The Effects of Ethanolic Extract of *Ferula Harmonis* and *Silybum Marianum* on Growth of *Entamoeba Histolytica* (Trophozoites) *in Vitro*

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Background*: Entamoeba histolytica* is a parasite that causes amoebiasis in humans. The common treatment includes different classes of drugs which were described to produce unpleasant side effects. Therefore the pharmacologist used plant that is frequently used in the popular medicine to treat gastrointestinal symptoms.

Objective: This study sheds light on the evaluation of the effects of crude extracts from *Ferula harmonis* and *silybum marianum* against *E. histolytica* on the trophozoite growth and morphology in vitro.

Material and Methods: Trophozoites of *E. histolytica* were cultured under xenic conditions in Locke's egg (LE) medium and then the parasite was treated with different concentrations of ethanol extract from *Silybum marianum* (0.125, 0.25, 0.5, and 1 %) and *Ferula harmonis* (0.25, 0.5, and 1 %) for 24, 48 and 72 h at 37º during this time they measure the Mortality rate for growth of the parasite.

Results The both ethanolic extract of plants showed inhibition activity of trophozoites growth at concentration 0.25, 0.5and1 % after 24, 48and 72 h of incubation, but the extract of *Silybum marianum* showed the best moderate activity on growth of trophozoite at all concentration and times. The morphological assays showed several alterations on plasma membrane surface of the parasite.

Conclusion Our results demonstrated anti- amoebic activity of two medical plants, indicating its potential value as therapeutic agent against *E. histolytica* infections.

Key words: *Entamoeba histolytica,* Amoebiasis**,** *Silybum marianum, Ferula harmonis*

Introduction

Amoebiasis caused by *Entamoeba. Histolytica* is ranked as the third highest parasite-associated cause of human mortality worldwide behind malaria and schistosomiasis. *E. histolytic* is estimated to account for 10 million cases of dysentery and liver abscesses every year, persisting as one of the principal causes of diarrhea in children under 5 years old in developing countries (1).

The common treatment for this illness includes different drugs such as metronidazole, furazolidone. In spite of their large use; these drugs can produce many side effects on patients including headache, vertigo, nausea, gastrointestinal disturbance, anorexia and dizziness (2).

Thus, the search for new antiprotozoal compounds with high activity, low toxicity, cheaper and more effective is still a necessary goal. The interest in the study of medical plants as a source of pharmacologically active compounds has increased worldwide.

It is recognized that in some developing countries, plants are the main medical source to treat infectious diseases.

Ferula harmonis (Shirsh el zallouh) is a small herb.It grows at high mountain areas of northern Lebanon, and on the biblical Mount Hermon in southern Lebanon. Recently much attention received due to its commercial value as an aphrodisiac, and as an herbal alternative to pharmaceutical drugs without the side effects (4).

Medical properties of ferula plant include antispasmodic, carminative, digestive expectorant, sedative, antihysteric, laxative, aphrodisiac, antiseptic and analgesic. Middle

East herbalists have used ferula hermonis for centuries as a folk remedy to treat frigidity in women, and sexual dysfunction in men by increasing blood flow to sexual organs with dazzling results.

The roots can also be soaked in wine or ground into powder and then taken in capsule or mixed with tea in Syria; the powder is mixed with honey (4).

Silybum marianum (milk thistle) has been used for centuries as a natural remedy since the time of ancient Greece. Pharmacokinetic studies have shown that there is rapid absorption of Silybin into the bloodstream after an oral dose (5).

Silymarin, a mixture of flavonolignanes from milk thistle, is a hepatoprotective herbal medicine with potent antioxidant, anti-inflammatory, and immuno-modulatory activities (6).

In the past decade, Silybin or silymarin has been in the spotlight owing to its multiple beneficial activities that are not directly related to its hepatoprotective and antioxidant activities, these include mostly anticancer and chemo protective behavior, as well as

hypocholesterolemic, cardio protective, neuroactive, and neuroprotective activities. Moreover, the scope of its application has been extended to organ systems other than the liver and gastrointestinal tract, e.g., the treatment of pancreatic problems, balancing glycemia, and the treatment and prevention of skin disorders, including a use in cosmetic preparations (4).

In vitro studies indicate that silymarin and Silybin may help to prevent and treat breast, prostate, skin and ovarian Cancers (6).

The present study was carried out to evaluate the *in vitro* effects of ethanolic extract of *Ferula harmonis* and *silybum marianum* on the growth and morphological change of *Entamoeba histolytica* trophozoites *in vitro*.

Materials and methods

1. ***Parasites culture***
   Trophozoites of *E. histolytica* were cultured under xenic conditions in Locke's egg (LE) medium modified by Boeck and Drobohlav (1925) at 37 °C, for 48–72 h. Subcultures were done twice a week. Tubes containing cells at log phase were used in the experiments.

2. ***Plant material***
   The plants were obtained from the field in different place, plant species, botanical name, family, voucher specimens and plant parts used to obtain the extracts. The dry seed of *Silybum marianum* (Iraq) and dry root of *Ferula harmonis* (Lebanon) were used.

3. ***Preparation of crude extracts***
   Fifteen gram (15 g) were extracted with 100ml of Solvent ethanol for each part of plants (seed of *silybum marianum* and root of *Ferula harmonis*), were extracted in a Soxhlet with ethanol solvents in succession , were collected and tested for their effects. Each solution was dried and the residue weighted to prepare stock solution in ethanol at concentration of 5%. Parasite was treated with different concentrations of ethanol extract from *Silybum marianum*(0.125,0.25, 0.5, and 1 %) and *Ferula harmonis* (0.25, 0.5, and 1 %) for 24, 48 and 72 h at 37º .The parasite number and morphology were determined using a Neubauer haemocytometer (7).

4. ***Growth inhibition assays***
   Trophozoites ($0.02x10^6$ cell/ml) were grown on LE medium in presence of the extracts. The concentrations of ethanol extracts employed were 0.25, 0.5, and 1 % in culture medium. Trophozoites were exposed to the extracts for 24, 48 and 72 h at 37 º ◦C in the presence of different concentrations.
   The total number of cells was obtained using a hemocytometer (Neubauer chamber).
   The experiments were performed in duplicate and repeated at least three times while the negative control determined by grown the trophozoites ($0.02x10^6$ cell/ml) on LE medium without any treatment.

5. ***Mortality rate***
   Growth rate of the parasite tested against propolis was calculated from the trophozoite count per ml, mortality rate of *E. hitolytica* with respect to propolis at various concentrations was obtained as follow (8):

$$\textbf{Mortality rate (\%)} = \frac{\text{Count/ml treated}}{\text{Count/ml (untreated control)}} \times 100\text{-}100$$

6. ***Statistical analysis***
   Analysis of variance was employed in order to evaluate plants extract activity on the growth of *E. histolytica* trophozoites, according to extract concentration and time of incubation. *F-test* and Duncan test was used to determine *P*-values for the differences observed between the test sample and the control. A *P*- value of 0.05 or less was considered indicative of a statistically significant difference.

Results

The results of testing the ant amoebic activity of the two crude ethanol extracts derived from two plant used for medicine treatment, *silybum marianum* seed and the *ferula hormones* root against trophozoite of *E. histolytica in vitro* , all tested are summarized in tables 1&2 .

The results showed the *S. marina* seed extract presented the highest inhibition activity against the multiplication of *E. histolytica* trophozoite in all the concentration and the time of incubation of extracts was recorder at different concentration (0.25 - 0.5 and 1 %), (24-48 and 72h), and growth reduction by 99.5% was observed in concentration 0.125 % *Samarium* treated cultures. While the effects the *F. harmons* root extract on the growth of *E. histolytica* trophozoite become more actively after 24h and this activity will be decreased after 48h and 72h.

Growth reduction by 85% was observed in concentration 0. 25% after 24-h incubation, while in 48 and 72-h incubation growth reduction by 50% and 28% respectively, and

the concentration of 1% was able to inhibit growth by more than 90% of trophozoites growth. Treatment of cultures with *F. harmons* at 0.5% inhibited growth by 78%, 87.6% and 55.6%, after 24, 48 and 72h incubation respectively. Besides *F. harmons* root extract effect on growth of *E. histolytica* by light microscope to observed the morphological changes showed that this extract caused several alterations on plasma membrane surface of the parasite and increased peripheral vesicles, apparently inducing the membrane disruption figure (1).

Table (1): The effects of different concentrations of *Silybum marianum seed extract* on the growth of *E. histolytica* trophozoites, after incubation for 24, 48 and 72h.

Concentration of extract	the mean of parasite numbers ± standard error×10^6\ml			Mortality rate (%)		
	24h	48h	72h	24h	48h	72h
control	٠.٣٨±٢.٣٠ a(B)	٠.٨٦±٧.٢٠ a(A)	٠.٢٥±٦.٦١ a(A)	0%	0%	0%
٠.١٢٥	٠.٠٠٣±٠.٠١ b(A)	٠.٠٣٩±٠.٠٦٦ b(A)	٠.٠٨٧±٠.١٨ b(A)	99.5%	99.0%	97.2%
٠.٢٥	٠.٠٠٢±٠.٠٠٢ b(A)*	٠.٠٠٢±٠.٠٠٤ b(A)*	٠.٠١٣±٠.٠٢ b(A)*	99.9%	99.9%	99.6%
٠.٥٠	٠.٠٠٤±٠.٠٠٤ b(A)*	٠.٠٠٢±٠.٠٠٢ b(A)*	٠.٠٠±٠.٠ b(A)*	99.8%	99.9%	100%
١.٠	٠.٠٠٢±٠.٠٠٢ b(A)	٠.٠٠±٠.٠٠ b(A)	٠.٠٠±٠.٠٠ b(A)	99.9%	100%	100%

*small letter in the same column refers there was no significant between the all concentrations in P>0.05 according to the Duncan test.

*capital letter in the same row means there was no significant between all the time in P>0.05 according to the F test.

Table (2): The effects of different concentrations of *Ferula harmonis root extract* on the growth of *E. histolytica* trophozoites, after incubation for 24, 48 and 72h.

Concentration of extract	the mean of parasite numbers ± standard error×10^6\ml			Mortality rate (%)		
	24h	48h	72h	24h	48h	72h
control	٠.٣٨±٢.٣٠ a(B) *	0%	0%	0%	0%	0%
٠.٢٥	٠.١١±٠.٣٤ b(B) *	٠.٤٧±٣.٦٠ b(A) *	٠.٦٣±٤.٧٤٥ b(A) *	85.0%	50.0%	28.2%
٠.٥٠	٠.٠١٦±٠.٥٠ b(B) *	٠.٢٩٧±٠.٨٩ c(B) *	٠.٤٠±٢.٩٣ c(A) *	78%	87.6%	55.6%
١.٠	٠.٠٠٢±٠.٠٠٤ b(A) *	٠.٠٠٥±٠.٠٠٨ c(A) *	٠.٢٠±٠.٢٧ d(A) *	٩٩.٨%	٩٩.٨%	95.9%

*small letter in the same column refers there was no significant between the all concentrations in P>0.05 according to the Duncan test.

*capital letter in the same row means there was no significant between all the time in P>0.05 according to the F test.

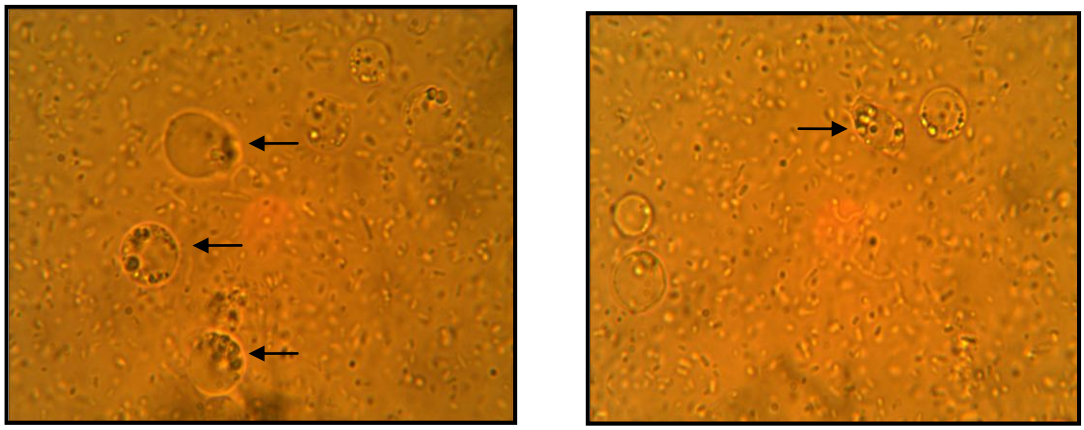

Figure (1): *Entamoeba histolytica* in culture media that treated with *Ferula harmonis root extract* showed alterations on plasma membrane surface of the parasite and increased peripheral vesicles (100X).

Discussion

The present study describes the ant amoebic activity of extracts from *Ferula harmonis* and *Silybum marianum*. The effects were observed affecting the multiplication and morphology of trophozoites.

All extracts were able to inhibit the multiplication of the trophozoites after 24, 48 and 72 h of exposure. The active extract from the seeds of milk thistle is silymarin, a mixture of the flavonolignans with many positive therapeutic properties and few adverse effects in animals and humans (9).

The flavonoids appear to be active as free radical scavengers and stabilizers of plasma membranes.

Silybin and silymarin are active components in numerous phytopreparations used in the prevention and treatment of various liver diseases and as protective against a number of hepatotoxins and mycotoxins (10).

Ferula hermonis roots was shown to be very rich in components, where most of the essential oils were at the state of traces, whereas there was an obvious predominance of α-pinene (38%-51%) in roots and seed extract respectively .

The evaluation of *F. hermonis* extract proves its antibacterial properties .Root extract of *F. hermonis* is highly effective against gram –negative bacteria while roots oil extracts is highly effective against gram positive bacteria. Fifty µl\ml of roots oil extract would inhibit within 10 minutes *Staphylococcus aureus*, and 10µl\ml would inhibit within 1 hour *Streptococcus fecalis*.

Ten µl\ml of resin extract would inhibit S*almonella typhi* within 1 hour, and within 24 hours 50µl\ml and 10µl\ml of resin extract would inhibit respectively *Escherichia coli* and germs of *Pseudomonas aeruginosa*. In general *F. hermonis* was found to be strongly bactericidal and its activity strongly exceeded that of usual antibiotic discs (11).

The antifungal activity of nine *F. hermonis* extracts against *Alternaria solani*, *Cladosporium* sp., *Colletotrichum* sp., *Fusarium oxysporum, Mucor sp., Penicillium italicum, Pythium sp., Rhizoctonia solani, Rhizopus stolonifer, Stemphylium solani*, and *Verticillium dahliae* is reported.

The strongest fungitoxic effects were found against *V. dahliae*, *P. italicum* and *R. stolonifer.*

The weakest effect was against *A. solani*. All extracts of *F. hermonis* had varying degrees of fungitoxicity against all the fungi tested, which makes it a potential source of antifungal compounds. Ferutinin and teferidine, two known sesquiterpenes, were isolated from the roots of *F. hermonis* and their structures were identified. The fungitoxic activity of the ethyl acetate extract might be due to the presence of ferutinin contained in it (12).

Ferutinine and tenuferidine have been show n to have estrogenic activity and may contribute to its aphrodisiac activity. zallouh root also contains naturally occurring vitamins (A, B1, B2, B6, C, Dand E) and minerals (iron, magnesium, selenium and zinc).

A recent study found that ferutinin, ferutidin, and tenuferidin increase cation permeability of lipid bilayers and mitochondria in a dose-

dependet manner suggesting that these sesquiterpenes may increase hormone levels. [9].

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