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ORIGINAL ARTICLES

Induced changes in the fatty acid profile of *Biomphalaria alexandrina* molluscan host to *Shistosoma mansoni* using two sublethal concentrations of selected plant molluscicides

Naema Zayed and Hanan F. Aly

Therapeutical Chemistry Department1, National Research Center, El Behooth Street, Dokki, Giza (Egypt).

ABSTRACT

The present study was undertaken to elucidate the efficacy of the crude powder of *Thymelaea hirsute, Sinapis arvensis*, *Callistemon Lanceolatus and Ambrosia martima* leaves to control human schistosomiasis through disturbances in fatty acid profile of intermediate host *B. alexandrina* snails. Two concentration of each plants were used (LC_{10} and LC_{25}) for one week. Snails treated with these plants were then collected and identification of fatty acids composition in snails tissue was carried out using gas liquid chromatography (GLC). The obtained results declared that, alteration in fatty acid profile post treatment of snail with various plant's powder, fluctuation in reduction percent of long chain and short chain fatty acid contributions either saturated or unsaturated one and decreased in total lipid content, that lead to disturbance in physiological adaption of parasite inside the host which in turn abolish its development. Hence these plant powders can be applied as potential candidate moluscicidal with more potent effect for *Callistemon Lanceolatus and Ambrosia martima* at high concentration.

Key words: *Shistosoma mansoni,* Biomphalaria alexandrina snail, Thymelaea hirsute, Sinapis arvensis, *Callistemon Lanceolatus* and *Ambrosia martima*

Introduction

Shistosomiasis a dreadful disease caused by parasitic trematode worm in both humans as well as in animals is widespread in the world specially in developing countries, causing high levels of morbidity and mortality in 74 countries in tropical and subtropical areas and most of these people are children. It is considered second only to malaria as a major target disease of the World Health Organization (Xiao *et al*., 2002).

Schistosomes as digenetic trematods have two hosts, a final mammalian hosts and a molluscan intermediate snail hosts. As intermediate hosts *Biomphlaria alexandrina (Mollusca; Gastropoda),* is widely distributed in Egypt and it plays a major role in the transmission of schistosomes; they are the sites of an intense multiplication of parasites. Thus, snail control strategies are considered a priority for the reduction of schistosomiasis transmission. Elimination of transmission should be the ultimate goal for control strategies. Specific measures such as chemotherapy and snail control have been developed in association with nonspecific measures aimed at the general improvement of sanitary and health conditions and the provision of safe water supplies (Brackenbury and Appleton,1997).

El –Ansary and Qurashy (1994) stated that the ability of the parasite to develop within snail host is correlated to the snail intrinsic biochemical composition rather than any regulatory immune response. Moreover, Thompson *et al*. (1991) reported that free living stages of schistosomes are completely dependent on the endogenous reserves acquired from their host in the previous parasitic stage. Cercariae for example, live on their endogenous glycogen and fatty acid stores that they build up while inside the snail host (Nabih *et al*., 1985).

It is well known that fatty acids are among the Snail Conditioned Water (SCW) signals needed by schistosome miracidiae to identify their snail host species (Harbel *et al*., 2000).

In the last few years there have been many investigations concerning lipids and fatty acids in molluscs, Bergmann (1993) summarized the fatty acids of the acetone –soluble lipids of *Helix pomatia* snail. Venugoplan (1996) reported some differences in the fatty acid composition of the *Oyster crassostrea* snails, beside given investigation of fatty acids composition of *Ananta arbustorum.* Voogt (1996) declared the fatty acids composition of *Succinla putris* snails. Moreover, Ackman and Hooper (2000) determined the distribution of saturated fatty acids in the lipids of three species of marine molluscs.

Fatty acids is very important, since anoxia is generally accompanied by a marked hydrolysis of membrane phospholipids. Free fatty acids (FFA) and in particular poly-unsaturated fatty acids (PUFA) play a vital role in the biochemical adaptation to hypoxia prevailing during host –parasite complex (Marcel *et al*., 1994).

Corresponding Author: Hanan F. Aly, Therapeutic Chemistry Department1, National Research Center, El Behooth Street, Dokki, Giza (Egypt).

El –Ansary *et al*. (2001) could induce *in vivo* attenuation of schistosome cercariae using sublethal concentrations of selected plant molluscicides which include, *Thymelaea hirsute*(Shaggy Sparrow-Wort, Spur flax), *Sinapis arvensis* (Wild mustard or charlock), *Callistemon Lanceolatus* (Lemon Bottlebrush or Crimson Bottlebrush) *and Ambrosia martima* (Sea Ragwood). Although, the reduced number of attenuated cercariae released from the treated snails showed normal skin penetration rate while, worm burden and egg count in the liver and intestine of mice infected with plant molluscicides –attenuated cercariae were remarkably lower compared to those infected with normal cercariae. Number and size of granulomatous reactions showed significant reduction in attenuated cercariae –infected mice.

The use of molluscicides has always been considered to be a major supportive procedure in integrated schistosomiasis control (Brackenbury and Appleton, 1997). Synthetic molluscicides have met with limited success in controlling the host snails, such as *Biomphalaria alexandrina Biomphalaria pfeifferi* and *Biomphalaria truncatus,* for several reasons one of which is their high cost which places them beyond the economic reach of developing countries. As, an alternative attention has focused on plants with intrinsic molluscicidal properties. The purpose of utilizing plant products is to provide infected rural communities with a cost–effective, locally available and biodegradable molluscicide (Brackenbury and Appleton, 1997). *Ambrosia maritime* (family, Asteraceae) is distributed in Senegal and is known to be molluscicidal with low toxicity to non-target organisms (Belot *et al*., 1993).The active compounds in the plant are thought to be sesquiterpenes and diterpenes (De Leo *et al*., 2010). All Ambrosia species are characterized by a high content of sesqiterpene lactones, which account for cytotoxicity, molluscicidal, antibacterial, antifungal and other pharmacological activities (Parkhomenko *et al*., 2006). While, Durkeet and Harborne (1973), Appelqvist *et al*. (1981), Onyilagha *et al*. (2003) and Agerbirk *et al*. (2008) reported that, the molluscicidal and biological activities of *Sinapis arvensis* (tribe Brassiceae, Brassicaceae), was related to flavonol aglycones, composition of sterols and 4-hydroxyphenylacetonitrile degrading enzyme activity. On the other hand, *Thymelaea hirsute* (Thymelaeaceae) was shown to have alcohols and phenols particularly benzene propanol, benzyl alcohol, nonanol, hexanol and 4-methoxyphenol (Odeh *et al*., 2007). Varma and Parthasarathy (1975) reported that, the molluscicidal and antifungal activities of *Callistemon Lanceolatus (*myrtaceae L.) related to triterpenoids.

These information initiated our interest to compare the fatty acid profile of control and molluscicides –treated snail in a trial to find out if different fatty acids are contributed to the previously reported remarkable reduction in snail compatibility to schistosome parasite (El-Ansary *et al*., 2003) which could easily correlated to the attenuation of cercariae released from molluscicide- treated snails.

Materials and Methods

Snails:

Stock culture of *Biomphlaria Alexandrina* snails were used in the present study. They were collected from Abou Rawash, Giza Governorate and were kept under standard laboratory conditions in the glass aerated aquaria, filled with dechlorinated water at 25 ± 2 ^{0}C, fed on fresh lettuce leaves *ad lib* and left for 45 days to ensure that they were free from infection. They were about 3 months old and their individual weight between 500 to 700 mg. *Thymelaea hirsute, Sinapis arvensis, Callistemon Lanceolatus and Ambrosia martima* are wild herbs. These plants were collected Egyptian country, dried and used as powder.
The chemicals used were of analytical quality and purchased from Merck, Germany.

Treatment:

B. alexandrina snails with a shell diameter of 10-15 mm were exposed to LC_{10} and LC_{25} values (LC_{10} of *Thymelaea hirsute, Sinapsis arvennsis, Callistemon lanceolaatus* and *Ambrosia martima* represented by the concentrations of 6, 3, 5 and 9 ppm of the plant powder while LC_{25} of the same mentioned plants represented by the concentrations of 15, 7.5, 12.5 and 22.5 ppm respectively) of *Thymelaea hirsute, Sinapsis arvennsis, Callistemon lanceolaatus* and *Ambrosia martima* plants as they were obtained from the toxicity lines statistically calculated according to the method of Finney (1952). LC_{10} and LC_{25} values were dissolved in dechlorinated water which has the snails for one week (El-Ansary *et al*., 2001). Whole snail bodies weighing 500-700 mg (wet weight) were collected from pools of 5 to 7 Thymelaea *hirsute, Sinapsis arvennsis, Callistemon lanceolaatus* and *Ambrosia martima* treated - snails. A total of three pools of each plants treated snails were analyzed in this study as recommended before by Higgs *et al*. (1990).

Isolation of native lipids:

Lipids were extracted from snails bodies with 10-14ml of choloroform –methanol (2:1), the extracts were filtered through a plug of glass wool contained in a pasture pipette and non- lipid contaminan*ts* were removed

by extraction with 8-10 ml of Folch wash (0.88% aqueous KCl soliution). The lipid –containing lower phase separated and evaporated just to dryness under a stream of nitrogen at room temperature. The total lipid sample were dissolved in approximately 30 ml of methanol and 0.5-1.0 ml of concentrated sulfuric acid was added. The mixture was refluxed for 1 hr, the formed fatty acid methyl esters was extracted with 30-40 ml of petroleum ether (40-60 ^{0}C), and the extract dried over anhydrous sodium sulfate. The fatty acid methyl esters were concentrated on a Rotor evaporator at 40 ^{0}C and the volume reduced to 1 ml. One microlitre of each concentrated test solution was injected into gas chromatography (GLC) using a 10 ul syringe (Fried *et al* ., 1991). The analysis GLC was performed in the National Research Center (Unit of central services) Dokki, Cairo, Egypt.

Lipid analysis by gas liquid chromatography (GLC):

Determination of saturated and unsaturated fatty acids in total lipids:

The GLC analysis of fatty acid methyl esters was carried out by using a Hewlett –Packard Model 5890-A gas chromatograph fitted with a polar (Supelcowax TM_{10}) fused silica capillary column (30m x 0.32mm) (Supelco, Inc.,Bellefonte, PA), flame ionization detector, and data processor. The helium carrier gas used at a pressure of 12 psig, and the injection port, column, and detector temperature were maintained at 220, 210 and 220 ^{0}C, respectively. GLC peaks were identified by comparison with the retention times of fatty acid methyl ester standards (obtained from Sigma Chemical Co., USA) and cod liver oil fatty acid methyl esters.

Identification of peaks by GLC representing lipids with different numbers of double bonds was confirmed by comparison of the R_F (R_F is the distance of compound / the distance of solvent.) values of standard and samples separated by argentation TLC (Morris, 1962). Silica gel layers containing 9% (w/w) silver nitrate were developed with diethyl ether –hexane (1:9) mobile phase, and lipid zones were detected by spraying the plate with 2,7–dichlorofluorescein and inspection under 254 and 366 nm UV light.

Quantitative results were determined by area normalization, in which the percentage of each component is calculated from the percent of total area which is represents.

Biochemical estimation of total lipid:

The level of total lipid of negative and treated snails was estimated according to the method of Zollner and Kirsch (1962).

Statistical analysis:

Analysis of data was carried out by one way analysis of variance with the Costat Computer Program, where the significance level at $p \leq 0.0001$.

Results:

Results presented in the table 1 and Fig 1 show the area percentage of fatty acid compositions which is the major components of the total lipid isolated from tissue homogenates of *B. alexandrina* intermediate host of *Schistsoma mansoni* species as a result of different plant treatments.

It can easily be noticed that free fatty acid (F.F.A) composition varies between different treatments. About 15 different fatty acids were consistently detected in *B.alexandrina* species. In general the major component of the FFA fraction were $C_{15:0,}$ $C_{16:0,}$ $C_{16:1,}$ $C_{17:0,}$ $C_{18:1,}$ $C_{18:2,}$ $C_{18:3}$ and $C_{20:0.}$

It was shown that, treatment of snails with LC_{10} and LC_{25} concentrations of Thymelaea *hirsue, Sinapis arvensis*, *Cllistemon lanceolatus* and *Ambrosia martima* produced remarkable alterations in the percentage of fatty acids contributions. These changes are summarized as follows:

control snails, have ten saturated fatty acids: $C_{8,}$ C_9 C_{10},C_{12}, C_{14}, $C_{15,}C_{16}$, $C_{17,}C_{18}$ and C_{20} and five polyunsaturated fatty acids :-$C_{14:1}$, $C_{16:1}$, $C_{18:}1$, $C_{18:2}$ and $C_{18:3.}$

Treated snails with *Thymealaea hirsute* (LC_{10}) have nine saturated fatty acids: $C_{8,}$ $C_{9,}$ C_{10}, C_{12}, C_{14}, $C_{15,}$ $C_{17,}$ C_{18}, C_{20} while C_{16} is not detected as a result of plant treatment. On the other hand, unsaturated profile of fatty acid shows three contributions of $C_{18:}1$, $C_{18:2}$ and $C_{18:3.}$

It can be demonstrated that, significant increase in capric ($C_{10)}$. louric ($C_{12)}$, myristic (C_{14}), stearic(C_{18})saturated fatty acid contributions as compared to normal control .While ,significant decrease in saturated caprylic ($C_{8)}$, pelargonic (C_{9}), pentadecylic ($C_{15)}$, unsaturated lenoleic and lenolenic fatty acids ($C_{18:2}$ and $C_{18:3}$) as compared to normal control .

Snails treated with *Thymealaea hirsute* (LC_{25}), have the same previous mentioned fatty acids with remarkable drastic effect, where capric fatty acid ($C_{10:0}$) was not detected and arachidonic (C_{20}) fatty acid contribution shows significant reduction as compared to normal control .

Treated snail with *Sinapis arvensis* (LC_{10}) have nine detected mono-saturated fatty acid C_8, C_9, C_{10}, C_{12},C_{14}, C_{15}, C_{17} ,C_{18} and C_{20},with significant increase in concentration percent of C_{10}, C_{12}, C_{14} C_{17} and C_{18} while, significant decrease in the others as compared to normal control. Considering, polyunsaturated fatty acids, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$; significant decrease was recorded as compared to control. It is obviously that, one saturated (palmatic C_{16}) and two unsaturated fatty acids ($C_{14:1}$ and $C_{16:1}$) were disappeared or not detected. In addition, upon treatment snails with LC_{25} of *Sinapis arvensis* the same pattern of both monosaturated and polyunsaturated fatty acids was recorded with sever drastic effect (dose dependent concentration relationship).

Treated snail with LC_{10} of *Callistemon lanceolatus* demonstrated nine saturated fatty acids C_8, C_9, C_{10}, C_{12},C_{14}, C_{15}, C_{17} ,C_{18} and C_{20}. Among them C_8, C_9, C_{15} and C_{20} recorded significant reduction, while significant increase was detected in others determined fatty acids(C_{14}, C_{17} and C_{18}) .With respect to unsaturated fatty acids ,three unsaturated contributions were observed, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$ and exhibited significant decrease upon treatment of snail with LC_{10} of *Callistemon lanceolatus*. LC_{25} have the same fatty acids pattern with more percentages of reduction was recorded (dose -dependent) and disappearance of C_8 and C_{10}.

Furthermore, snails treated with LC_{10} of *Ambrosia martima* showed also the same fatty acid profile of nine saturated fatty acids with fluctuated significant percent, where C_8, C_9, C_{15}, and C_{20} exhibited significant reduction, while C_{10} shows insignificant change and C_{12}, C_{14}, C_{17}, C_{18} demonstrated significant elevation respectively as compared to the normal control group. On the other hand, the three detected polyunsaturated fatty acids $C_{18:1}$ and $C_{18:3}$ showed significant reduction, while $C_{18:2}$ shows insignificant change as compared to the normal control. Concerning, LC_{25} of *Ambrosia martima,* it demonstrated identical both saturated and unsaturated profile of fatty acids with remarkable effect (dose –dependent) in addition to, disappearance of C_8, C_9 and C_{20} fatty acids contributions. Hence fatty acids contributions were affected by different plants in a dose – dependant fashion.

The current data indicate that, the mean chain lengths and unsaturation index are significantly reduced in *B-alexandrina* snail post various plant treatments and these low levels are more obvious upon using high concentration of the selected plants.

The present results declared also, significant reduction in total lipid upon treatment *B. alexandrina* snail with different plants in a dose dependent manner using LC_{10} and LC_{25} concentrations of the selected plants.

Table 1: Area percent (%) of fatty acid contributions in control and treated fresh water snail *B. alexandrina* intermediate host of *Schistosoma mansoni* parasite.

Fatty Acid	Control	*Thymelaea hirsute*	*Sinapis arvensis*	*Callistemon Lanceolatus*	*Ambrosia martima*	*Thymelaea hirsute*	*Sinapis arvensis*	*Callistemon Lanceolatus*	*Ambrosia martima*
		LC_{10}				LC_{25}			
Caprylic ($C_{8:0}$)	0.62±0.22 [a]	0.40±0.03 [b]	0.30±0.02 [c]	0.25±0.01 [d]	0.19±0.02 [e]	0.23±0.02 [d]	0.20±0.03 [f]	N.D.	N.D.
Pelargonic ($C_{9:0}$)	0.12±0.06 [a]	0.10±0.006 [b]	0.09±0.005 [b]	0.085±0.003 [b]	0.070±0.001 [c]	0.04±0.001 [d]	0.025±0.001[e]	N.D.	N.D.
Capric ($C_{10:0}$)	0.14±0.03 [a]	0.23±0.05 [b]	0.29±0.04[c]	0.31±0.05[c]	0.148±0.06[a]	N.D.	0. 36 ±0.001 [d]	0.58±0.03 [e]	0. 40±0.005[d]
Louric ($C_{12:0}$)	0.27±0.01 [a]	0.98±0.05[b]	4.44± 0.99 [c]	8. 00±1.33 [d]	3.00±0.19 [e]	2.9±0.10 [e]	12.65±0.50 [f]	16.0 ±3.16 [g]	3.16±0.33 [e]
Myristic ($C_{14:0}$)	0.23±0.11 [a]	0.81±0.05 [b]	30.76±3.98 [c]	5.100±0.67 [d]	5.0± 1.00 [d]	3.44±0.54 [e]	43. 43±3.90 [f]	13.56±3.00 [g]	3.00±0.89 [e]
Myristolic($C_{14:1}$)	3.97±0.50	N.D.	N.D.	N.D	N.D	N.D	N.D	N.D	N.D
Pentadecylic ($C_{15:0}$)	10.90±0.822 [a]	7.40±0.92 [b]	6.55±0.98 [c]	2.90±0.56 [d]	2.50±0.45[d]	3.734±0.04 [e]	3.367±0.02 [e]	0.054±0.004 [f]	0.123±0.01 [g]
Palmatic $C_{16:0}$	19.65 ±4.80	N.D.	N.D	N.D.	N.D	N.D	N.D	N.D	N.D
Palmitoleic $C_{16:1}$)	8.47±0.79	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Margaric ($C_{17:0}$)	27.78±2.38 [a]	33.20±4.90 [b]	36.00±2.90 [c]	40.23±2.90 [d]	44.0±4.90 [e]	56.90±6.90 [f]	69.89±9.22 [g]	109.90±23.98 [h]	145±14.90 [i]
Stearic ($C_{18:0}$)	2.753±0.20 [a]	8.50±1.20 [b]	5.00±1.09[c]	5.52±1.88 [c]	7.10± 0.90[d]	16.642±2.3 [e]	11.483±2.12 [f]	12.53±2.10[f]	9.500±0.90 [g]
Oleic ($C_{18:1}$)	11.60±3.10 [a]	12.60±2.23 [a]	6.70±1.30 [b]	3.66±0.22 [c]	2.092±0.80 [c]	11.703±2.7 [a]	2.14±1.90 [c]	0.09±0.005 [d]	0.04 ±0.001 [e]
Lenoleic ($C_{18:2}$)	11.89±2.18 [a]	6.30±1.70 [b]	4.00 ±1.00 [c]	5.40±1.10 [b]	11.00±1.90 [d]	3.90±0.67 [c]	0.50±0.001 [e]	0.64±0.02 [f]	8.00±1.34 [g]
Lenolenic ($C_{18:3}$)	9.08±1.3 [a]	5.90±0.90[b]	4.10±0.50 [c]	3.00±0.80 [d]	2.30±0.5 5 [e]	1.33±0.03 [f]	0.942±0.04 [g]	1.067±0. 23 [f]	0.45±0.05 [h]
Arachidonic ($C_{20:0}$)	5.98±0.80 [a]	5.20±0.60 [a]	3.00±0.32 [b]	1.50±0.40 [c]	0.10±0.006 [d]	3.045±0.22 [b]	1.743±0.33 [e]	0.3±0.01 [f]	N.D.
Chain length	14.43±0.58[a]	10.55±0.71 [b]	10.23±0.43 [b]	10.99±0.99 [b]	10.67±0.87 [b]	9.18±0.56 [c]	8.86±0.92 [c]	8.54±0.32 [c]	9.23±0.10 [c]
USI	64.16± 11.58 [a]	52.50±10.89 [b]	48.02±12.09 [c]	44.90±12.30 [d]	45.45±10.10 [d]	32.78 ±6.32 [e]	21.34 ±3.89 [f]	15.89±4.10 [g]	12.32±2.80 [h]
Total lipid	0.45±0.01 [a]	0.13±0.002[b]	0.06±0.001 [c]	0.09±0.002 [d]	0.09±0.003 [d]	0.08±0.001 [d]	0.03±0.001 [e]	0.06±0.002 [e]	0.04±0.001 [e]

Values represents mean ± S.D of three independent experiments and are expressed as moles percentages.
Total lipid is expressed in mg /dl.
Mean chain length: is defined as ∑fi ci, where fi is the mole fraction and ci is the number of carbon atoms of fatty acids.
USI: Unsaturation index and is defined by∑ mi ni, where mi is the mole percentage and ni is the number of carbon –carbon double bonds of fatty acids .
Statistical analysis is carried out using one way analysis of variance with Costat Computer Program, where unshared letters is significance at p≤0.0001.

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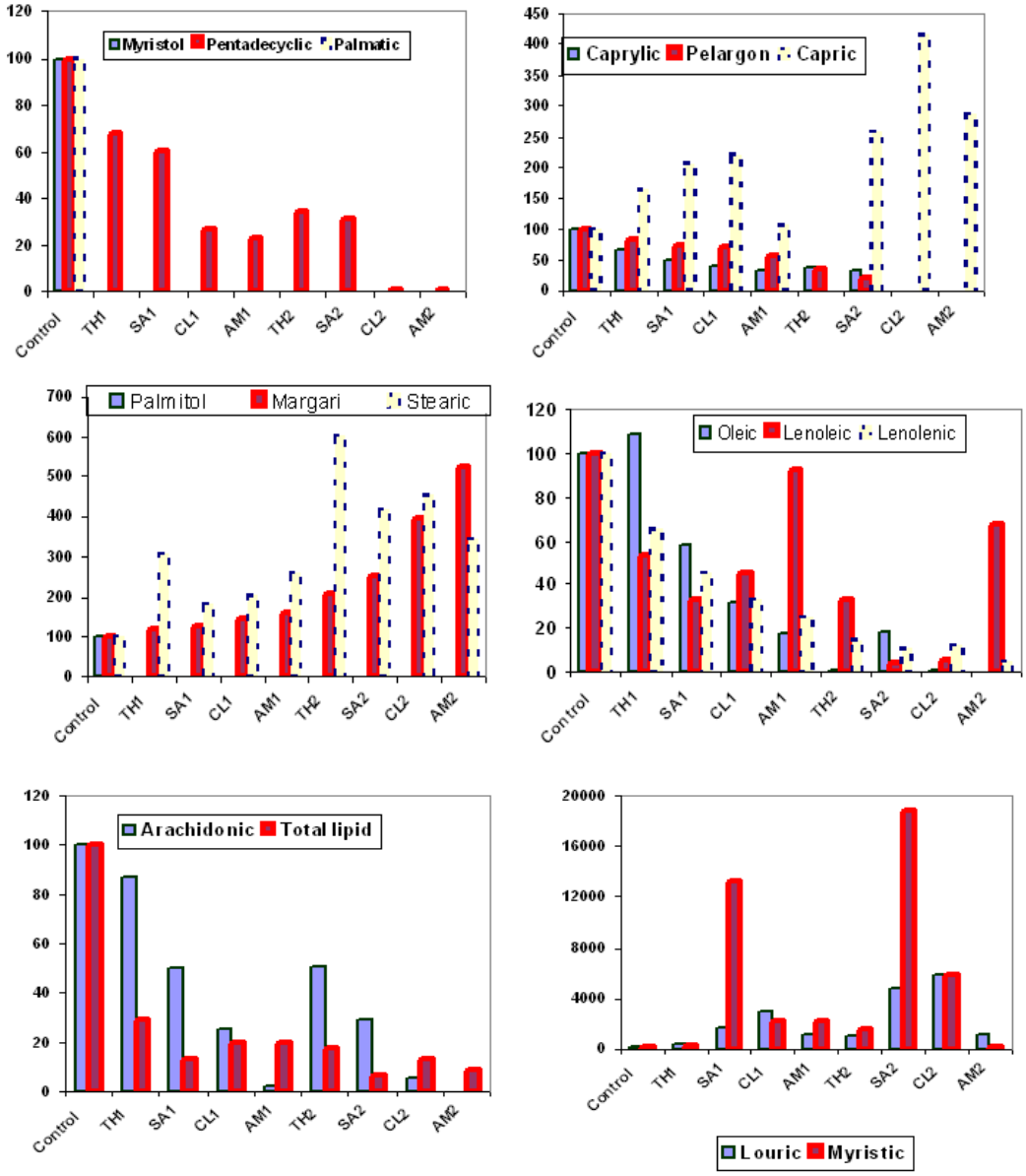

Fig 1: Percentage change in short and long chain fatty acid compositions of *b-alexandrina* snails post treatment with *Thymelaea hirsute, Sinapis arvensis, Callistemon Lanceolatus and Ambrosia martima.*
TH1,SA1,CL1 and AM1 : LC_{10} of *Thymelaea hirsute, Sinapis arvensis, Callistemon Lanceolatus and*

Discussion:

Little information is available on tissue free fatty acids (FFA) patterns of freshwater *B. alexandrina* snails. The present study demonstrated that FFA composition of *B. alexandrina* treated snails vary between the selected plant treatments.

Fatty acid profile of control *B. alexandrina* snails reported in the present study is more or less similar to that reported in the Digestive Gland –Gonad complex (DGG) of *Biomphalaria glabrata* (Fried *et al*., 1991).

Ambrosia martima respectively, where TH2,SA2,CL2 and AM 2: LC_{25} of the same previous plants respectively.

Quantitative analysis of the present study revealed the presence of, 15 different fatty acid contributions upon treatment *B. alexandrina* with different molluscicidal plants. In general, the major components of the FFA fraction were $C_{15:0}$, $C_{16:0}$, $C_{16:1}$, $C_{17:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$ and $C_{20:0}$.

The present results are concerned with marked depletion in the level of long chain fatty acids($C_{14:1}$, $C_{15:0}$, $C_{16:0}$, $C_{16:1}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$ and $C_{20:0}$), while enhancement of $C_{17:0}$ and $C_{18:0}$ in either saturated or unsaturated of *B. alexandrina* snail post various plant treatments. Moreover, the short chain fatty acids $C8:_0$ and $C_{9:0}$ observed in the tissue homogenates of the various plants –treated snails were detected in traces values, although $C_{10:0}$ $C12:_0$ and $C_{14:0}$ were stimulated. Depletion of some long chain and short chain fatty acids may be explained on the basis that reduction in rates of glucose metabolism in the snails was balanced through the stimulation of triglyceride hydrolysis and fatty acid oxidation. The snails can tolerate the lower concentration (LC_{10}). The ability of the snails to tolerate the reduction in rates of glucose metabolism which induced by plant-treatments was decreased by increasing the concentrations of the plants, i.e. at LC_{25} less lower chain fatty acids were detected and lower concentration for that detected (Ahmed and El –Ansary ,1994).Altered fatty acids spectra recorded in the present study may lead to abnormal signals which in turn could disturb the snail –finding mechanisms by schistosome miracidiae (Korner and Hass,1998).

Mahmoud *et al*. (1999) supporting the present results by declared that *Ambrosia maritime* L. (*Asteraceae*) showed molluscicidal and ovicidal activity ,and hence it used for control of bilharziasis and was proved to have lethal effect on snail miracidiae and cercaria. The adverse effect of *Ambrosia maritime* was found related to its active compounds sesquiterpenes and diterpenes (De Leo *et al*., 2010). However, Soliman and El –Ansary (2007)found that, *Ambrosia maritime* showed only slight alteration in amino acid levels compared to *hirsute, Sinapis arvensis, Callistemon Lanceolatus*. The present data declared that, the four selected plants are effective by different degree (Fig.1), and these may explained on the basis that, these effective plants could have immunostimulatory effect through induced lysine amino acids which is considered as a critical importance in inducing parasite killing by hemocytes of molluscicides –treated snails (Soliman and El –Ansary, 2007).The significant alterations in fatty acid pattern in the present study could be used to explain the decrease in snail compatibility previously recorded by El-Ansary *et al*. (2000a, 2000b and 2001), as reduction in the mean total number of cercariae shed each *B. alexandrina snails* treated with the same molluscicides. These could be easily correlated to the reduction or indictable levels of the major component of fatty acids and hence fatty acid cycle and all biochemical process.

Intermediate host *B. alexandrina* snail was shown to have high contribution of poly-unsaturated fatty acids (PAFA) $C_{18:1,}$ $C_{18:2}$ and $C_{18:3}$. This high contributions of PUFA in *B. alexandrina* may be explained by the presence of considerable elongation and instauration activities in the snail. Treatment of the intermediate host with the different plant species produced obvious reduction in these fatty acid contributions which is considered as index of disturbances in elongation, instauration process of fatty acid and inhibition of activity of intermediate host (Fried *et al*., 1993). In addition, $C_{18:2\ (linoleic\ acid)}$ availability is considered as an aspect of biochemical adaptation. Being in an environment or medium rich with linolic acid may be considered as a prerequisite for the schistosoma parasite to be transformed into cercariae more efficient for penetration and development in the final host. Thus the reduction in the percentage contribution of these fatty acid inhibited the transformation of schistosoma parasite into cercariae (Zanotti *et al*., 1995). However, Hara *et al*. (1993) proved that oleate ($C_{18:1}$) and linleate ($C_{18:2}$) fatty acids induced strongly tail removal in *S. mansoni* cecariae and calcium enhanced the cercarial tail –loss rate. These findings suggested that the decreased percent of these fatty acids contributions caused inhibition of calcium influx into cercariae, resulting in preventing tail loss and hence abolish the transformation process of schistosoma parasite into schistosomula .

On the other hand, caprylic, capric and margaric were not reported to have any biochemical significance (Zanotti *et al*., 1995).

Randall *et al*. (1992) and Marcel *et al*. (1994) suggested that, polyunsaturated fatty acids and prostaglandins play a role in the physiological response to hypoxia. reduced level of these contributions ($C_{18:1}$, $C_{18:2}$, $C_{18:3}$) and lower unstaturation index(USI) in snail - treated plants may be an important to un-adapt condition that prevent aerobic- anaerobic transition induced by the schistosoma parasite.

It is well known that, fatty acid pattern of the molluscan hosts is of great importance for developing parasite, in this concern, Fukushima *et al*. (1993) reported that arachidonic acid ($C_{20:0}$) metabolized to prostaglandin E_2 (PGE_2) by intermediate host *B.alexandrina* snail . PGE_2 is known to suppress the functions of mononuclear cells and immune system of the intermediate host to enable the development of pararsite inside the host . Low percentage contribution of arachidonic acid post different treatment of plants leads to decease in the level of PGE_2 and enhancement in immune system of the host that in turn overcome parasite development.

In addition, the detected low level of saturated arachidonic acid in *B-alexandrina* snails post treatments with various plants indicting disturbance of many enzymes including those involved in fatty acid oxidation (acetyl CoA carboxylase, fatty acid synthase and citrate ligase (Jump, 2002), TCA cycle functioning (pyruvate dehydrogenase, citrate synthase (Pastural *et al*., 2009), α-ketoglutarate dehydrogenase (Lai *et al*., 1993), glutamate dehydrogenase (Pastural *et al*., 2009), and oxidative phosphorylation (Ventura *et al*., 1996). Accumulation of intermediates of fatty acid oxidation may be considered as toxic mechanisms. In addition, lactate is accumulated and glycogen, lipid are depleted (Crabtree effect) confirming inhibition of aerobic respiration and stimulation of anaerobic glycolysis.

The current results demonstrated significant reduction in the total lipid of *B. alexandrina* snails post various plant treatments. In a parallel results Fried *et al*. (1993) reported that the fatty acid difference in *B. alexandrina* snail post various molluscicidal treatments probably reflect differences in their available lipid pools and their metabolic activity. The reduction in total lipid may confirm the disturbance in fatty acid metabolism, oxidative phosphorylation, transformation process of lipids into glucose and aerobic-anaerobic transition induced by developing parasite (Crabtree effect) which is a vital for intermediate host to withstand under stressed condition (Tielens, 1997).

El –Ansary *et al*. (2003) showed that sublethal concentrations ((LC_{10} and LC_{25}) of the plant molluscicides used in the present study were effective in reducing fecundity of the treated snails, normal cercariae penetration rate in spite of their attenuation and decrease of their pathogenicity to the mammalian hosts. This could find support in the present study, since the changes in the major fatty acid fractions are correlated with disturbances in the major biochemical pathways previous reported (Tielens,1997).

In, conclusion, treatment of *Biomphalaria alexandrina* snails with sublethal concentrations of *Thymelaea hirsute, Sinapis arvensis, Callistemon Lanceolatus and Ambrosia martima* can be applied safely for non-target organism and were effective in altering the fatty acids profile of this snail species which could be contributed to disturbance in biochemical mechanisms, abolish the developmental process of schistosome parasite inside the host ,impairment of snail egg laying capacity and snail –schistosome miracidiae finding mechanisms. Hence these plants are shown to have potential candidate moluscicidal with more potent effect for Callistemon *Lanceolatus and Ambrosia martima at high* concentration.

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