

Histopathological and Ultrastructural Studies on *Biomphalaria alexandrina* Snails Infected with *Schistosoma mansoni* miracidia and Treated with Plant Extracts

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ABSTRACT

Background: *Biomphalaria alexandrina* snails are the intermediate host of *Schistosoma mansoni* in Egypt. **Aim of the work:** this study aimed to evaluate the molluscicidal activity of the methanol extract of the plants *Anagallis arvensis* and *Viburnum tinus* against *B. alexandrina* (Normal and *S. mansoni* infected). **Results:** the present results proved high activity for both plant extracts (LC₅₀ & LC₉₀ which reached 45 & 60 ppm and 38 & 59 ppm for *A. arvensis* and *V. tinus*, respectively). The effect of sub-lethal concentration, ½ LC₅₀, of the two plant extracts (26 and 11 ppm, respectively) affected *B. alexandrina* survival rate to be in the following order, control > *V. tinus* treated > *A. arvensis* treated > infected > infected-*A. arvensis* treated > infected-*V. tinus* treated. On the other hand, exposure to those sub-lethal doses caused considerable reduction in the infection percentages. In addition, the histopathological effects of the examined sub-lethal concentrations on hepatopancreatic tubules of the treated snails showed cells vacuolation, presence of hyaline substances filled the lumens of the tubules and necrotic focal areas in case of *A. arvensis* and vacuolar degeneration with the necrotic changes in case of *V. tinus*. While, alterations in the hermaphrodite glands of the treated snails included: degeneration and necrotic changes in the acini. The severity of lesions was progressed with infection as a result of invading of snail tissue by developmental stages of the *S. mansoni* cercariae. The ultrastructural micrographs were used to explain and confirm the recorded histopathological alterations in the hermaphrodite glands of the infected-treated snails. In comparison with the control and infected snail groups, infected-treated snails showed degeneration with severe deformation and destruction in their reproductive units, degeneration in developmental stages tissues of *S. mansoni* cercariae and accumulation of the toxic agents. **Conclusion:** the two examined plants, *A. arvensis* and *V. tinus* plant extracts showed high activity against *B. alexandrina* and provide a considerable scope in exploiting local indigenous resources for snail's molluscicidal agents. The sub-lethal concentrations, ½ LC₅₀, of the two plant extracts caused a considerable reduction in survival rate and infection rate among *S. mansoni* infected snails. Histopathological changes in the digestive glands showed cells vacuolation, hyaline substance filled lumens of the tubules and necrotic focal areas in the digestive glands. Histopathological effects explained and confirmed by TEM images showed degeneration with severe deformation and destruction in the reproductive units.

Keywords: *Biomphalaria alexandrina*, snail control, *Anagallis arvensis*, *Viburnum tinus*, *Schistosoma mansoni* infection, histology, Transmission Electron Microscope (TEM).

INTRODUCTION

Biomphalaria alexandrina snails are the intermediate host of *Schistosoma mansoni* in Egypt, they had colonized The River Nile from the Delta to Lake Nasser then extended their distribution from the Nile Delta throughout the country resulted in an increase in schistosomiasis transmission^[1]

Use of molluscicides in snail control showed a significant effect in reducing both incidence and prevalence of schistosomiasis. Plant molluscicides are inexpensive and have a potential to be biodegradable in nature and appropriate technology for focal control of the snail vectors^[2]. There are other chemical

compounds that may reach water sources during the agricultural

activities such as herbicides, fungicides and pesticides which may kill snails or make their environmental conditions unsuitable for their life^[3]. For about two decades, increasing trails are given to the study of plant molluscicides in hope that may prove less toxic, cheaper, readily available and easily applicable by simple technique, but the ideal applicable molluscicide did not proposed yet^[4-6]. Also, El Emam^[7] used relatively high concentrations of dry powder of *A. arvensis* (125 and 100 ppm) to induce death of snails in two field trials carried out in Sharkia

Governorate to control vector snails of schistosomiasis and fascioliasis.

Abdel-Gawad^[8] proved strong molluscicidal activity of saponins isolated from the plant *A. arvensis* against schistosome intermediate hosts, *Biomphalaria glabrata* and *Oncomelania quadrasi*. From the leaves of *Viburnum tinus* L. (Adoxaceae) two acylated iridoid glucosides (viburtinoside A and B), a coumarin diglucoside scopoletin 7-O-β-D-sophoroside and a natural occurred dinicotinic acid ester 2, 6-di-C-methyl-nicotinic acid 3, 5-diethyl ester were isolated. Toxicity of the investigated extract was determined (LD₅₀ = 500 mg/kg). Their highly elevated levels were significantly reduced by treatment with the investigated aqueous methanol extract in dose-dependant manner^[3]

The present work was planned to search for ideal source alternative to synthetic molluscicides. So we investigated the effect of extracts of two natural plants, *A. arvensis* and *V. tinus*, as molluscicidal agents against *B. alexandrina*. Also, effects of their sub-lethal doses were studied on *S. mansoni* infected and non-infected *B. alexandrina* through histological and ultrastructural investigations.

MATERIAL AND METHODS

Plant extracts

Anagallis arvensis (Family Agavaceae) and *Viburnum tinus* (Caprifoliaceae) plants were collected at the flowering stages during March–April 2015 from Giza Governorate, Egypt. Plants were identified then shade dried and finely powdered using an electrical grinder. The dry powder of the experimental plants was stored in clean, dark and dry conditions at room temperature till used. The dry powder of the plants *A. arvensis* and *V. tinus* (200 g) was extracted in Medicinal Chemistry Laboratory, Theodor Bilharz Research Institute (TBRI) by using 95% methanol for 5 days at room temperature. The solvent was filtered and evaporated under vacuum for dryness by using rotatory evaporator at temperature >50°C. The extraction process was repeated several times and the dried extracts were kept for bioassay tests.

Snail samples

B. alexandrina snails were collected from irrigation canals in Giza Governorate, transferred to the Environmental Research Laboratory, (TBRI) where they were thoroughly washed and maintained under laboratory conditions in plastic aquaria and fed on green lettuce leaves for 4 weeks before being used. During this period snails were

examined weekly for their natural infection by exposure to a light source for one hour to detect any cercarial shedding and exclude positive ones. Adult healthy negative snails were selected of uniform size of 10–12 mm. Each 50 snails were maintained in plastic aquaria containing 5 liters of de-chlorinated tap water. Water changed weekly and snails were fed on boiled lettuce twice a week.

Experimental infection of snails

Laboratory breeding of *B. alexandrina*: the offsprings of the collected field snails, of size 5–7 mm were exposed individually to 7–10 freshly hatched *S. mansoni* miracidia that supplied by Schistosome Biological Supply Program (SBSP) at TBRI. Snails were allowed to remain in contact with miracidia overnight and then snails were washed thoroughly and maintained in a separate aquarium under laboratory conditions. Water temperature was maintained between 24–26°C throughout the period of the experiment. The snails were tested weekly for shedding cercariae starting from 21 day post-exposure by exposing them, individually, to fluorescent light in 2 ml of water for 1 hours at 25°C.

Evaluation of molluscicidal activity of *Anagallis arvensis* and *Viburnum tinus* extracts against adult *Biomphalaria alexandrina* snails:

Serial concentrations of each plant extracts (5, 10, 30, 60, 80, and 100 ppm per liter in glass beakers) were done in 2 replicates and 10 snails / replicate were added. Another set with 2 replicates was done using de-chlorinated tap-water only as a control. Exposure and recovery periods were 24 hours each and then mortality counts were recorded and corrected according to **Abbott**^[9]. Mortality regression lines were established by SPSS Computer Program 20.0.

Exposure to of sublethal concentrations of *A. arvensis* and *V. tinus* plant extracts:

Effects of the ½ LC₅ of the methanol extracts of *A. arvensis* and *V. tinus* (13 & 5.53 ppm, respectively) on six different snail groups three non-infected (1, 2 & 3) and three infected (4, 5 & 6) were studied. Group I included unexposed snails and used as control. Groups 2 and 3 were exposed to *A. arvensis* and *V. tinus*, respectively. Group 4 included the infected snails and used as the infected control. Group 5 and 6 included infected and exposed snails to *A. arvensis* and *V. tinus*, respectively. Each group was represented by five

replicates of small plastic aquaria (500 ml capacity) each contained 10 *B. alexandrina*. Water temperature was adjusted to 24 ± 2 °C. Groups of non-infected snails were exposed for 6 weeks, while groups of infected snails were exposed in the first day of infection till the cercarial shedding. The experimental snails were fed fresh lettuce leaves and extract solutions of each aquarium were changed twice a week. At the end of the experiment, the survival rate, infection rate and prepatent period were calculated. Also, the histopathological and ultrastructural changes were investigated.

For histopathological study, the snail samples were randomly selected from the experimental groups. The soft parts of snails were dissected out from the shells after gently crushing between two glass slides and the shell fragments were removed using pointed forceps under the dissecting microscope then fixed in 10% neutral buffered formalin solution, washed, dehydrated, cleared and paraffin sections (5 µm) were prepared. Serial sections were cut at 5 µm thickness using rotary microtome. Sections were hydrated, stained with dyes; hematoxylin and eosin (HE) according to the method of **Bancroft and Stevens**^[10] then microscopically examined and photographed to record the histopathological observations

For ultrastructural study

Snail specimens collected from groups; control, infected control and infected treated groups (1, 4, 5 and 6). The present technique had been achieved according to **Reynolds**^[11]. This technique included: anesthetizing the target snails with 30% ethyl alcohol, dissection to obtain the hermaphrodite glands and cutting it into small pieces, fixing with 2.5% paraformaldehyde-3% glutaraldehyde (pH 6.7) and post-fixed with 1% phosphate buffered 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 specimens were rinsed in 0.2 M phosphate buffer (pH 7.3), dehydrated in ethyl alcohol and embedded in epon 812 mixture. Thin sections for transmission electron microscopy were prepared by 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 by using TEM (JEM 100CX II transmission electron microscope operated at 80 kV).

Statistical analysis

Data were expressed as means \pm SD. The results were computed statistically significant by used A one-way analysis of variance (ANOVA).

The study was approved by the Ethics Board of Al-Azhar University.

RESULT

Molluscicidal activity of *Anagallis arvensis* and *Viburnum tinus* extracts against adult *Biomphalaria alexandrina* snails:

Results presented in **table 1** showed values of $\frac{1}{2}$ LC₅, LC₅₀ & LC₉₀ obtained by probit analysis of the two plants against *B. alexandrina* by using SPSS program. It has been shown that *V. tinus* was more potent than *A. arvensis* especially in the lower doses till LC₅₀ then the efficiency of the two plant extracts gradually approaching each other and approximately coincided at LC₉₀ (LC₉₀ values of *A. arvensis* and *V. tinus* were 60 ppm and 59 ppm, respectively).

Effect of sub-lethal concentrations of plant extracts were done on the survival rate of the six examined groups three non-infected (one control and two treated groups with *A. arvensis* and *V. tinus* and three groups were studied and their results were illustrated in **fig. 2**. The survival rate of the infected snails were significantly lower than that of the non-infected snails (The survival rate of infected control, infected treated with *A. arvensis* and infected treated with *V. tinus* were 62, 57 & 51 and of control un-treated, treated with *A. arvensis* and treated with *V. tinus* were 89, 83 & 87, respectively).

The results presented in **table 2**: exposure of *B. alexandrina* snails to $\frac{1}{2}$ LC₅ of *A. arvensis* and *V. tinus* extracts was significantly lower ($P < 0.05$) in the infection rates with *S. mansoni* meracidia than in the experimental groups 5 & 6 compare to the infected control group. The infection rate of *B. alexandrina* snails exposed to of *A. arvensis* and *V. tinus* extracts were 69% and 56%, respectively, while the infection rate of the control group was 82%. All snail groups started shedding at 21 day post infection however infected groups (however the treated groups) had significantly shorter means of prepatent periods than the control group (32 ± 9.37 , 34 ± 7.23 and 43 ± 12.5) infected snails exposed to *A. arvensis*, infected snails exposed to *V. tinus* extracts and infected control group, respectively).

Hepatopancreatic gland (Digestive gland):

Sections from the control *B. alexandrina* [Group 1] showed normal histological structure of the

digestive gland, hepatopancreas included glandular tubules interspersed with connective tissues. The hepatopancreatic epithelium is rested on thin basement membrane consists of two types of cells; the excretory cells which contain granular cytoplasm and digestive cells which have basal nuclei [Fig. 1a]. Alterations exhibited in hepatopancreatic tubules treated with *A.arvnsis* plant and non infected [Group 2] were cells vacuolation, presence of hyaline substances filled the lumens of the tubules and necrotic focal areas [Fig. 1b]. In addition in the treated snails with *V. tinus* [Group 3] the hepatopancreatic cells showed vacuolar degeneration with necrotic changes [Fig 1c]. The severity of lesions was progressed with infected and non treated snails. The hepatopancreas of snail infected and non treated [Group 4] showed severe vacuolar degeneration with developmental stages of the cercariae parasite [Fig. 1d]. These hepatopancreas histological alterations were more evident in snails infected and treated with *A.arvnsis* [Group 5], in addition to necrosis [Fig. 1e]. Group six [infected and treated with *Viburnum tinus*] showed the same lesions in obvious group [Fig. 1f].

The hermaphrodite gland: normal hermaphrodite gland of the adult *B. alexandrina* snails was consisted of number of vesicles known as acini separated from each other by thin vascular connective tissue [Figs. 2a, 2b]. Each acinus was enveloped in a sheath of squamous epithelium. In each acinus both male and female reproductive gametes were produced where mature ova were located at the periphery of the acini and bundles of male sperms were arranged in the center. Various

stages of sperm and ovum development (simultaneous) were evident.

Alterations exhibited in the hermaphrodite of snail treated with *A.arvnsis* [Group 2] included degenerative and necrotic changes in the acini [Fig. 2c]. Sever necrotic changes were realized[Group 3] in the hermaphrodite of snail treated with *Viburnum tinus* [Fig. 2d]. Moreover, in the infected and non treated [Group 4] developing schistosoma cercariae were filled hermaphrodite acini [Fig. 2f], the same lesions were noticed in group 5 [Infected with *A.arvnsis*] and group 6[Infected +nontreated] [Figs. 2e,2g] respectively.

Ultrastructure examinations of the hermaphrodite gland of the non-treated "control" of the present snails had acini with normal architecture shape. All the mature ova had nucleus and identical shape and also, their yolk layers were found (Fig. 5a). The sperms consisted of head, neck and the glycogen helix region, (Figs. 5b,c&d). On the other hand, the hermaphrodite gland of the infected target snails had obvious developmental stages of the cercariae of parasite, (Fig.6a) degenerative ova, (Figs. 6a&b) and destruction of sperms was shown in fig. 6c. The induced histopathological changes in the hermaphrodite gland of the infected snails treated with sub-lethal dose of $\frac{1}{2}$ LC₅ of *A. arvnsis* and *V. tinus* included degeneration and disappearance in gonadal cells with severe deformation, destruction in the reproductive units (Figs. 7a&b and 8a&b, respectively), presence of some vacuoles in cells of all tissues besides degeneration in tissues of the developmental stages of cercariae, (Figs. 7c and 8c, respectively) and accumulation of the toxic agents of the target plants (Figs. 7d and 8d).

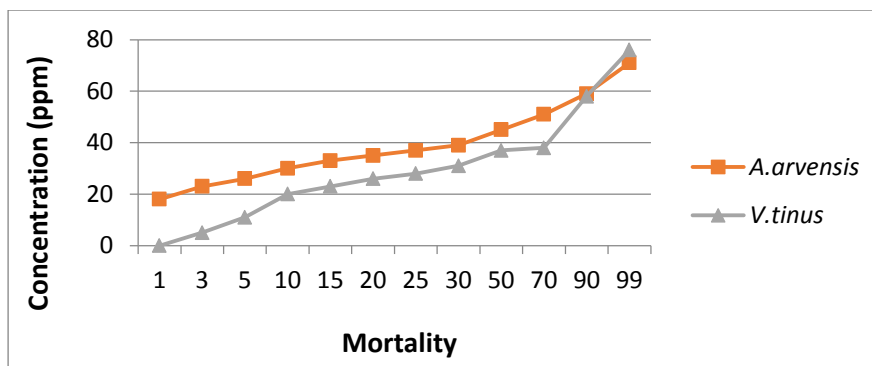


Fig. 1: comparison of *Anagallis arvensis* and *Viburnum tinus* extracts toxicity on adult *B. alexandrina* snails

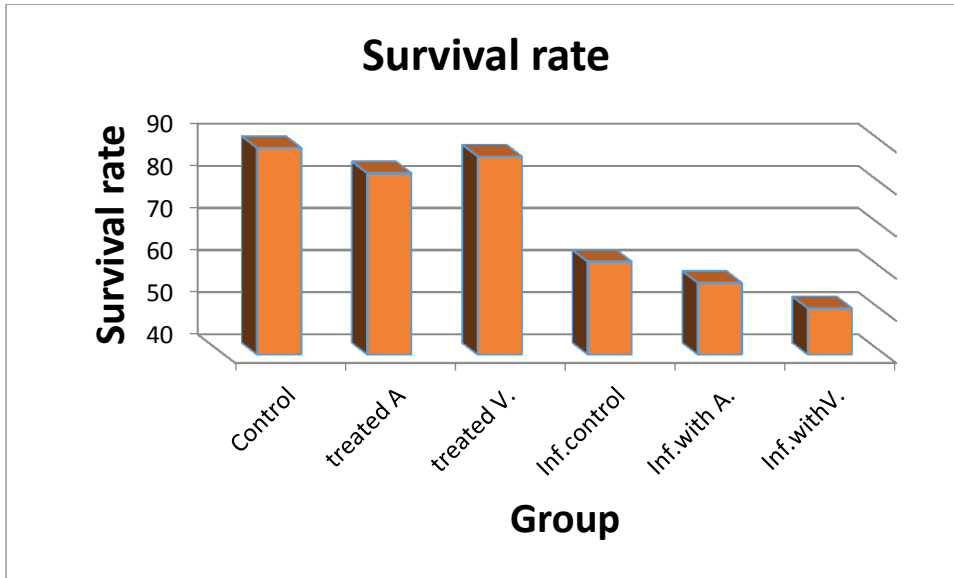


Fig. 2: effect of $\frac{1}{2}$ LC₅ of *Anagallis arvensis*(A) and *Viburnum tinus* plant extracts on survival rate of *Biomphalaria alexandrina* snails.

Table 1: molluscicidal activity of *Anagallis arvensis* and *Viburnum tinus* against adult *Biomphalaria alexandrina* snails after 24 hours exposure period

Lethal Dose Values		
	<i>Anagallis arvensis</i> (ppm)	<i>Viburnum tinus</i> (ppm)
$\frac{1}{2}$ LC ₅	13	5.5
LC ₅₀	45	38
LC ₉₀	60	59

Table 2: effect of $\frac{1}{2}$ LC₅ of extract of *A. arvensis* and *V. tinus* plants on the infection of *Biomphalaria alexandrina* snails with *Schistosoma mansoni* miracidia

Groups	No of examined Snails	Prepatent (days)		Infected Snails	
		Min –Max (days) Range	Mean± SD	NO.	%
4 (inf.) Control	50	21-58	48±0.52	25	82
5 (inf.)Treated with <i>A. arvensis</i>	50	21-48	38±0.64***	19	69
6 (inf.)Treated with <i>V. inus</i>	50	21-45	31±0.34***	14	56

*** significant compared to control value at p<0.001

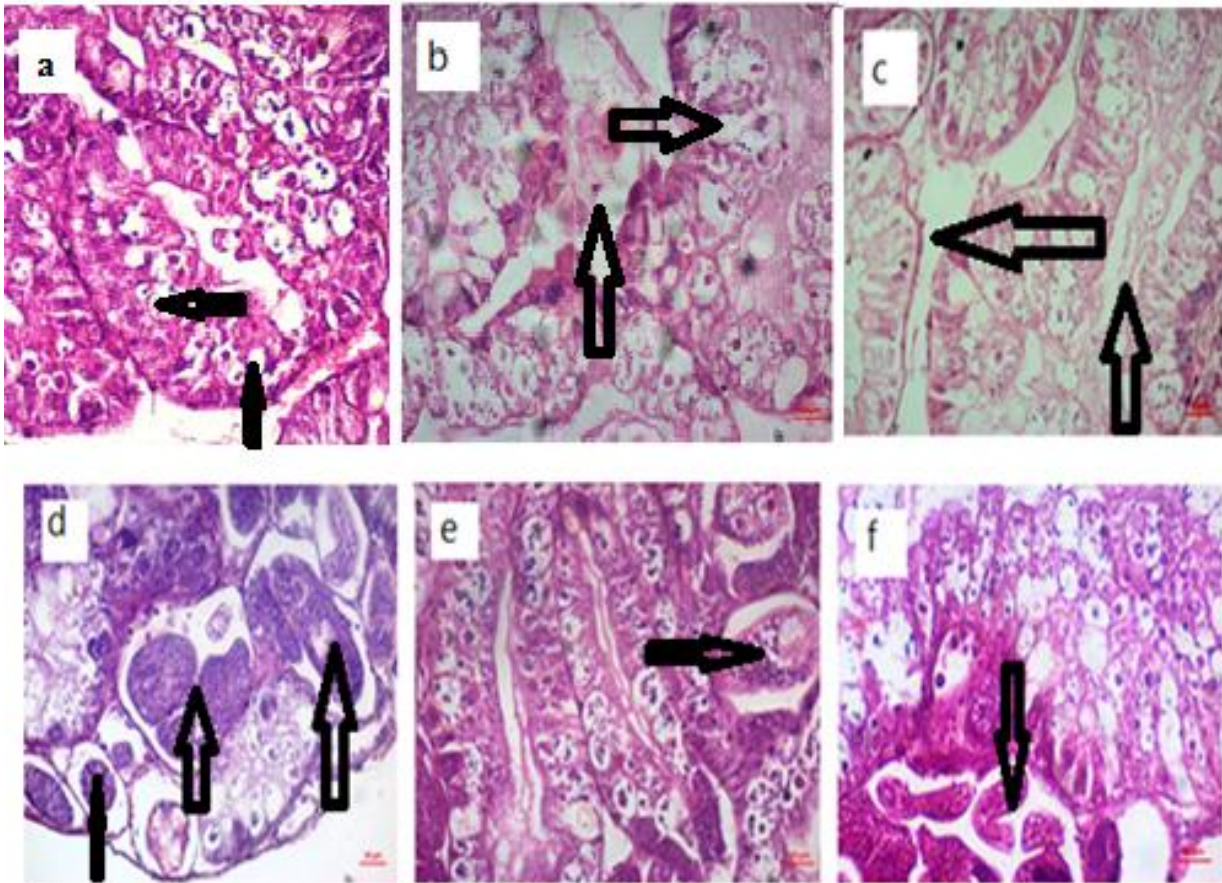


Fig 3:photomicrographs of transverse sections (T.S.) in the digestive gland of *B. alexandrina* snails (Hematoxylin and eosin) showing:
a- control normal digestive gland of *B. alexandrina* snails showing secretory cells and digestive cell (400 X);
b- treated *B. alexandrina* snails with sublethal dose of *A.arvensis* showing necrotic changes of the secretory cells (400 X) ;
c- *B. alexandrina* treated with *V.tinus* showinghyaline substances filled the lumens of the tubules (x400);
d-tubules filled with different cercariae stages and cells disappear (X400);
e-infected *B. alexandrina* treated with *A.arvensis*, necrotic changes of the secretory and digestive cells (X400);
f- infected *B. alexandrina* treated with *V.tinus* showing necrotic changes of the secretory and digestive cells with different stages of cercariae filled tubules (X400) .

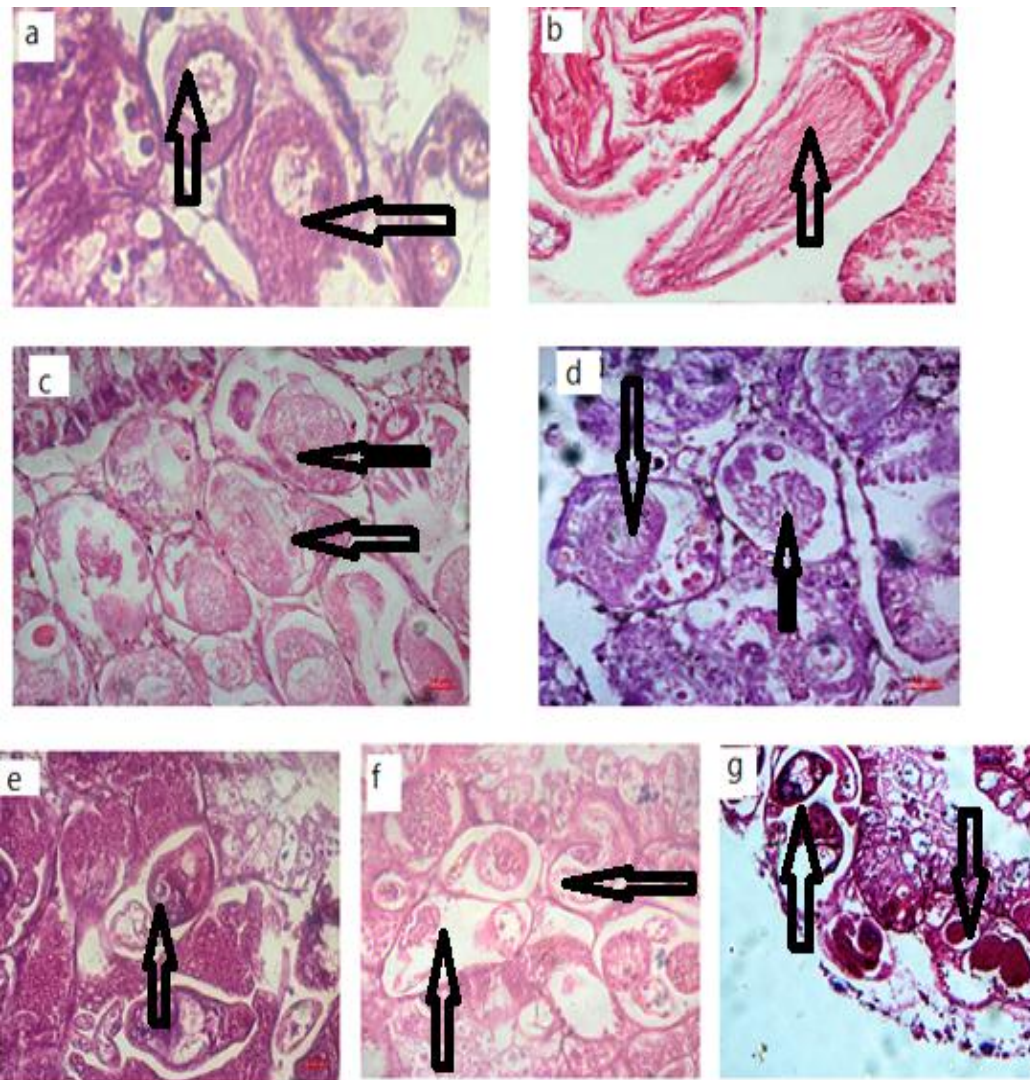


Fig. 4: transverse sections (T.S.) in hermaphrodite gland of *B. alexandrina* snails (Hematoxylin and eosin stained) showing

a,b-hermaphrodite gland of *B. alexandrina* snails showing the control, oocytes and spermatocysts (X400); c- treated *B. alexandrina* snails with sublethal dose of *A.arvensis*, showed degenerated oocytes (X400); d-treated *B. alexandrina* snails with sublethal dose of *V.tinus*; e-severe degenerated oocytes and sperms and necrotic change (X400); f- developing schistosoma cercariae in snails treated with *A.arvensis* and treated with *V.tinus* (X400) and infected control(X100) (g) .

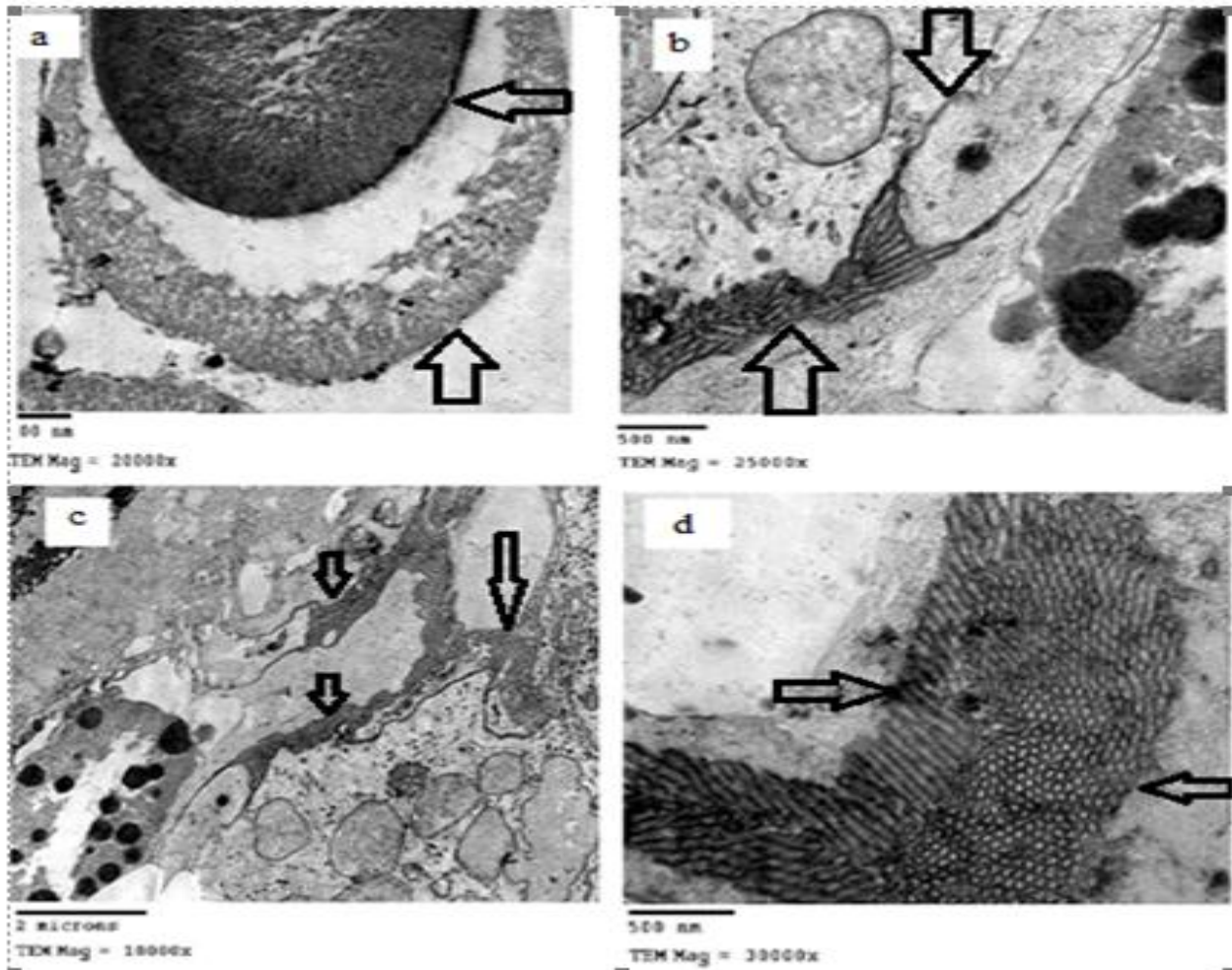


Fig 5: electron micrographs showing the hermaphrodite gland of non-infected non-treated *Biomphalaria alexandrina* (control); a: showing the mature ova with nucleus, yolk layer and identical shape (X20000); b&c: normal sperm (X25000 &X10000, respectively) and d: transverse and longitudinal sections in a sperm bundle (X30000).

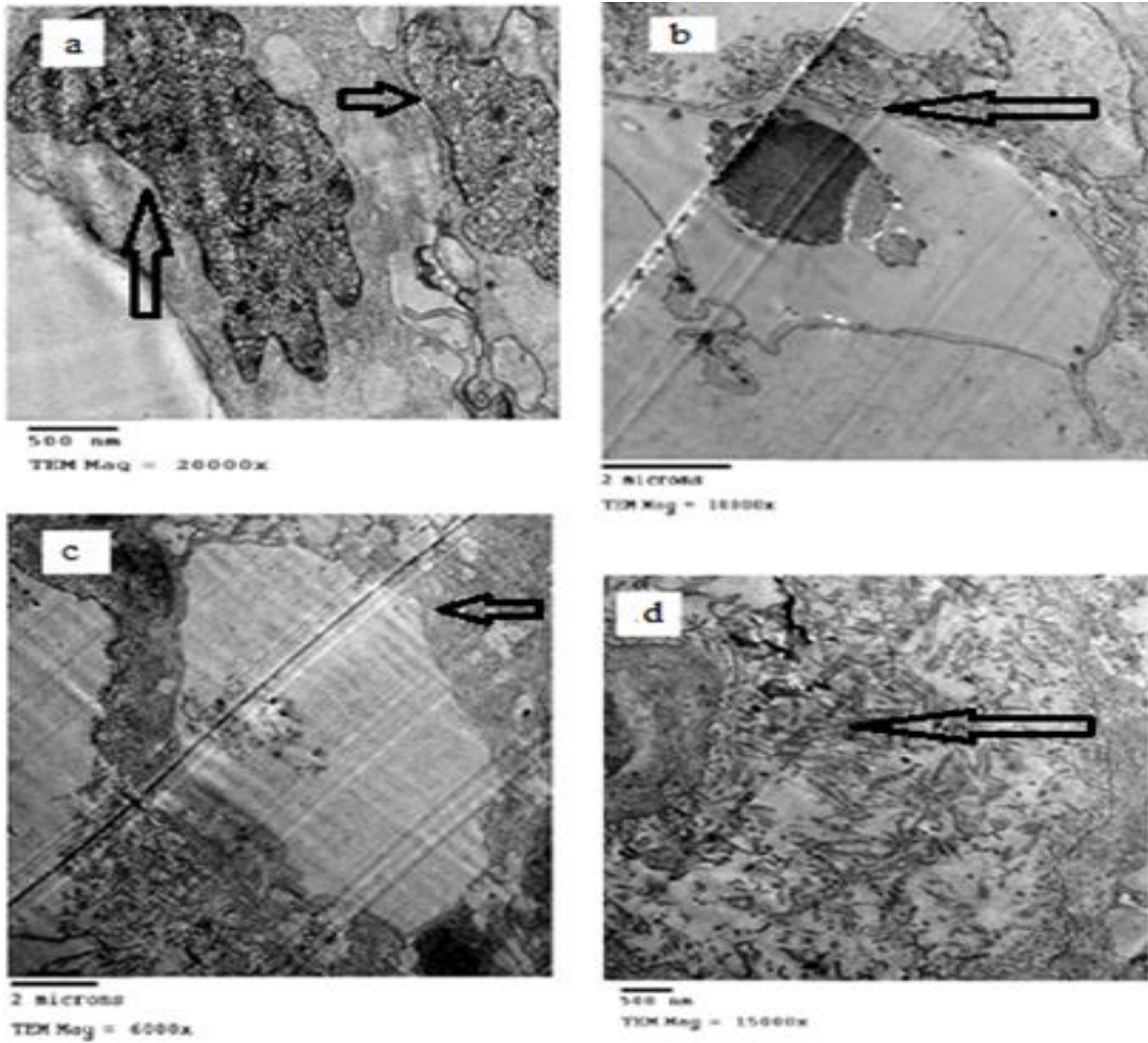


Fig 6: electron micrographs showing the hermaphrodite gland of *Biomphalaria alexandrina* infected with *Schistosoma mansoni* (non-treated); a: showing obvious normal developmental stages of cercariae (20000X); b&c: showing degenerated ova (X6000 & X10000, respectively), and d: showing destroyed sperms (X15000).

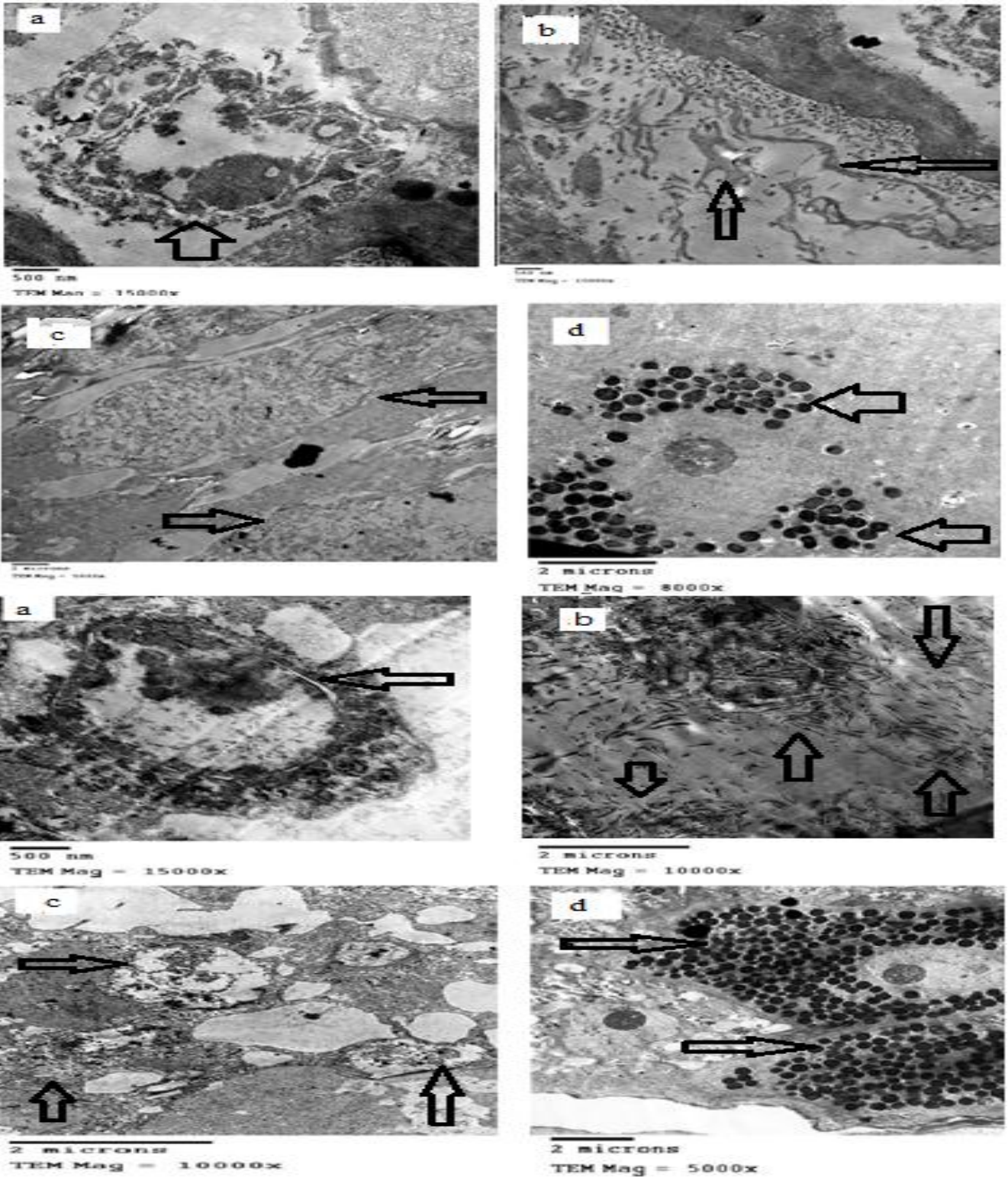


Fig 7: electron micrographs showing the hermaphrodite gland of *Biomphalaria alexandrina* infected-treated with *Anagalis arvensis*; 1: showing degenerated ova (X15000), 2: showing damage and destruction of sperms (10000X), 3: showing degenerated tissues of the developmental stages cercariae (X5000) and 4: showing accumulation of the toxic agents of the target plants and degeneration at nuclear membrane of secretory cells (8000X).

Fig 8: electron micrographs showing the hermaphrodite gland of *Biomphalaria alexandrina* treated-infected with *Vibrunum tinus*; a: showing degenerated ova (X15000), b: showing damage and destruction of sperms (15000X) c: showing degeneration in tissues of developmental stages of cercariae (10000X) and d: showing accumulation of the toxic agents of the target plants and degeneration at nuclear membrane of secretory cells (X5000).

DISCUSSION

In view of need to search for natural products with molluscicidal activity and low operational cost, the present screening for molluscicidal activity *A. arvensis* and *V. tinus* methanolic plant extracts showed high molluscicidal activity against *B. alexandrina* (LC₅₀ values were 45 and 38 ppm and LC₉₀ values were 59 and 58 ppm, respectively). Several authors studied the water extract of *A. arvensis* and confirmed the plant potency against *B. alexandrina* [12,13,14,16]. They estimated LC₅₀ ranged between 78-85 ppm and LC₉₀ between 88-135 ppm. The present tested extracts showed a significant reduction on the survival rate of both non-infected and *S. mansoni*-infected *B. alexandrina* snails. Such reduction of snail's survival may arise from metabolic disorders as a results of saponine compounds present in the two plant extracts. This study findings is in a harmony with the resuts obtained [15] who tested the low dose of methanol extract of *Oreopanax reticulatum*, *Azadirachta indica*, *Dizygotheca kerchoveana*, *Oreopanax reticulatum* and *Dizygotheca kerchoveana* plants on *Biomphalaria alexandrina* snails and *Schistosoma mansoni* stages and recorded reduction in the snails survival rate, infection rate and number of shedding cercariae. On the other hand, **Hasheesh** [16] found reduction in survival rates of *Bulinus truncatus* snails as well as in the infectivity of *Schistosoma haematobium* miracidia to the snail when used methanol extract of *Sesbania sesban* plant (LC₀, LC₁₀ and LC₂₅). In the same consequence, the present results confirmed reduction in the infection rates after exposure to *A. arvensis* and *V. tinus* extracts. This may be attributed to the activity of compounds in the extracts of the two tested plants that have weakened the ability of the penetrated miracidia to proliferate and established their developmental stages within different snail tissues. **Bakry** [17] observed reduction in the infection rate of *B. alexandrina* snails infected by *S. mansoni* miracidia and subjected to LC₂₅ methanol extracts of *Euphorbia lacteal*. These results also are in accordance with many investigations that used various chemical and plant molluscicides and revealed similar conclusions, **Mohamed** [3] examined *Abamectin*, **Tantawy** [18] examined *Solanium dubium*, **Bakry** [19] examined *Agava franzosin*, **Sharaf El-Din** [20] examined *Zygophyllum simplex* and **Bakry** [21] examined methanol extracts of *Oreopanax reticulatum* and *Furcraea selloea*.

In the present study, the two tested plant extracts induced histopathological changes in the digestive

and hermaphrodite glands. *A. arvensis* plant caused cells vacuolation, hyaline substance filled the lumens of the tubules and necrotic focal areas in the digestive gland. While, *V. tinus* caused vacuolar degeneration with necrotic changes. These findings agree with those recorded by **Yousef and El-Kassas** [22] who observed histopathological effects of of three Egyptian wild plant-extracts, as botanic toxic agents; *Euphorbia splendens*, *Ziziphus spina Christi* and *Ambrosia maritima* on the digestive gland of the infected-target snails, they showed numerous vacuoles in the digestive and excretory cells. Also, the present study demonstrated alterations in the hermaphrodite glands of the treated snails. These changes included degeneration and necrotic changes in the acini. The severity of lesions was progressed with infection as a result of invading of snail tissue by developmental stages of the *S. mansoni* cercariae. In the same consequence, [23] found histopathological changes in the hermaphrodite gland of *B. alexandrina* and *Lymnaea cailliaudi* snails after two weeks post exposure to LC₂₅ of the ethanolic extract *Euphorbia aphylla*, *Ziziphus spina-christi* and *Enterolobium contortisiliquum*. The same authors recorded degenerative changes in the hermaphrodite acini and their contents of ova and sperms. Also, [24] recorded severe changes in the sperms and ova besides degeneration in the gonadal acini structure of *B. alexandrina* snails post exposure to sub-lethal concentrations of *Sesbania sesban* plant. In addition, [25] found that the molluscicidal activity of *Asparagus densiflorus* and *Oreopanax guatemalensis* plants and Difenoconazole fungicide caused degeneration in the hermaphrodite gland tissue of *Biomphalaria alexandrina* snails. Furthermore, [26] showed that the hermaphrodite gland of treated *B. alexandrina* snails with diethyldithio-carbamate exhibited destruction of oocytes. Moreover, mature ova appeared to be necrotized and few sperms were represented.

The present ultrastructural study on hermaphrodite gland of *B. alexandrina* infected-treated with the plant extracts of *A. arvensis* and *V. tinus* showed that plant treatments induced degeneration and disappearance in the gonadal cells with severe deformed, destruction in the reproductive units. Also, degeneration in developmental stages tissues of *S. mansoni* cercariae and accumulation of the toxic agents of the target plants were demonstrated inside the hermaphrodite gland. The same observations are observed [27] who studied the effect of low

concentrations of *Euphorbia milii* plant extract against on the spermatozoon ultrastructure. The authors showed that examination of the longitudinal and transverse thin sections revealed that the genital cells gonadal follicles and the head and the neck regions of the sperm were the most affected ones. This indicated that these snails became nearly sterile where the gonads became unable to produce spermatozoa or the liberated sperms became immotile and they had damaged mitochondrial derivatives with loss of their viability. They concluded that this effect on sperms can be considered as a new way in snail control. [28] studied the ultrastructural of the hermaphrodite gland of *B. alexandrina* and *B. truncatus* exposed to LC25 revealed that some ova organelles were distorted and empty ova appeared. Moreover, this concentration caused fragmentation of convoluted membrane of some sperms with change in the axonemal microtubules, other sperms were suffered from fragmentation of the entire sperm structure and their contents were released. Other sperms were totally disfigured without any definite contents. Also, acinar epithelium showed necrotic changes in the form of invagination and partial destruction. Degenerative changes were observed in most of the ova, where some of them had faintly stained nuclei and others lost their nucleolus. Reduction in the number of sperms was also observed and some acini appeared more or less evacuated. Furthermore, these observations agreed with results of [29] who found that exposing *B. alexandrina* snails to sublethal concentrations of the photosensitizer hematoporphyrin coated gold nanoparticles revealed injuries in spermatocytes, oocytes, several degenerations of *B. alexandrina* hermaphrodite gland then evacuations in many gonad cells which severely suppressed their capacity for egg-laying. [30] applied *Euphorbia aphylla* against *B. alexandrina* and showed that the acini lost their normal architecture and their separating connective tissues were almost damaged.

From the histological and ultrastructure studies it was noticed that there were great differences between the normal infected snails and treated snails with *A. arvensis* and *V. tinus*. It was obvious that little number of cercariae was found in the tissue of the treated snails compared to the normal infected snails with number of residual sporocysts.

Conclusion

In conclusion the two examined plants, *A. arvensis* and *V. tinus* plant extracts showed high activity against *B. alexandrina* and provide a considerable scope in exploiting local indigenous resources for snail's molluscicidal agents. The sub-lethal concentrations, $\frac{1}{2}$ LC₅₀, of the two plant extracts caused a considerable reduction in survival rate and infection rate among *S. mansoni* infected snails. Histopathological changes in the digestive glands showed cells vacuolation, hyaline substance filled lumens of the tubules and necrotic focal areas in the digestive glands. Histopathological effects explained and confirmed by TEM images showed degeneration with severe deformation and destruction in the reproductive units.

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