

Effect of *Mentha Longifolia L* (Family Lamiaceae) ethanol extract on Cercaria, miracidia of *Schistosoma mansoni* by Scanned electron microscopy and *Biomphalaria alexandrina* Hanan S. Mossalem

ABSTRACT

Objective

Effect of ethanol extract of *Mentha Longifolia* as antischistosomal factor on:

1- cercaria, miracidia of *Schistosoma mansoni* by Scanning electron microscopy

2- Survival rate of *Biomphalaria alexandrina* snails

3- Infection rate of *Biomphalaria alexandrina* snails with *Schistosoma mansoni*

Ethanol extract of *Mentha Longifolia L* (Family Lamiaceae) as poisonous stuff from plant has antioxidant activity against cercariae and miracidia of *Schistosoma mansoni*. Cercaria of *Schistosoma mansoni* represents the infective stage for human and miracidia of *Schistosoma mansoni* represents the infective stage for *Biomphalaria alexandrina* snails so that throw away two infective stage help in control of *Schistosoma mansoni* without say publicly of *Biomphalaria alexandrina* snails which represents intermediate host for *Schistosoma mansoni* and in other side food source for another organisms. Screening in different concentrations of ethanol extract *Mentha Longifolia L* (Family Lamiaceae) against cercaria and miracidia record that $LC_{25} = 24.420$, 3.431 , $LC_{50} = 79.242$, 9.855 and $LC_{90} = 183.404$, 142.513 ppm respectively. Examination morphology shape changes, and mobility of cercaria and miracidia before and after exposure for LC_{25} , LC_{50} and LC_{90} by light microscopy and scanning electron microscopy found that secure and miracidia lake, mobility and morphology shape by increasing concentrations and time of exposure as shown in figures. Survival rate of *Biomphalaria alexandrina* snails significant increase at $p < 0.05$ in continues exposure of 500 ppm ethanol extract of *Mentha Longifolia L* (Family Lamiaceae) for two months has no any toxic effect against existence *Biomphalaria alexandrina* snails so spot light on survival rate in presence of 500 ppm ethanol extract *Mentha Longifolia L* (EMLL) (Family Lamiaceae) 100% in compared with 80 % normal control. The infection rate of *Biomphalaria alexandrina* snails with *Schistosoma mansoni* significant decreased at $p < 0.05$ to 142.513 ppm with continues exposure by 20 % in compared with 95 % in infected control due to the miracidicidal effect of the extract.

Key words: Ethanol extract *Mentha Longifolia L* (Family Lamiaceae) (EMLL), *Biomphalaria alexandrina*, Cercaria of *Schistosoma mansoni*, Miracidia of *Schistosoma mansoni*, scanning electron microscopy.

1 INTRODUCTION

The disease of schistosomiasis is a chronic and endemic disease in the Arab Republic of Egypt and it has many complications that lead to death, so many researchers have eliminated it according to its specialization. Many researchers have tried to eliminate the snails that represent the intermediate host for parasite development and growth by many ways, some of them with chemicals and other ways by the plant have molluscicides properties (Ibrahim et al 2004, Mossalem, 2006, 2007,2008). Some researches spot light on some plants has antioxidant properties to control schistosomiasis (Mossalem et al., 2013, Mossalem and Mossa, 2014, Mossalem and Labib, 2014). However, snails are not the only intermediate host for the parasite, but represent a grade of food tree and have other medical importance. This research has focused on the presence and development of snails in a less polluted environment using a plant extract that has an antioxidant property in the parasite elimination

(Mossalem et al., 2018). By eliminating the infectious stages of snails and humans, which are represented in the miracidia and cercariae. There are many types of research that use an anti - schistosomal plant-like. Some of them have been used in the fight against many diseases such as malaria, typhoid, and fever such as. In addition, it can be used in the production of cream for the full cover of the skin and the work of a deadly protective layer of the score without any side damage to the person or the aquatic environment to deal with. World health organization in 2017 stated that there are an estimated 207 million people with schistosomiasis worldwide. The disease is endemic in tropical and subtropical regions. Is the most World health organization in 2017 stated that there are an estimated 207 million people with schistosomiasis worldwide. The disease is endemic in tropical and subtropical regions. Is the most

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prevalent in sub-Saharan Africa, where more than 90% of people live with it. In the Eastern Mediterranean Region, Somalia, Sudan, and Southern Sudan remain the most affected countries. The disease has been eradicated in the Islamic Republic of Iran, Lebanon, Morocco, and Tunisia. A reduction in endemic disease was achieved in Egypt, Iraq, Jordan, Libya, Oman, Saudi Arabia and the Syrian Arab Republic. Three mass drug treatment campaigns were implemented using Praziquantel in the project, which began in 2010 and is scheduled to continue until 2015.

Synthetic insecticides have shaped a number of environmental evils, such as the expansion of different to insect strains, environmental imbalance, and injure to mammals. Therefore, there is a stable need for developing biological active plant materials as larvicides, which are expected to reduce the hazards to human and other organisms by minimizing the accumulation of destructive residue in the environment. Innate products are usually preferred because of their fewer harmful nature to non-aim organisms and due to their accepted biodegradability. Essential oil (EOs) and plant extract may be a choice of synthetic insecticides because they are successful, ecological, and easily biodegradable and reasonably priced (Govindarajan 2010a). The EOs and extracts of edible and medicinal plants, herbs, and spices constitute a class of very strong natural bioactive compounds used in cosmetics, pharmaceutical, and food industries. As a result, many researchers were intrigued to use EOs as a possible source for the identification of novel natural pest control agents (Mossalem et al., 2017 and Govindarajan et al. 2011a). Labiatae is a large family containing many useful plants with latent beneficial activity, especially due to their EOs. An earlier investigation has indicated that various *Mentha* plant extracts have displayed larvicidal effect on *C. pipiens*, *C. quinquefasciatus*, *A. aegypti*, *A. stephensi*, and *Aedes tessellatus* (Ansari et al. 2000). More than a few phytochemicals have been reported to exhibit disadvantageous effects on mosquitoes (Samidurai et al. 2009). *Mentha* has chronological meaning as a therapeutic and an insecticidal plant in the traditional knowledge

system. *Mentha* (commonly known as mint or pudina) is a famous genus (family Lamiaceae) for therapeutic and perfumed value. The genus *Mentha* includes 25–30 species that grow in the temperate regions of Asia, Australia and South Africa (Khanuja 2007). The look for novel effective drugs for treating schistosomiasis has directed studies towards extracts and compounds isolated from plants that can supply schistosomicidal activity (Mossalem et al.,2018). The present study evaluates ethanol extract of *Mentha Longifolia* L (Family Lamiaceae) has larvicidal activity against cercaria and miracidia of schistosoma mansoni and this toxic activity increase by time and dose consentrated. Many extracts from plant origin were studied by several authors as ant parasitic and distorted the morphology and feasibility of *Giardia lam-blia* in vitro (Vidal et al., 2007). Essential oils of *M. aquatic* L., *M.longifolia* L. and *M. x piperita* L. showed strong bactericidal activity, chiefly against *Escherichia coli*. These oils also showed important fungicidal activity (Neda et al., 2003). The use of inhaled *M. x piperita* L. oil concurrently with multiple drugs therapy in patients with pulmonary tuberculosis led to a decrease in the number of bacilli (Shkurupi et al., 2002). *Mentha* species have been known for their curative and aromatherapeutic properties since early times. The prehistoric Egyptians, Romans, and Greeks used peppermint as a flavoring agent for food and as a medicine, while mint essential oils have been used as perfumes, food flavors, deodorants and pharmaceuticals (Lorenzi et al., 2002). During the Middle Ages, powdered mint leaves were used to whiten the teeth (Sousa et al., 2009). Leaves, flowers, and stems of *Mentha* spp. are often used in herbal teas or as additives in commercial spice mixtures for many foods to offer aroma and flavor. In addition, mints have been used as a folk medicine for an action of nausea, bronchitis, flatulence, anorexia, ulcerative colitis and liver complaints, due to their anti-inflammatory, carminative, antiemetic, diaphoretic, antispasmodic, analgesic, stimulant, emmenagogue and anticatarrhal activities. Different mint species are also used for rheumatism, dysentery, indigestion, skin allergy, chills, jaundice, throat infections, constipation, spasms, bladder stones, gall stone, diarrhea, toothache, stomach aches, dyspnea, gastrodynia, and as stimulant, diaphoretic, diuretic, constituent, stomach tonic, anti-infective, sedative,

insect repellent, antimycobacterial, antifungal, antiallergic, virucidal, radioprotective, cyclooxygenase inhibitor, anti-inflammatory and hemostatic agents (Silva et al., 2011).

Lately, vital oils and a variety of extract of plants have aggravated attention as the source of accepted products. They have been the screen for their possible uses as alternative remedies for the treatment of many transmittable diseases and the conservation food from the toxic effects of oxidants (Gawish et al., 2008; Bakery et al., 2011). Research on plants from diverse regions has led to pioneering ways to use the essential oils. Chiefly, the antimicrobial activities of plant oils and extracts have formed the foundation of much application, including untreated and processed food preservation, pharmaceuticals, alternative medicine and accepted therapies. Hydrodistilled oils of the fresh aerial parts of MS cultivated in Egypt were characterized by carvone and limonene as the main constituents (El-Kashoury 2012), while the cultivated material from Beheira (Egypt) show supremacy of linalool (35.32%), p-menth-1-en-8-ol (11.08%) and geranyl acetate (10.86%) (Aziz and Craker, 2010, Zekri, et al., 2013). The wild material investigation from the Alexandria-Cairo desert road (Egypt) showed PO (35.14%), germacrene-D (22.65%), o-menth-8-ene (8.98%), trans- β -farnesene (6.92%), veridiflorol (7.67%) and L-limonene (5.89%) as the chief constituent (Elansary, and Ashmawy 2013). According to a literature survey, dissimilar mint species have been investigated in search of antimicrobial behavior (Işcan, et al., 2002, Hajlaoui, et al., 2009), inclosing several analyses perform on MS with an aim to examine it's sterile, antifungal or antiviral effects. Investigation of the aerial parts of MS growing in Egypt showed moderate inhibitory activity alongside the tested human pathogenic bacteria. Antimicrobial transmission of the ethanolic extract and its subfractions were performed (El-Kashoury et al., 2014). The oil of the new aerial parts shows a strong antifungal activity against *Candida albicans*, *Saccharomyces cerevisiae* and *Aspergillus niger* (El-Kashoury, et al., 2012). Other studies on the Egyptian plant showed a physically powerful antibacterial activity of the essential oil, especially against *Staphylococcus aureus* (Elansary et al., 2013). This study aimed to evaluate the ethanol extract of *Mentha Longifolia L* (Family Lamiaceae) as

larvicidal agent against miracidia and cercariae of *Schistosoma mansoni* and estimated that 200 ppm of ethanol extract of *Mentha Longifolia L* (Family Lamiaceae) cause morphological commotion progressively initiate death after two hours from exposure at $25 \pm 1^\circ \text{C}$ this changes clearly seen by scanning electron microscope.

2 MATERIALS AND METHODS

2.1. PLANT MATERIAL

A plant used in this study is *Mentha Longifolia L* (Family Lamiaceae) as the whole plant.

2.2. PLANT EXTRACTION ETHANOL EXTRACT

Dry powder of the whole plant was totally extracted by soaking at ethanol alcohol (0.5 kg/liter) for seven days. Then the solvent was filtered and distilled under vacuum and the crude extract residues were used in preparing series of concentrations in terms of weight/volume.

2.3. EFFECT OF *MENTHA LONGIFOLIA L* (FAMILY LAMIACEAE) AGAINST LARVAE STAGE (CERCARIA AND MIRACIDIA) OF *SCHISTOSOMA MANSONI*

The same used concentrations in case of snails were used in cercaria and miracidia to investigate crecaricidal and miracidicidal activity five millimeters of water containing about 100 freshly shed *S. mansoni* cercaria were mixed with five millimeters of double concentration from each used concentration, 100 ppm, 200 ppm, 300 ppm, 400 ppm, and 500 ppm in a divided Petri dish and ten millimeters of dechlorinated water containing 100 cercaria was kept as control. At 10, 15, 20, 30, 40, 60, 90, and 120 minutes the cercaria were investigated under a dissecting microscope to observe the change in movement, morphology shape and the number of dead i.e. motionless cercaria and mortality rate recorded (Lahlou 2002). For miracidia, 5ml of water containing about 100 freshly hatched miracidia were mixed with 5ml of double used concentration as in cecaricidal examination. Motionless miracidia were dead and their mortality was recorded.

2.4. ULTRASTRUCTUREAL STUDY OF EXPOSED SCHISTOSOMA MANSONI CERCREA AND MIRACIDIA SCHISTOSOMA MANSONI

100 cercera Schistosoma mansoni, 100 Schistosoma mansoni miracidia exposed to LC90= 183.404, 142.513 ppm respectively of ethanol extract Mentha Longifolia L (Family Lamiacea) for one hour then collect exposed cercera and miracidia each of them in ependwarf 2ml and control fresh cercera, miracidia processed according to Glauert, (1974) they were fixed for 30 min in 2.5%glutharaldehyde in PBS buffer at room temperature. All samples were centrifuged gently, washed 3 times in PBS, postfixed for min in 2%osmium tetroxide in PBS buffer at 4°C, and dehydrated in 4 changes of graded alcohol (50, 70, 90, and 100%) at 5 min gaap. Finally pellet was examined on fornvar coating grids by Scanning Electron Microscope (Inspect S; FEL, Holland) illustrating the shape of cercera and miracidia in treated and control samples at Electron Microscopy Unite OF Theodor Bilharz Research Institute (TBRI).

2.5. SURVIVAL RATE AFTER CONTINUES EXPOSURE OF BIOMPHALARIA ALEXANDRINA SNAILS TO ETHANOL EXTRACT MENTHA LONGIFOLIA L (FAMILY LAMIACEA)

180 snails divided into 6 groups each group contains 10 snails/1Litre dechlorinated water each with three replicates. Group one continuously treated with 100 ppm concentration, 200 ppm, 300 ppm, 400 ppm, 500 ppm for 2nd, 3rd,4th, 5th

groups and 6th group for a control group. All six groups are kept at 25±1 ° C in Malacology lab for two months taking into account changing the dosage used every three days to avoid any defects in storage. After that calculate the number of dead snails and percentage of snail's survival rate.

2.6. INFECTION RATE OF BIOMPHALARIA ALEXANDRINA SNAILS WITH SCHISTOSOMA MANSONI IN PRESENCE OF ETHANOL EXTRACT MENTHA LONGIFOLIA L (FAMILY LAMIACEA)

Two groups of cleaned Biomphalaria alexandrina snails first group represents control group with three replicate exposed for Schistosoma mansoni miracidia 10 miracidia /snail and 50 snails were kept in 1 liter of dechlorinated water. Second group represents infected group 50 snails with Schistosoma mansoni miracidia in presence of 142.513 ppm of ethanol extract Mentha Longifolia, renewed every 4 days to avoid any defects, two groups were kept at lab for 21 days till first shedding at 25±1°C, and then calculate number of shedding snails in compare with control to calculate percentage of infection rate of snails by divide number of shedding snails by total number of exposed snails to Schistosoma mansoni miracidia.

3 STATISTICAL ANALYSIS:

Mortality percentages of miracidia and cercera were analyzed by chi - square values of contingency tables (Southwood 1978). Spss (IBM) version 20 windows 7 Microsoft word 2010.

4 RESULTS

Table (1). Toxicity effect of ethanol extract *Mentha Longifolia L* (Family Lamiacea) on cercaria and miracidia of *Schistosoma mansoni*

A CONCENTRATION OF ETHANOL EXTRACT MENTH LONGIFOLIA L (FAMILY LAMIACEA)	LC25 95% CONFIDENCE LIMITS (LOWER BOUND - UPPER BOUND)	LC50 95% CONFIDENCE LIMITS (LOWER BOUND -UPPER BOUND)	LC90 95% CONFIDENCE LIMITS (LOWER BOUND - UPPER BOUND)
CERCREA SCHISTOSOMA MANSONI	24.420 PPM (16.733- 47.884)	79.242 PPM (98.309- 57.654)	183.404 PPM (153.85 - 239.255)
MIRACIDIA SCHISTOSOMA MANSONI	3.431PPM (113.58 - 113.587)	9.855PPM (53.488 - 38.774)	142.513PPM (113.980 - 204.532)

Miracidicidal effect of ethanol extract of Mentha Longifolia L (Family Lamiacea)

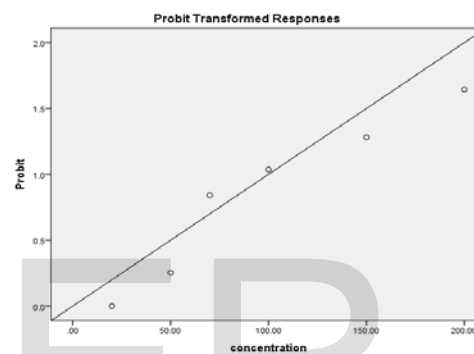
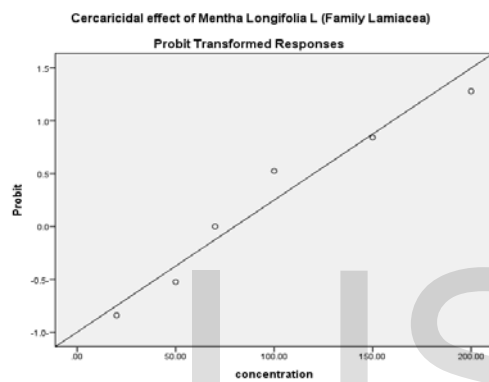


Figure. (1) cercaricidal and miracidicidal activity of ethano extract Mentha Longifolia L (Family Lamiacea)]

Survival rate after continues exposure of Biomphalaria alexandrina snails to ethanol extract Mentha Longifolia L (Family Lamiacea) 100 ppm, 200 ppm, 300 ppm, 400 ppm, 500 ppm for two months there are no dead snails in all treated snails with various concentrations compared with control group, so survival rates of treated snails are 90%, 92%, 94%

with significant at $p < 0.05$, 96% with high significant at $p < 0.01$ and 100% very high significant at $p < 0.001$ respectively in compared with control 85% as shown in table (2) with no any significant can be recorded so ethanol extract Mentha Longifolia L (Family Lamiacea) has no molluscicidal activity against Biomphalaria alexandrina snails. Infection rate record high significance decrease as shown in the table (2) in compared with control infection rate decreased by increasing exposed concentrations.

TABLE (2). SURVIVAL RATE AND INFECTION RATE AFTER CONTINUES EXPOSURE OF BIOMPHALARIA ALEXANDRINA SNAILS TO ETHANOL EXTRACT MENTHA LONGIFOLIA L (FAMILY LAMIACEA) FOR TWO MONTHS

CONCENTRATION PPM	100	200	300	400	500	CONTROL
SURVIVAL RATE	90	92	94 *	96 **	100 ***	85
INFECTION RATE	20 ***	10 ***	5 ***	0 ***	0 ***	95

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ IN COMPARED WITH CONTROL.

Effect of *Mentha Longifolia L* (Family Lamiacea) LC90= 183.404, 142.513 ppm against larvae stage (cercarea and miracidia) of *Schistosoma mansoni*

respectively for one hour by light dissecting microscope shows that first in case of cercarea movement and swimming activity became less

than control by exposed time. Continues exposure of cercaria for 183.404 ppm ethanol extract of *Mentha Longifolia* showed that killing for cercaria exceeds by increasing the time of exposure as shown in the table (3) with very high significant at *** $p < 0.001$ in compared with control. Second, in

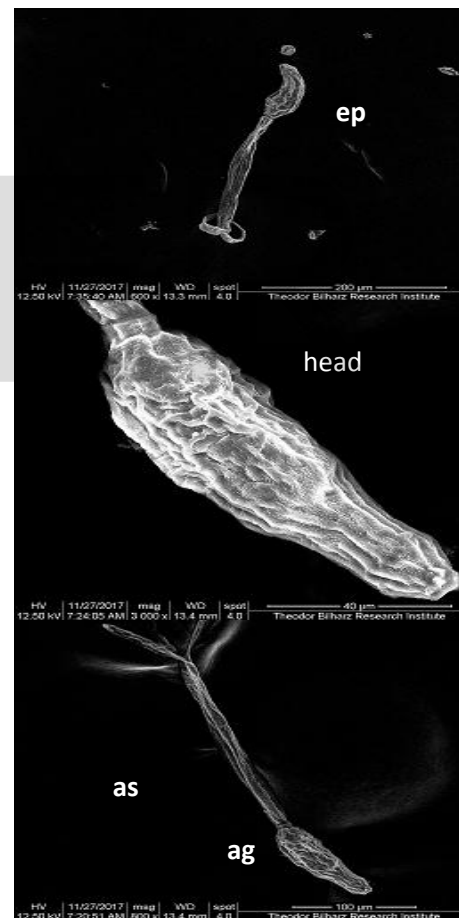
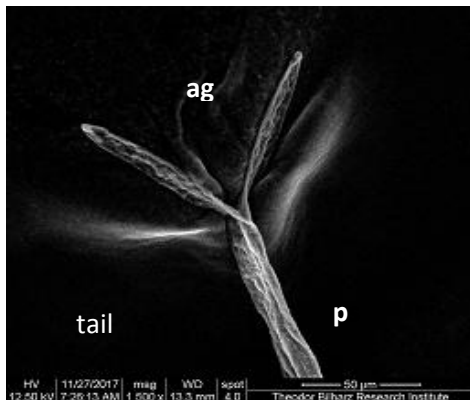
case of miracidia of *Schistosoma mansoni* light dissecting microscope showed that also morphological, activity changes in compared with control till completely death after one hour of exposure as in table (3) with very high significant at *** $p < 0.001$ in compared with control.

TABLE (3). EFFECT OF LC₉₀ MENTHA LONGIFOLIA L (FAMILY LAMIACEA) AGAINST LARVAE STAGE (CERCARIA AND MIRACIDIA) OF SCHISTOSOMA MANSONI FOR ONE HOUR.

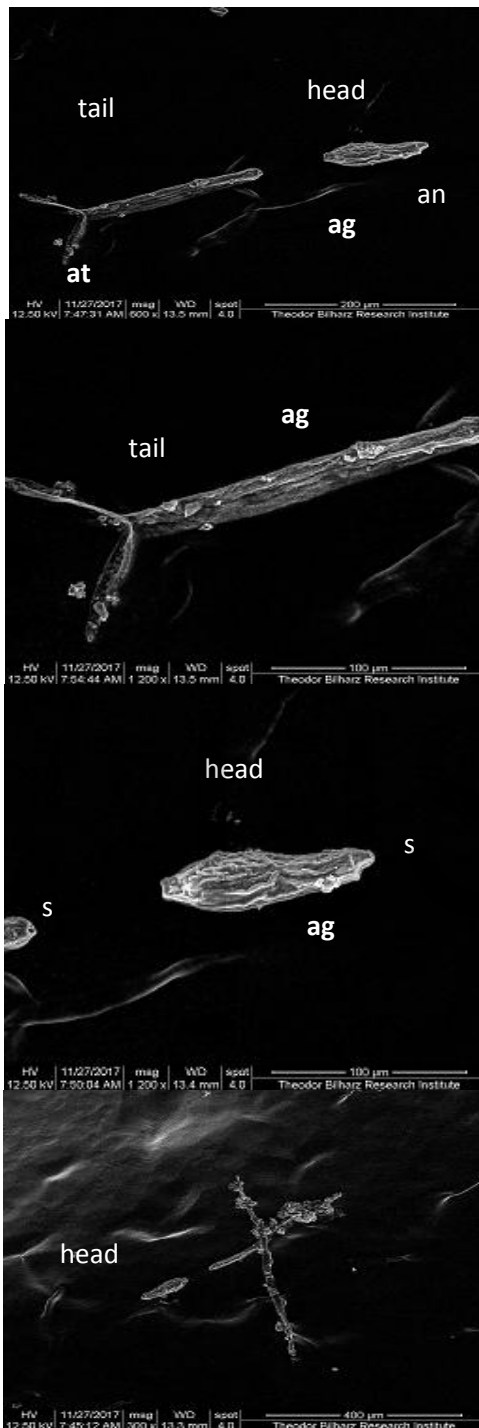
TIME OF EXPOSURE MINUTES	15	30	45	60	CONTOL
CERCARIA % OF MORTALITY	25 ***	50 ***	70 ***	90 ***	4
MIRACIDIA % OF MORTALITY	30 ***	45 ***	80 ***	93 ***	5

*** $p < 0.001$ IN COMPARED WITH CONTROL VERY HIGH SIGNIFICANT.

Effect of *Mentha Longifolia L* (Family Lamiacea) LC₉₀ ppm against larvae stage (cercaria and miracidia) of *Schistosoma mansoni* for two hours by scanned electron microscope illustrated that progressive morphological changes observed at 15 minutes post exposure. Gross changes were observed, including killing of exposed cercaria by splitting their head than a forked tail, degenerative changes including granules into the tegument that resolution, vacuolation, apoptotic phenomena and disturbance in internal organelles of larvae stages as shown in figure (2).



A : Control schistosoma mansoni cercaria

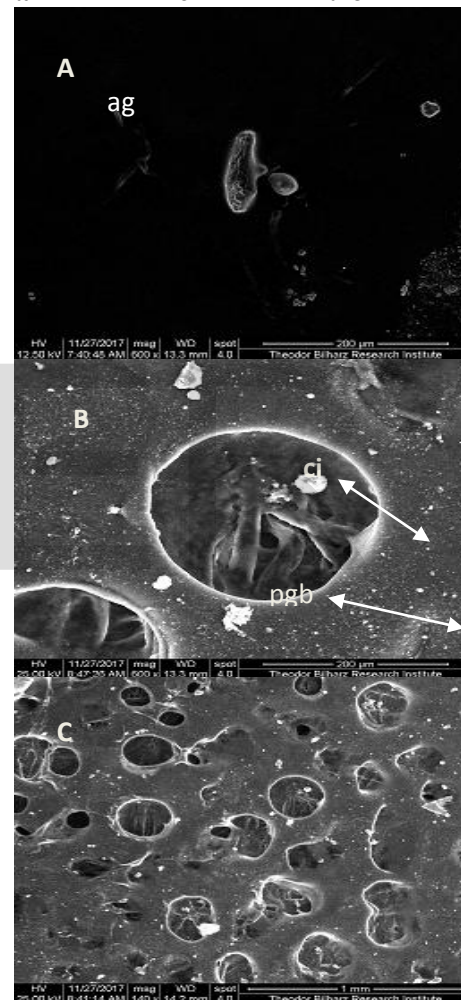


B: Exposed for 60 minutes

Figure (2): Morphological changes observed in larvae stage cercaria of Schistosoma mansoni for one hour Effect of LC90 Mentha Longifolia L (Family Lamiaceae) by the scanned electron microscope showed:

A: Micrograph of control schistosoma mansoni cercaria at 60 minutes, showing complete cercaria with head and tail (as) anterior sucker; (ps) posterior sucker; (ud) urinal duct; (s) spine; (ag) acetabular glands and urinary opening

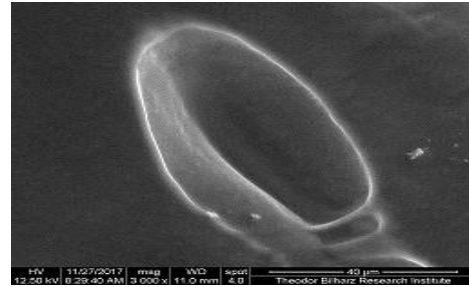
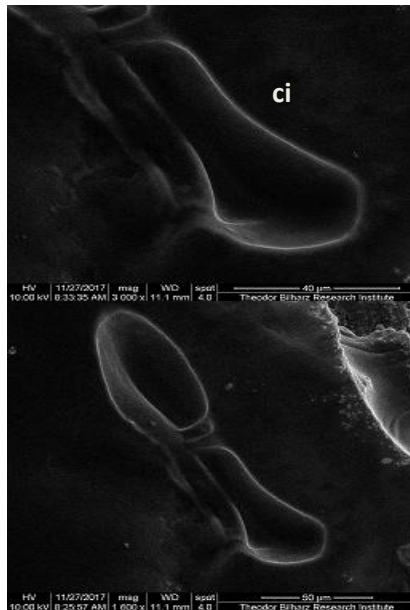
B: Morphological changes of schistosoma mansoni cercaria after exposed to 200 ppm Mentha Longifolia L (Family Lamiaceae) for one hour illustrated that partial degeneration of the acetabular glands with external protrusion (ep); severe edema leading to rupture head far from a tail of the cercaria.



1: control schistosoma mansoni miracidia

Fig. (3) A: show mature egg of scistosoma mansoni, an apical gland, papilla, pc parenchymal cell, ls lateral spine, u u-shaped body on posterior loop of collecting tubule. B: show free hatched miracidium swimming with ci cilia over ovate body, ap apical, e eyespot, ci cilia, pgb posterior granular body

C: show a large number of hatched control miracidia



2: Exposed miracidia with complete resolution of internal organelles and disappearance (ci) cilia Figure (3). Morphological changes of schistosoma mansoni miracidia before and after exposed to LC90 *Mentha Longifolia* L (Family Lamiacea) ethanol extract

1: Micrograph of control *Schistosoma mansoni* miracidia A, B, and C

2: *Schistosoma mansoni* miracidia after exposed for LC90 of *Mentha Longifolia* L (Family Lamiacea) ethanol extract

5 DISCUSSION

According to antioxidant activity of ethanol extract of *Mentha Longifolia* L (Family Lamiacea). The present study documented that ethanol extract of *Mentha longifolia* has antischistosomal activity. Several studies show that natural products or compounds isolated from the *Mentha longifolia* show antibacterial, antimicrobials and antischistosomal activity (Gawish et al., 2008; Magalhães et al., 2009, Fahmy et al., 2009, Neveset al., 2011, Morease et al., 2011. Magalhães et al., 2010, Lima et al., 2011, Miranda et al., 2012). Many researches have also shown that some medicinal plants have been used clinically against schistosomiasis (Abdel Ghani et al., 2009; El sayed et al., 2011). Studies have shown that plants of the genus *Mentha* have significant antimicrobial actions (Mimica-Dukic et al., 2003). Pharmacological and therapeutic effects of *Mentha Longifolia* L. and its main constituent, menthol *M. longifolia* be a possible accepted source for the development of new drugs. However, further studies are required to determine the accurate excellence and security of the plant to be used by clinicians (Saljoqi et al., 2006). In 2013 scientists recorded that menthe has many advantages (Gulluce et al., 2013). This study Evaluate ethanol extract of *Mentha Longifolia* L (Family Lamiacea) as poisonous stuff from plant has antioxidant

activity against cercariae and miracidia of schistosoma mansoni. Cercaria of schistosoma mansoni represents the infective stage for human and miracidia of schistosoma mansoni represents the infective stage for *Biomphalaria alexandrina* snails so that throw away two infective stage help in control of schistosoma mansoni without declaring widely of *Biomphalaria alexandrina* snails which represent intermediate host for schistosoma mansoni and in other side food source for another organism. Screening different concentrations of ethanol extract *Mentha Longifolia* L (Family Lamiacea) against cercaria and miracidia record that LC25= 24.420, 3.431, LC50= 79.242, 9.855 and LC90= 183.404, 142.513 ppm respectively. Examination morphology shape changes, and mobility of cercaria and miracidia before and after exposure for LC25, LC50 and LC90 by light microscope and scanned electron microscopy found that cercaria and miracidia lake mobility and morphology shape by increasing concentrations and time of exposure as shown in figures. Survival rate of *Biomphalaria alexandrina* snails significant increase at $p < 0.05$ in continues exposure of 500 ppm ethanol extract of *Mentha Longifolia* L (Family Lamiacea) for two months has no any toxic effect against existence *Biomphalaria alexandrina* snails so spot light on survival rate in presence of 500 ppm ethanol extract *Mentha Longifolia* L (EMLL) (Family Lamiacea) 100% in

compared with 80% normal control. An infection rate of *Biomphalaria alexandrina* snails with *Schistosoma mansoni* decreased with significant at $p < 0.05$ in 142.513 ppm continues exposure is 20% in compared with 95% in infected control. However, this change increased by increasing time exposure also treated miracidia exhibited gradual resolution in internal organelles, decrease in movement speed, and changes in morphology shape till completely dead after one hour of continuous exposure, investigators show that some extracts have schistosomicidal activity of essential oils of some medicinal plants against *Schistosoma mansoni* (Parreira et al., 2010, Melo et al., 2011, Caixeta 2011, Kozan et al., 2006). Biological studies reported that essential oils of the plant show schistosomicidal activity of adult worms on a *Schistosoma mansoni* with different concentrations and different periods of the exposed time (Melo et al., 2011). Similarly, the results of the present study were 200 ppm of EML shows anticercaria and antimiracidia of *Schistosoma mansoni* and also results from schistosomicidal activity of *Plectranthus neochilus* (Caixeta, et al., 2011). Treatment of cercaria and miracidia which represents larval infective stages of *Schistosoma mansoni* with 200 ppm of ethanol extract of *Mentha longifolia* cause 100% of mortality after one hour continues exposure and investigations by scanned electron microscope for treated cercaria showed that gradually degradation in mobility speed, disturbance in internal shape, changes in morphology shape obvious in internal glands and ventral, posterior sucker and renal duct in a different way of another study in which found that essential oils more effective than ethanol extract against adult worms of *Schistosoma* (Kozan et al., 2006). Also studying the schistosomicidal effect of EOAc, evaluated the two major compounds of the essential oils have schistosomicidal effects (Mossalem et al., 2018; Caixeta, et al., 2011). The essential oil of *M. longifolia* has shown attractive antimicrobial activity against *Escherichia coli*, *Salmonella typhimurium* (Hafedh et al., 2010). On the other hand, another study showed results differing from the above in an evaluation of the biological effect of essential oil of on adult worms of *S. mansoni* and did not show any schistosomicidal effect on the adult worms at any of the concentrations tested tradition of *M. longifolia* in the deed of throat irritation, a sore throat and mouth is extensive. (Al-Bayati, 2009), mostly due to the attendance of oxygenated monoterpenes in their chemical composition

(Hussain et al., 2010, Karaman et al.2003, Kitic et al., 2002, Sahin et al., 2003, Hafedh et al., 2010). Ethanolic and aqueous extracts from *M. Longifolia* demonstrate important anthelmintic activity in conjunction with pinworms, *Syphacia obvelata*, and *Aspicularis tetraoptera*, in mice (Kozan et al., 2006). In one study, *M. longifolia* was established highly effective (>88%) in the spore germination test adjacent to some fungi (Abou-Jawdah et al., 2002). A lot of information record that the insecticidal activity of *M. Longifolia*. feed on this plant was set up to cause bereavement in *Chrysolina herbacea*. Piperitenoneoxide is the major integral that is credited to the insecticidal activity of the plant (LC50, 9.95 mg /L) (Cordero et al., 2012). It is similarly shown that *M. Longifolia* essential oil has 100% repellents in opposition to *Sitophilus zeamais* (10, 15, 20 days old) (Odeyemi et al., 2008, Kumaret al., 2009) and *Tribolium castaneum* (25 days old) (Pascual - Villalobos et al., 1998). Two studies contain report higher effectiveness of the ethanolic extract of *M. longifolia* next to third - and fourth - instar larvae of house mosquito *Culex pipiens* (LC50 - 26.8 ppm) (Cetinet al., 2006) and against *Sitophilus oryzae* (24.2 % repellency) (Saljoqi et al., 2006).

6 CONCLUSION

The ethanolic extract of *M. longifolia* recorded higher toxic effectiveness against cercaria and miracidia of *Schistosoma mansoni* within one hour of exposure with LC90 concentration. An infection rate of *Biomphalaria alexandrina* with *Schistosoma mansoni* miracidia decreased in presence of high dose of ethanolic extract of *M. longifolia*. In correspondence has no any molluscicidal effect against intermediate host within *Biomphalaria alexandrina* snails by any treated dose for various periods of time so this extract safe in aquatic media and their environment.

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Conflicts of Interest

The authors declare no conflict of interest.

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