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Impact of Some Plant Extracts on Histological Structure and Protein Patterns of *Biomphalaria alexandrina* Snails

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Abstract: Histological changes following exposure to LC_{25} from the plants *Guayacum officinalis*, *Atriplex stylosa* and *Euphorbia splendens* methanol extract on the digestive and hermaphrodite glands of *Biomphalaria alexandrina* snails were studied. Exposure of snails to LC_{25} of the three plant's extract for 2 weeks caused a great damage in the epithelial tissues of *B.alexandrina*, cells lost their regular shape, appear empty from cytoplasm, having several vaculations, disappearance of secretary cells from the digestive tubules and connective tissue between shrinked acini was damaged. The present results showed severe damages, obvious degeneration of most gametogenic stages and Inhibition of spermatogenesis and oocytes in the hermaphrodite gland of *B. alexandrina* post 2 weeks of exposure to LC_{25} of the tested plant's extract. Qualitative and quantitative effects on the protein patterns have been revealed for methanol extract from the three tested plants. The electrophoretic pattern of total protein showed differences in number and molecular weights of protein bands. DNA concentration was investigated by measuring the intensity of the genomic bands and it showed an increase in the treated snails. Degradation of protein and histological signs after treatment with LC_{25} of *G. officinalis*, *A.stylosa* and *E. splendens* extracts introduce these plants effective molluscicidal agents.

Key words: *Biomphalaria alexandrina*, Plant molluscicide, Histological study, Molecular biological studies

INTRODUCTION

B.alexandrina snails are essential for transmitting intestinal schistosomiasis since they are intermediate hosts for this disease. The high costs of synthetic molluscicides, used for the control of such snails, along with the possible built-up snail resistance to these compounds and their toxicity to non-target organisms, have drawn much attention during recent years for the use of plant molluscicides. These represent cheap, locally produced, biodegradable and effective control agents in rural areas of developing countries; whereas schistosomiasis is endemic [1].Screening of local plants for molluscicidal activity has received increasing attention by several authors [2-6].

Several plant species were proved to have molluscicidal properties against different snail species e.g.: *Dyzygotheca elegantissima* and *D. kerchoveana* [7], *Ammi majus* [8], *Anagallis arvensis* [9], *Solanum dubium* [10]and *Commiphora molmol* [11].

The digestive gland of mollusks is involved in extracellular and intracellular digestion of food material, absorption of nutrients, storage of lipids, glycogen and minerals and it plays also a major role in detoxification [12]. The molluscicidal activity of plant extract on the histological structure of the digestive gland of snails acting as intermediate hosts have been suggested in scattered cases in the literature [13, 14].

DNA is the fundamental building component of all living cells. It regulates the production of specific proteins and enzymes via the Central Dogma Theory [15].Therefore, it was useful to study.

In a previous work, Bakry [16] found *G. officinalis*, *A.stylosa* and *E. splendens* extracts had molluscicidal activity against *B. alexandrina* snails. Moreover, the effect of LC_{25} of three plant extracts on snail survival, egg production, growth rate, infection and production of cercariae has been investigated. The present work was planned to investigate the histological changes in the tissues of digestive and hermaphrodite glands of *B. alexandrina* snails following exposure to LC_{25} of *G. officinalis*, *A. stylosa* and *E. splendens* extracts. The effect of these plant extracts on the pattern of native proteins was monitored. Also, DNA concentration was investigated by measuring the intensity of the genomic bands.

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MATERALS AND METHODS

Snails: Laboratory bred *Biomphalaria alexandrina* snails (5-6 mm) from the stock reared in Medical Malacology Department, Theodor Bilharz Research Institute (TBRI), Egypt,were used.

Plants: *Guayacum officinalis* (Zygophyllaceae) was collected from Fayoum governorate desert, *Euphorbia splendens* (Euphorbiacae) from Giza governorate and *Atriplex stylosa* (Chenopediaceae) from Borg El-Arab on the Mediterranean Coast, Egypt. These plants were collected during May 2005 and kindly identified by the Botany Department, Faculty of Science, Cairo University, Egypt.

The molluscicidal activities (LC_{25}, LC_{50} and LC_{90}) of these plants were previously determined by the author in another manuscript. $LC_{90's}$ were 62, 54 and 27 ppm for *G. officinalis*, *A.stylosa* and *E. splendens,* respectively.

Histological Study: *Biomphalaria* snails (5-6 mm) were continuously exposed to LC_{25} of methanol extract of *G. officinalis, A. stylosa* and *E. splendens* for 2 weeks. Another group (control) was not exposed to the tested plants.

The digestive tract and hermaphrodite gland of treated and control snails were removed from their shells, fixed in Bruin's fluid for 5 hrs, then transferred to 70% alcohol. Further procedures included dehydration in 100% alcohol, clearing in xylol and paraffin embedding were followed. Five ìm sections were stained with hematoxylin and eosin. Stained slides were also examined under polarized light microscope [17].

Molecular Biological Studies: Snails (5-6mm) were continuously exposed to LC_{25} of methanol extract from *G. officinalis, A. stylosa* and *E. splendens* for 2 weeks to evaluate their effect on the protein and DNA content of these snails. The snail's soft tissues were homogenized in double volume of 10 mM potassium phosphate buffer (pH 6.8) and centrifuged for 20 minutes at 25000 g [18]. The supernatant was frozen at -20 °C for electrophoresis. SDS-Page electrophoresis was done according to the method of Laemmli [19]. The wide range SDS-Page molecular weight pre-stained standard mixture (Bio-Rad) was applied to the first well. Scanning was applied using Gel pro software (Ver.3.0, USA, 1998), Media Sci. Image densitometry 700 Biorad).

The total DNA was extracted from soft tissues of *B.alexandrina* exposed to $LC_{25\ of}$ methanol extract of *G. officinalis, A. stylosa* and *E. splendens* preserved in ethanol according to the method of Kocher *et al.* [20] with minor modifications using chloroform-isoamyl alcohol and ethanol precipitations twice [21]. The DNA extract has been separated by agarose gel electrophoresis and detected by UV at 260nm using ethidium promide.

RESULTS

The snail's digestive gland (hepatopancreas), is a tubuloacinar gland which occupies a considerable part of the visceral hump. By light microscope, the digestive tract composed of bilobed tubulo acinar gland, attached with a connective tissue. Each tubule is surrounded by a thin basement membrane and is separated from the neighbouring tubules by a thin sheet of vascular connective tissue rich in blood spaces, connective tissue cells and pigment cells. In the control group. Normal digestive tract (Fig. 1) shows different types of normal columnar epithelil cells (Ec) as well as secretary cells (Sc). The hermaphrodite region (Fig. 5) shows mature ova (o), sperms (sp) and spermatocytes (sp).

The results obtained in Figs. 2, 3 & 4 of snails treated with methanol extract from the three plants showed great histological changes in digestive and hermaphrodite glands. Exposure of snails to LC_{25} of *G. officinalis* extract caused great damage in the epithelial region of *B. alexandrina*, the cells seemed to be empty, secretary cells disappeared and connective tissue between shrinked acini was damaged (Fig. 2).

Inhibition of spermatogenesis and detachment of germinal epithelial layer of the hermaphrodite gland of treated *B.alexandrina* were detected (Fig. 6).

*B. alex*andrina treated with LC_{25} of *A.stylosa* extract (Fig. 3) showed enlargement of Ec, cells lost their regular shape, appear empty from cytoplasm and nuclei could not be detected. Disappearance of secretary cells from the digestive tubules was realized. While slight damage was observed within the hermaphrodite region (Fig. 7). In spite of the presence of mature ova and sperms, absence of oocytes and spermatocytes was noticed.

Tteatment with LC_{25} of *E. splendens* extract have also a great effect (Fig. 4). Treated *Biomphalaria.* snails showed elongation of epithelial gut cells, its apical margin appeared empty from cytoplasm, margined chromatin of the nuclei appeared.

Hermaphrodite follicles lost its normal architechture with degeneration of all stages of spermatogenesis (Fig. 8). More degeneration of gametogenic stages and cells evacuation was seen.

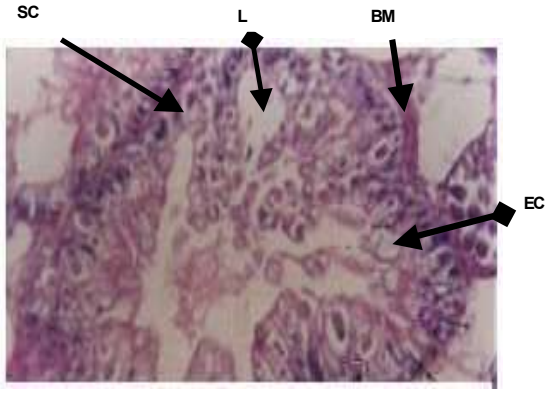

Fig. 1: T.S. in normal *Biomphalaria alexandrina* showing the digestive gland Ec: epithelial cells, SC: secretory cells, L: lumen, bm: basement membrane

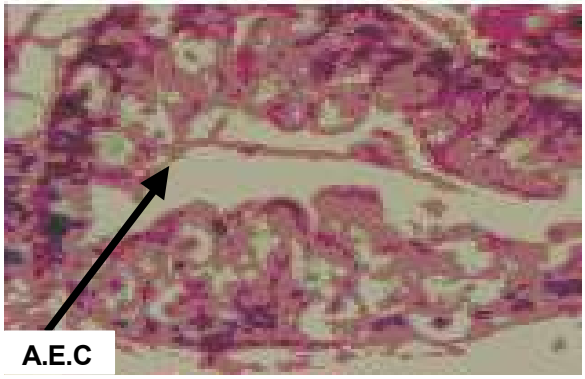

Fig. 2: T.S. in treated *Biomphalaria alexandrina* with methanol extract of *Guayacum officinalis* (digestive gland), A.E.C:evacuated epithelial cells.

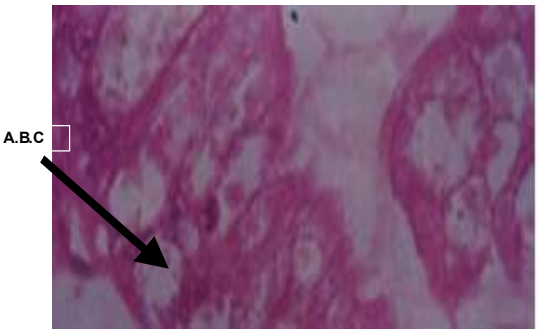

Fig 3: T.S. in treated *Biomphalaria alexandrina* with methanol extract of *Atriplex stylosa* showing digestive epithelia. A.E.C: evacuated epithelial cells

The pattern of protein profile identified by SDS-PAGE electrophoresis for *B. alexandrina* snails that exposed to the extracts of the three selected plants

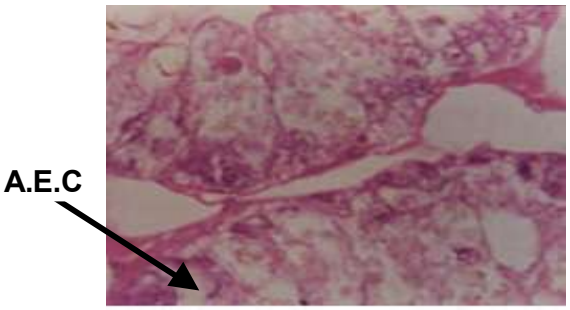

Fig. 4: T.S. in treated *Biomphalaria alexandrina* with methanol extact of *Euphorbia splendens* (digestive gland), A.E.C: evacuated epithelial cells

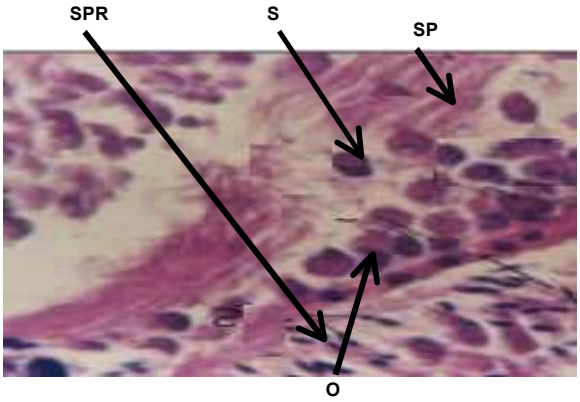

Fig. 5: T.S. in normal *Biomphalaria alexandrina* showing the hermaphrodite gland. Sp=sperms, S=spermatoeytes, O=oocyte SPR: heads of sperms.

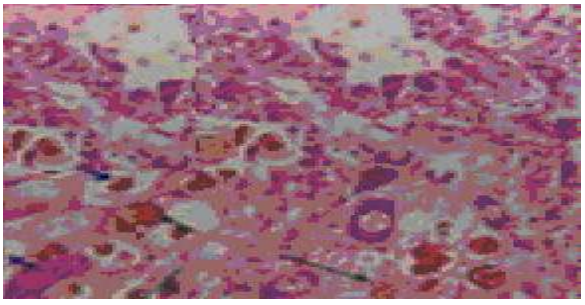

Fig. 6: T.S. in treated *Biomphalaria alexandrina* with methanol extract of *Guayacum officinalis* Showing damage hermaphrodite region.

was shown in Fig. (9). Data in tables 1 and 2 as well as illustrated in fig 9 show that the protein profile of untreated *B. alexandrina* and treated snails with *G. officinalis* composed of 9 protein bands. This profile reduced to 7 bands after treatment of *B. alexandrina* with *A. stylosa* and *E. splendens*. The molecular weights of these bands for snails treated with *E. splendens* ranged

Fig. 7: T.S. in *Biomphalaria alexandrina* treated with methanol extract of *Atriplex stylosa* showing the damage hermaphrodite follicle

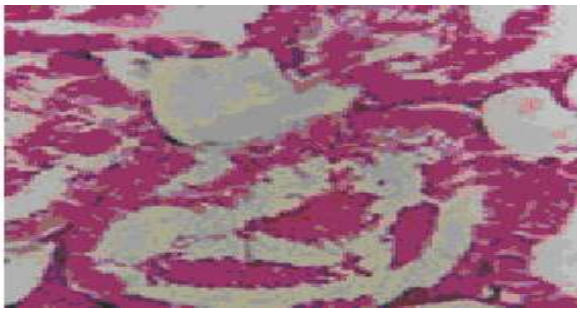

Fig. 8: T.S. in *Biomphalaria alexandrina* treated with methanol extract of *Euphorbia splendens* (damageHermaphrodite gland).

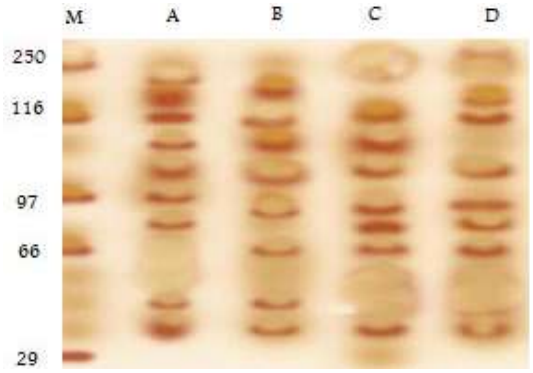

Fig. 9: Protein fraction of *Biomphalaria alexandrina* snails treated with methanol plant extract. A= Cotrol; B = snails treated with *Guayacum officinalis*, C = snails treated with *Atriplex stylosa* and D = snail treated with *Euphorbia splendens* and M=Marker KDa

from 231 to 35 KDa. Those for snails treated with the *G.officinalis* extract ranged from151 to 35 KDa.

The present data (Table 1 and Fig. 9) showed the appearance of few bands in treated snail groups and disappearance of others in comparison with control group. The disappeared bands are 171 and 141 KDa, while two bands appeared in snails treated with each

Table 1: Protein fractionation of *Biomphalaria alexandrina* snails treated with plant extracts (methanol extract) for two weeks

Marker KDa	Control	*Guayacum officinalis*	*Atriplex Stylosa*	*Euphorbia splendens*
–	–	–	–	231
205	–	–	–	–
–	171	–	–	–
–	–	151	–	–
–	141	–	–	–
116	116	116	116	116
–	109	109	109	–
–	102	102	102	102
97	–	–		–
–	95	95	–	95
–	–	85	88	–
–	79	–	79	79
66	–	–	66	66
–	–	64	–	–
–	43	43	–	–
–	35	35	35	35
29	–	–	–	–

Table 2: Dice's similarity coefficient (*S) of the protein profile bands between control snails and snails treated with LC_{25} of methanol extract of *Guayacum officinalis, Atriplex stylosa and Euphorbia splendens*

	Control	*Guayacum officinalis*	*Atriplex stylosa*	*Euphorbia splendens*
Control	1	0.8	0.71	0.71
Guayacum officinalis	0.8	1	0.63	0.53
Atriplex stylosa	0.71	62.5	1	0.71
Euphorbia splendens	0.71	53.3	0.71	1

S = 2 a / 2 a + b + c, where: a = the number of shared bands between two individuals; b = the bands present in the 1st and not in the 2nd and c = the bands present in the 2nd and not in the 1st.

*determining the similarity coefficient as described by [35]

experimental plant.e.g. 231 and 66 KDa for *E. splendens* group,151 and 85 KDa in *G.officinals* snails and 88 and 66 KDa for *A.stylosa* treated snails.

The protein concentration for the shared bands among the examined groups is shown in Fig10 (a, b & c). An increase in this parameter for the band 35KDa was observed in snails treated with *A.stylosa* and *E. splendens*, while a general decrease was recorded in the band 102KDa compared to control snails. The band 116 KDa exhibited an obvious increase in snails treated with *A.stylosa*, but an obvious decrease was observed for *G. officinalis* treatment.

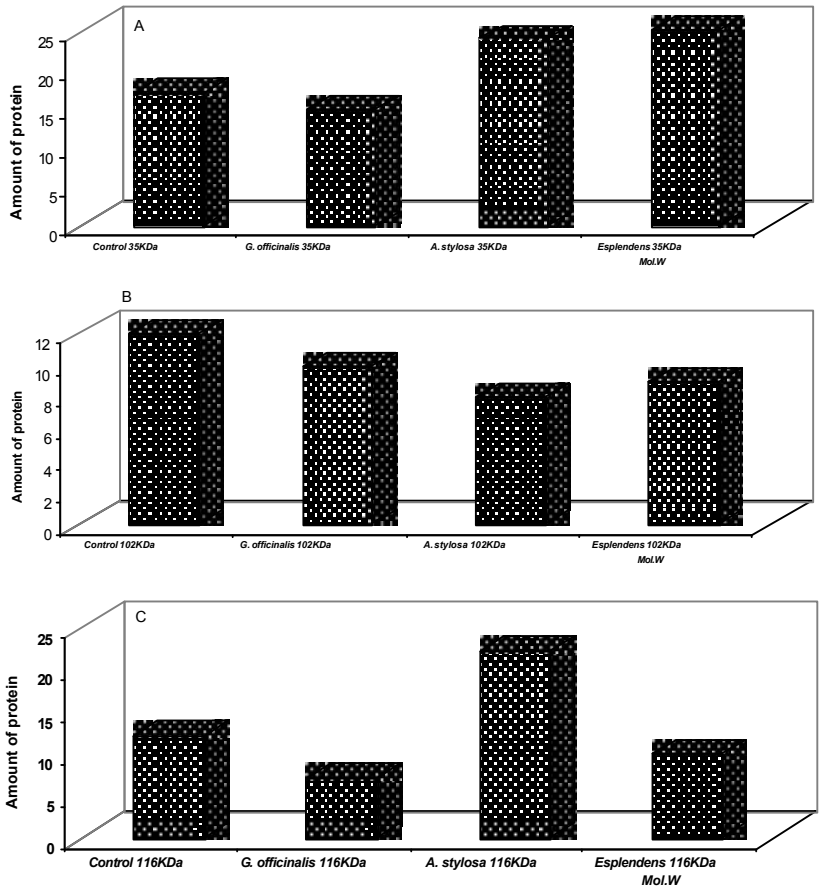

Fig. 10: The amount of protein for the shared bands of *Biomphalaria* snails after treatment with methanol extracts of the three tested plants

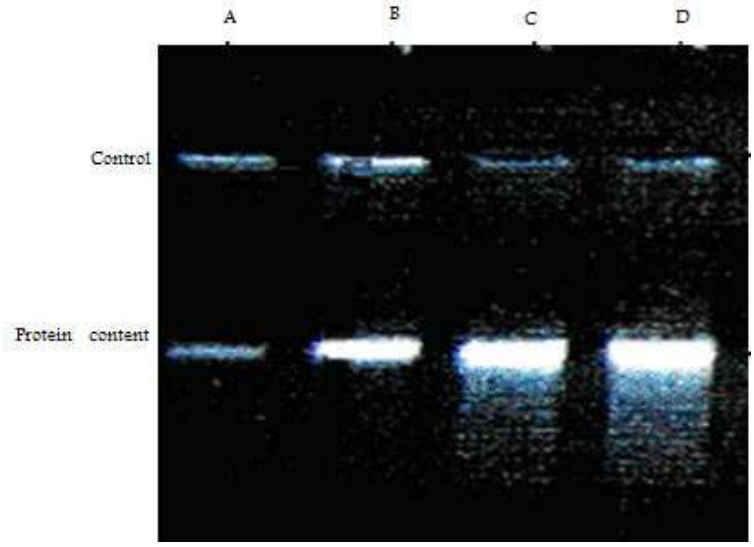

Fig. 11: The total DNA content of the snails treated with the three different methanol plant extract. A= control; B = snails treated with *Guayacum officinalis*; C = snails treated with *Atriplex stylosa* and D = snails treated with *Euphorbia splendens*

The present results (Table 2) indicated that the similarity index (S) was higher in case of snails treated with *G.officinalis* than those treated with *E. splendens* indicating that *E. splendens* had strong effect on protein profile of treated snails.

DNA extracted from snails treated with LC_{25} of the three plant extracts is shown in Fig (11). The DNA intensity was obviously increased in the treated snails than that of control.

DISCUSSION

Selection of the digestive and hermaphrodite glands of *B. alexandrina* snails in this study was based on the fact that they are the target tissues for molluscicides [13]. In addition, previous studies have shown that both of them are strongly affected by molluscicides [22,23].

The present results showed that exposure of snails for 2 weeks to LC_{25} of methanol extract from the tested plants caused great damages in the digestive gland of *B. alexandrina*. Thus, epithelial cells lost their regular shape appear empty from cytoplasm, having many vaculations, disappearance of secretary cells from the digestive tubules and connective tissue between shrinked acini was damaged. These finding agree with those recorded by Brackenbury [14] who found that a graded series of cellular injuries to the epithelial layer was observed along the length of the digestive tract of *B. africanus* treated with *A. attenuate.* These included the loss of cilia and brush border, disruption of the epithelial layer, cellular vacuolation, swelling and rupture and the discharge of secretory products from mucous gland cells. Similar signs were detected after treatment of snails with other plant extracts [24- 26]. The histologic changes observed in the current study in *B. alexandrina* digestive gland in a response to treatment with three plant extracts are comparable to those obtained from other molluscicides on fresh water snails. Adewunmi and Ogbe [27] declared that the overall appearance of the tissues of *Bulinus globosus, B.glabrata* and *Physa watterlotti* exposed to methalonic extract of *Tetrapleura tetraptera* was the swelling of the epithelial cells with disruption of the epithelial lining. They attributed this condition to the accumulation of fluid in these tissue cells suggesting that the molluscicide may either have acted on the membranes of these cells in some way as to alter their permeability or interfered with the regulatory or metabolic processes within them.

The present results showed severe damages in the hermaphrodite gland of *B. alexandrina* post two weeks of exposure to LC_{25} of the tested plants, obvious degeneration of most gametogenic stages and inhibition of spermatogenesis and oocytes These findings agree with Mossalem [28] who found the same results post *B. alexandrina* exposure to the LC_{50} of the plants *Dyzygotheca kerchoveana*, *Solanum nigrum* and *Panicum repens* for 24 and 48 hours. Atlam [29], also, found harmful effects in the hermaphrodite gland cells and gametogenic stages of *B. alexandrina* treated with sublethal concentrations of *E. peplus* dry powder and El-Izzi *et al.* [30] mentioned that the plant *Tetrapleura tetrapleura* extract has an antigonadotrophic agent due to triterpenic saponin action on the gonadotrophic hormone. Rizk [31] 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*.

The present results indicated that the tested plant extracts had qualitative and quantitative effect on the protein patterns of the studied snails. The shared bands of 116, 102 and 35 KDa seemed not to be affected by the tested plants in spite of the variation shown in their concentrations. The electrophoretic pattern of the native proteins revealed difference in the number and molecular weights of protein bands compared to the control snails. These differences indicated that these plant extracts caused intensive molluscicidal effects which induced fractionation of the native protein. Furthermore, treated groups with *A. stylosa* and *E. splendens* showed less number of protein bands indicating that the plant extracts were thought to induce damage for these snails. It seemed appropriate to suggest that snails that gave similar type of protein bands to that of the control were able to resist molluscicidal effect. Protein changes due to snail treatment with plant extracts was previously detected by Rawi *et al.* [32], Aly *et al.* [33] and El-Sayed [34]. Accordingly, the fractionation of native proteins into bands different from that of the control may be attributed to changes occurred in DNA of the treated snails. This suggestion is confirmed by the increase of the DNA concentration shown in the treated snails.The DNA intensity was obviously increased in the treated snails than that of control. This finding agrees with El- Sayed [34].

In general, the use of plant extracts demonstrates a promising molluscicidal activity against *B. alexandrina* snails. The plant species *G. officinalis, A. stylosa* and

E. splendens exhibited a promising effect in this respect. Degradation of protein and the histo-pathological changes in tissues of snails treated with three plants added to their potencies as molluscicidal agents.

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