 40 snails tested in each concentration / 4 liter. * (Significant at P ≤ 0.05).

\(^b\) At a water temperature of 27 ± 2 °C, pH 7.4 ± 1, total number of 40 eggs /each concentration is (in average) for each replicates.

A significant increase in ALT of digestive gland of exposed snails (P ≤ 0.05) but insignificant in AST activity, where the rates of increase were 62.7% and 18.4%, respectively.

Figure (5): Effect of C. molmol water suspension on mortality rate of newly hatched B. truncatus snails at standard laboratory conditions\(^*\).

* At a water temperature of 27 ± 1 °C, pH = 7.4 ± 1 and no. of 40 snails offspring tested in each concentration / 4 liter.

- **Effect on lactate dehydrogenase (LDH):**

Exposure of B. truncatus snails to plant water suspension caused a high significant increase in LDH activity in the digestive gland (PS 0.05) (Table 2), where the recorded value was 3.94 ± 0.45 u /mg protein compared with 1.9 ± 0.33 u/mg protein in the control.

- **Effect on alkaline phosphatase:**

Table (2) shows that the activity of alkaline phosphatase insignificantly decreased in the digestive gland of exposed snails to C. molmol plant suspension compared to the control group, where the percentage of inhibition was -1.07%.

**Table (2).** Effect of sublethal concentration\(^a\) of C. molmol water suspension on the total protein, glycogen, Lactate dehydrogenase (LDH), Transaminases (AST, ALT) and alkaline phosphatase activities in the digestive gland tissue of B. truncatus snails.

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>Control</th>
<th>C. molmol</th>
</tr>
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<tbody>
<tr>
<td>Total protein gm %</td>
<td>7.55 ± 0.59</td>
<td>3.9 ± 0.81*</td>
</tr>
<tr>
<td>Glycogen mg/ gm/ wet tissue</td>
<td>25.20 ± 1.3</td>
<td>17.7 ± 0.78</td>
</tr>
<tr>
<td>LDH U/mg protein</td>
<td>1.9 ± 0.33</td>
<td>3.94 ± 0.45*</td>
</tr>
<tr>
<td>Transaminases U/L</td>
<td>AST 27.1 ± 2.5</td>
<td>33.2 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>ALT 17.5 ± 1.12</td>
<td>46.9 ± 4.6*</td>
</tr>
<tr>
<td>Alkaline Phosphatase U/L</td>
<td>21.9 ± 2.2</td>
<td>10.9 ± 1.6</td>
</tr>
</tbody>
</table>

\(^a\) Sublethal concentration of C. molmol water suspension = 1/10 (23.2 ppm) * (Significant at P ≤ 0.05).

5. **Histological studies:**

5.1. **Light microscopy:**

The digestive gland of normal untreated B. truncatus is composed of a large bilobed tubuloacinar gland showing bundles of tubules, surrounded by a connective tissue. The epithelia of the ducts consist mainly of columnar epithelial cells covered by integument of visceral mass. The main cell types present in the epithelia are digestive cells, excretory cells and excretory or crypt cells (Fig. 6 a). The digestive cells dominate the epithelium; they exhibit four different shapes with different cytoplasmic inclusions. The secretory cells are present in few numbers than the latter ones. They are long spherical cells with basal or lateral small nuclei (Fig. 6 a). Each excretory cell contains brownish secretory granules released into the lumena of...
hepatic tubules by the rupture of the apical membrane of the cell.

Fig. 6 (a & b): Histoarchitecture of digestive gland of (a) control Bulinus truncatus showing the acini of the normal architecture of the gland and two cell types of (digestive and excretory cells). (b) the digestive gland of exposed snails showing vacuolation and focal degeneration of the epithelial cells. Stained with H & E. Scale bar = 0.1 mm.

Abbreviations: Ct: Connective tissue, d: digestive cell, de: degeneration of the epithelial cells, e: excretory cell, lu: lumen of tubule, s, secretory granules, V: Vacuole.

Treatment of snails with C. molmol water suspension induced extensive vacuolation of the epithelial cells of the digestive tubules (Fig. 6 b). That vacuolation finally led to the rupture of the apical membranes of some cells and focal degeneration of the epithelial cells (Fig. 6 b). The secretory cells increased in number than the digestive cells, besides an irregular shape of the tubuli and the border of the epithelial cells.

5.2. Transmission electron microscopy:

The ultrastructural investigation of the digestive gland of unexposed B. truncatus showed that the digestive cell has elongate body with long and heavy microvilli at the apex (Fig. 7 a). The cytoplasm contains large number of glycogen particles, different lysosomes and many large vacuoles (Figs 7 a & b). Rough endoplasmic reticulum is represented by tubules and long cisternae with intensive aggregation of ribosomes around the nucleus (Fig. 7 b). Golgi complex is also observed in the cell cytoplasm as well as lysosomes (Fig. 7b). Its nucleus is oval, has eccentric nucleolus and granular outer membrane. Heterochromatin material is condensed adjacent to the inner nuclear membrane and karyoplasm is full of scattered chromatin matrix (Fig. 7c). The mitochondria are large and spherical to oval with long cristae (Fig. 7d).
Chronic exposure of snails to the sublethal concentration of *C. molmol* water suspension showed some alterations in the digestive cell as reduction and deformation of microvilli (Fig. 8 a) and cytotic channels could be observed at the apical cell membrane. Increase of vacuoles at the apical part of the cell (Fig. 8 b) and also heavy aggregations of rough endoplasmic reticulum, phagosomes (Fig. 8 b). The nucleus increased its electron density. Mitochondria are elongate with heavy long cristae (Fig. 8 c).

The excretory cell is a long broad cell with different types of vacuoles and lysosomal contents (Fig 9 a). The apical cell membrane is wavy bearing long microvilli. The wavy margin may be resulted from the rupture of lysosomes (exocytotic vesicles) at the surface during exocytosis (Fig. 9 a). Lysosomes have two types, transparent and lucent lysosomes (Fig. 9 a). The lucent type is very large multivesicular body and occur in large number in the basal portion of the cell. Also, large number of mitochondria was observed. Few rough endoplasmic cisternae and elongate vesicles and tubules were observed in the cell (Fig. 9 b). The excretory cell has spherical basal nucleus with transparent central nucleolus and scattered chromatin material. Heavy condensation of ribosomes appear attached to the nuclear membrane (Fig. 9 b).

Exposed *B. truncatus* to the plant water suspension, showed many electron-dense lysosomal like vesicles in the apical portion of the excretory cell (Fig. 10 a). The endoplasmic reticulum proliferates in cytoplasm forming a tubular system (Fig. 10 c).
electron-dense with presence of crystalline inclusions in the karyoplasm (Fig. 10 c).

Fig. 9 (a & b): Transmission Electron Microscopy micrograph of an excretory cell of control Bulinus truncatus showing (a) normal shape of excretory cell containing microvilli, exocytotic vesicle, lysosomes and heavy long mitochondria. Scale bar = 1 μm. (b) The basal cell part showing the nucleus, nucleolus, rough endoplasmic reticulum, Golgi apparatus and glycogen. Scale bar = 1 μm.


Fig. 10 (a, b & c): TEM of an excretory cell of exposed Bulinus truncatus showing (a) electron dense lysosomes, microvilli and some vacuoles. Scale bar = 1.4 μm. (b) the nucleus shranked, elongated with electron dense bodies and phagosomes. Scale bar = 1 μm. (c) large vacuoles with electron dense bodies, enlarged mitochondria and ruptured lysosomes. Scale bar = 1 μm.


DISCUSSION

Certain snails are known to act as vectors for S. haematobium and S. mansoni, hence molluscicidal activity can play a role in the prevention of bilharziasis (schistosomiasis). The present study showed that the plant water suspension of Commiphora molmol (myrrh) had molluscicidal activity against B. truncatus and B. alexandrina snails. The 24 hour LC50 and LC90 values recorded 46.4 and 145.8 ppm, respectively. The molluscicidal properties of the oil, alcohol and petroleum ether extracts of Commiphora molmol were tested against the Egyptian vector snails B. alexandrina, B. truncatus, and Lymnaea caillaudi. The impact of the extract on the egg clutches of B. alexandrina and L. caillaudi was also evaluated. Allam et al. (2003) showed those snails and their eggs when exposed for 24 and 48 hours at 22 ºC to 26 ºC to various concentrations of the oil extract exhibited different susceptibilities. B. alexandrina showed higher LC50 and
LC$_{90}$ (155, 195 ppm) than $B$. truncatus (50, 95 ppm) and $L$. caillaudi (50, 85 ppm) after 24-hour exposure. One hundred percent mortality was obtained for the egg clutches of $B$. alexandrina and $L$. caillaudi at concentrations of 100 ppm and 75 ppm, respectively. Myrrh has mollusccidal effect on infected $B$. truncatus and $B$. alexandrina snails at low concentrations of 10 and 20 ppm, respectively after 24 hours exposure. The number of dead-snails increased with prolongation of exposure time (Massoud & Habib, 2003). In a recent study, Almathal & Fouad (2006) found that $C$. molmol has a mollusccidal effect on Biomphalaria arabica snails at low concentration (40 ppm) after 48 hours exposure. However, the eggs were more resistant to $C$. molmol effect than adult snails, where embryogenesis began to stop at 20 ppm and eggs were all dead at 60 & 80 ppm. Furthermore, $C$. molmol showed a mollusccidal effect on Bithynia connollyi at concentration (80 ppm) after 72 hr exposure. The mortality rate increased with the increasing of exposure period (death 100% at 72 hr, Shoukry, 2006). Massoud et al. (2004) showed that sub-lethal exposure to myrrh decreased the compatibility of $B$. alexandrina to S. mansoni infection, thus playing an important role in the control of schistosomiasis. Abu-Naser (2003) showed that the water solution of $C$. molmol was more toxic to L. natalensis infected and non-infected snails, where the 24hr-LC$_{50}$ values were 34.95 ppm and 66.117 ppm, respectively. It was also found that the mollusccidal activity of plant suspension was time dependent.

The present study is yet the first attempt to investigate the toxicity of the $C$. molmol aquous suspension in vitro against non-target aquatic organisms coexisting with the snails in their habitat. The results obtained revealed that the plant water suspension was more toxic at the mollusccidal levels to the crustaceans Daphnia sp, Cardina nilotica, Cypris sp and Cyclops sp., where 100% mortality after 48 hr exposure was obtained at concentrations of 20, 40, 50 and 50 ppm, respectively. In addition, the plant suspension was toxic to the tested fish G. affinis, which were all dead (100 % mortality) at a concentration of 50 ppm after 48 hr of exposure. Culex larvae were more tolerant to the plant suspension than other organisms. In contrast, the plant molluscsicide Ammi majus fruit did not cause mortalities among all the studied non-target aquatic species when applied at a concentration equivalent to that used in the control of snails (Al-Sharkawi & Rizk, 1996). Recently, Massoud et al. (2005) showed that myrrh has been shown in vitro to be toxic to the fowl tick Argas persicus and also it was larvicidal against Culex pipiens and Aedes caspius mosquitoes (Massoud and Labib, 2000; Masoud et al., 2000 & 2001). On the other hand, Roe et al (2005) found no toxicity symptoms upon chronic treatment with $C$. molmol during 30 days in mice and biochemical investigation showed no significant differences as compared to the control except mild reduction in CK-MB and serum GOT, which was statistically non-significant. In contrast, Oliveira & Paumgarten (2000) showed that the lyophilized latex of Euphorbia millii killed the target snails’ B. glabrata and B. tenagophila by very low concentrations (0.12 and 0.09 ppm respectively). However, latex was less toxic to other nontarget aquatic species i.e. the oligochaete Tubifex tubifex, 0.31 ppm; planktonic crustacean (Daphnia similis, 0.38 ppm; and considerably less toxic to amullariid snail (Pomacea sp., 10.55 ppm) and other insect larvae. The toxicity of $C$. molmol against the target snails and nontarget organisms may be attributed to the presence of high contents of volatile oils that are known to have depressant action on the central nervous system (Ahmad et al., 1993). Recently, Mills & Bone (2005) found that myrrh is composed of a volatile (essential) oil (two to ten percent), including sesquiterpenes, an alcohol-soluble resin (25 to 40%) containing commiphoric acids and a water-soluble gum (30 to 60%). In addition, Evans, (1989) and Rao et al. (2001) found that $C$. molmol (oleo–gum–resin) contains volatile oils (up to 17%), resins (up to 40%), and gum (up to 60%). In the volatile oil fraction different terpenes, sesquiterpenes, esters, cinnamaldehyde, ciminaldehyde, cumicalcohol, eugenol, heerabolene, limonene, dipentene, pinene, m-cresol and cadinene were identified. The resins were found to contain α-, β- and Y - commiphoric acids, commiphoronic acid, α-,β-herrabomyrhol, heeraboressene, commiferin, ketosteroids, compesterol, -sitosterol, cholesterol, α-amyrone and 3-epi- α -amyrin. The gum on hydrolysis yielded arabinose, galactose, xylose and 4-O-methylglucuronic acid.

The present study revealed that chronic effects of sublethal concentrations of $C$. molmol water suspension on some biological parameters of $B$. truncatus showed a significant decrease in food consumption. Massoud et al. (2000) showed that feeding of $B$. alexandrina and L. natalensis was inhibited from the first day of treatment with both the oil and oleo-resin extracts of $C$. molmol. In addition, these observations coincide with that of Abd El-Meeged (1999) on L. caillaudi exposed to C. micrantha officinalis plant extract. This may be due to the potential of allergic contact dermatitis, inflammation of the mouth leading to mouth ulcers, where the feeding of snails was stopped (Scientific Committee of ESCOP, 1999 and Saeed & Sabir, 2004).

The inhibitory effect of $C$. molmol on reproduction, which appeared in the decreased number of eggs from the 2nd to 6th weeks of exposure or delay in the sexual maturation of experimental snails, was also manifested. Several parameters of ovulation were affected.
by the tested compound due to its activity on the genital organs of the snails (Joose, 1988; Wilbrink, 1991). Firstly, gametogenesis was regulated by dorsal bodies on the nervous system that produce dorsal body hormone (DBH) which is responsible for egg – cell maturation in ovotestis (effects on number of eggs). Secondly, at the level of the nervous system, where ovulation hormone producing cells induce oviposition (effects on number of egg mass). These findings were explained by Abu-Nasr (2003) that the histological structure of the caudo-dorsal cells and dorsal body cells of exposed L. natalensis to C. molmol showed complete damage and denaturation of the neurosecretory cell nuclei and the surrounding cytoplasm. In general, presence of volatile oils in C. molmol is known to have depressant action on the central nervous system (Ahmad et al., 1993).

The present results also revealed that at LC50, no hatching was observed after 17 days of exposure. After 24 hour of exposure to C. molmol water suspension 70% mortality of newly hatched snails (3-days old) was observed at 50 ppm. The eggs were less sensitive to plant solutions toxicity than the juveniles which may be due to the envelope surrounding the eggs. This result agrees with Olivares et al. (1982) and Tang et al. (1995) who showed that extracts of Millettia thonnigii seeds contain considerable concentrations of isoflavonoides such as alpinumisoflavone and robustic acid, which have molecular weights of less than 400 and might therefore, be able to penetrate into the local environment of embryos in B. glabrata egg masses.

Biochemical investigation of exposed snails to C. molmol water suspension showed significant differences as compared to the control in the concentrations of total protein, transaminases (AST, ALT), lactate dehydrogenase (LD), except mild decrease in glycogen concentration and alkaline phosphatase activity. These results are in accordance with those obtained by Abu-Nasr (2003) who found that the water solution of C. molmol was more toxic to L. natalensis with significant increase (P<0.05) in activity of transaminase enzymes, a highly significant increase (P<0.05) in lactate dehydrogenase and decrease in alkan phase in hemolymph and hepatopancreas-gonad complex tissue. Tiwari et al. (2004) stated that after exposure to sub-lethal concentrations of Euphorbia royleana latex fraction there were significant time and dose dependent alterations in pyruvate, lactate levels, ALAT, AAT, AChE and cytochrome oxidase enzyme activities in different body tissues of Lymnaea acuminata.

Up to the present, detailed information are lacking concerning the mode of action even of the plant molluscicides especially the tested C. molmol. Nowadays a great attention is paid to evaluate the toxic action of plant molluscicides on the snail’s tissues and their structure and function (Brackenbury, 1999). The present study showed that the ultrastructural investigations of exposed snails to C. molmol water suspension showed some alterations, extensive vacuolation, increased lysosomal activity, and the mitochondria appeared swollen with consumption of stored lipid and glycogen. Triebskorn (1991) reported cells damage, mucous activation, damage of nuclei and mitochondria as well as reduction of storage products after L. stagnalis was treated with a carbamate molluscicide and metaldehyde. Moreover, Bode et al. (1996) followed the effect of subchronic dose of Tetrapleura terpleura on the digestive cells of the hepatopancreas of B. glabrata. They stated that there was a significant increase in the number of secretory cells and decrease of digestive cells. Furthermore, the ultrastructural investigation of the digestive epithelium showed autolysis of membranous structures like Golgi and endoplasmic reticulum. Hepatopancreas and reproductive system of Oncemelania hupensis were examined by Song-Geng et al. (1997) using transmission electron microscopy after exposure to Camellia sinesis. They reported necrosis of parenchyma cells of the digestive gland and deformation of sperm and ova. Eissa, et al. (2002) showed that chronic exposure of B. alexandrina, and L. carinatus. Snails to Euphorbia peplus water suspension, caused deformation and haemolysis of haemocytes. TEM study revealed that the plant has high action on different cell organelles, leading to nuclear karyolysis, lysosomal degeneration, swelling and damage of mitochondria and endoplasmic reticulum.

On the other hand, Brackenbury (1999) observed a graded series of cellular injuries to the epithelial layer along length of the digestive tract of Bulinus africanus exposed to aqueous extracts of Agave attenuata. Also, Abd-El-Megeed (1999) showed great destructive effect on stomach epithelial cells as degeneration of some cells and shrinkage of others, besides swelling in the cell size and their nuclei of digestive gland cells when Lymnaea caillaudi was exposed to sublethal concentration of Calendula micrantha officinalis.

From the above interpretation and based on the toxicity of C. molmol on aquatic ecosystem at the molluscidal levels, is not yet recommended as an herbal molluscicide against the vector snails.
REFERENCES


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الوفيات للفوقات حديثة النفس (عمر 3 أيام) هو 70% عند تعرضاً لتركيز 50 جرء في المليل للملعقة المانعة لللصفي. كذلك أظهرت بعض المفاعلات البكوبكيمائية تغييرات ذات إصابة غير قوية بالرقم المقابل في تركيزات كل من الحيوانات الريثياني وفي نشاط أنزيمي ترانزيمير (Lactate dehydrogenases) وآنثيم للكيتي ديدروجين (ALT alkaline) ونفاك متزن لإمزج الريثياني الفلفي (phosphatase) وكذلك في محتواج الجليكوجين في سرطان عددها القلبي المكون في تركيز 0.75 مليمول/لتر. وقد أظهر التركيب المجاري الدقيق بالميركوسكوب الإلكتروني الباليد بعض التغييرات في النسيج البلوي للعدة الهامة للفوقات مثل زيادة في النشاط المذكور لخلايا العدد زيادة في النشاط الديمومي وحذف إنتاج للميزوكوندريفاستركت الخليطة محتواها من الدهون والجليكوجين. وبناء على النتائج المقدمة للملعقة المانعة كوميفورا مولمول (أم) ضد الأحياء المانعة غير المستهدفة، يوصى البحث بعدم استخدامها كمبيد للملاحة.

المتحكم:

1. كليه علم عين شمس.
2. كليه العلوم - جامعة النهضة.


http://www.egyptseb.org