Therapeutic attempt of amoebic dysentery with water extract of Eruca sativa L. in laboratory mice and its histopathological effect

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Abstract

471 fecal samples were collected and percentage of infection with amoeba dysentery was 13.375% for 63 positive samples for all age groups. All samples were taken from Salahuddin General Hospital in Tikrit and its suburbs for period from beginning of October 2018 until end of March 2019. The highest incidence of parasite was recorded in December at 16.091%, the lowest in October was 8.823%, the highest infection rate was in males at 18.627% and in females 9.363%. The highest incidence of males in age group 1-10 years was 40.476%, while the lowest incidence in 21-30 years was 7.142%. The highest infection rate for females was in age group 1-10 years by 16.393%, and lowest rate was among age group 12-30 years with a percentage of 3.448%. The results showed a variation in concentration of lipids, total proteins, triglycerides and some liver enzymes in laboratory mice infected with dysentery amoeba and treated with different concentrations of water extract of Eruca sativa L. using three concentrations (0.5, 1, 2) ml. The microscopic examination of tissue sections showed revealed a dissociation of some lining cells of colon in infected mice, presence of renal glomeruli in cortical tissue in kidney that filled each Bowman purse and on surface of each glomerulus of lymphocytes, and presence of leukocyte infiltration at base of mucous glands and observed extension of this infiltration to smooth muscle layer forming colon wall, colon cavity contained mucus and a number of cysts parasite. In liver, there was an enlargement of hepatic cells and presence of degeneration of nuclei of these cells, and observed lymphocytic infiltration around these blood vessels.

Key words: Entamoeba histolytica, water extract, biochemical test, histopathology changes.

Introduction:

Entamoeba histolytica is an intestinal parasite that causes amoebic dysentery and is responsible for 100,000 deaths annually. It is commonly observed in tropical and subtropical regions of the world and in developed countries (Tengku & Norhayati, 2011).
Amoebiasis was described by Greek scientists between 460-377 BC in a patient suffering from bloody diarrhea (Tanyuksel & Petri, 2003), and the *E. histolytica* parasite was first discovered by Losch in 1875 in Russia in the faeces of a dysentery patient. Bloody (Roberts et al, 2009).

The parasite is classified within Kingdom of Protista, Division Protozoa, class Lobosea (Panikers, 2013). *E. histolytica* parasite has four stages during its life cycle (Trophpzoit, Precyst., Cyst, Metacyct), and during life cycle mucous cells of the intestines dissolve down to submucosal layer and other layers below it, analyzing host cells and forming separate pin-like sores and edges. Elevated and are more numerous in mucosa and mucosa while ulcers are intact (Panikers, 2018). The disease may develop to infect the liver and liver abscess arises from proliferation of active blood vessels. liver abscess may be mono or multiple and is usually located in the upper right lobe of liver. Cerebral or cavity reaches any other organ, and lung abscess may burst directly into lungs or outward under skin to lead to cutaneous amoebic inflammation (Mahmud et al, 2017).

Medicinal plants attracted the attention of scientists after they found more than 5000 species of these plants for purpose of medical use, while plant extracts are used to treat many diseases when it shows the speed of its therapeutic effect and few adverse effects when compared with chemically manufactured drugs (Adebayo et al, 2010). Medicinal plants are more capable than complex medicines in treating many incurable diseases (Cacerves et al, 1991).

The study aimed to conduct a parasitological study of amoebic dysentery parasite and to identify the rate of infection according to age, sex for patients attending Salahuddin General Hospital and private laboratories, and study some biochemical variables such as cholesterol, triglycerides, total protein and some liver enzymes AST and ALT in infected mice treated with water extracts of *Eruca sativa* with different concentrations compared to control group.

**Materials and methods:**

1-Sampling area: *E. histolytica* samples were taken from patients attending Saladin General Hospital/Tikrit who suffer from severe to moderate diarrhea and most often those with bloody diarrhea, during period from October 2018 to March 2019. Samples were placed in sterile plastic bottles, and samples were examined within half an hour and taken from parts containing mucus or blood (Clark & Diamond, 2002).

2-Examination of fecal specimens: The samples were examined by direct wet wiping to diagnose Trophozoite. And mixed with physiological saline and dye with lougle iodin on the slide (Singh et al., 2009).

3-Purification of the polycystic phase of parasite: was prepared Amoeba suspension dilute the fecal sample containing by 1:15 and then filtered, and took 2 ml of the filter and add 2 ml of physiological saline solution and discard the centrifuge at a speed of
2500 rotations/Min, then neglected the precipitate and took the leachate containing the cysts and was used in the mice (Clark & Diamond 2002).

4-Animals Experience:

55 male Mus musculus mice of the Balb-C strain, aged 8–6 weeks and weighing 10–20 g, were taken from the animal house of College of Veterinary Medicine / University of Tikrit and placed in clean plastic cages for breeding laboratory animals. In Animal House of Biology Department at College of Education for women / Tikrit University, divided into eight groups:

Group I (negative control group): 5 mice dosed with natural saline solution.

Group II (positive control group): 20 mice infected with amoeba suspension.

Group 3: 10 mice treated with water extract of Eruca sativa at 0.5 ml daily for 10 days.

Group 4: 10 mice treated with water extract of Eruca sativa at a dose of 1 ml daily for 10 days.

Group E: 10 mice were treated with water extract of Eruca sativa at a dose of 2 ml daily for 10 days.

5 -Adminstation of laboratory animal: Laboratory mice were immunized with amoeba (1000 cysts) orally using a modified infectious syringe, as well as negative control animals with natural saline solution, and for two weeks after infection, parasite cysts were investigated in faeces of infected animals to make sure The occurrence of infection by preparing swabs and examined directly under the microscope and watching parasite and its various phases. Laboratory animals were then dosed with plant extracts, and mice were explained 10 days after treatment with the extracts.

6-Obtain blood samples: After expiry of time limit for experiment mice explained, then blood samples were withdrawn from heart and placed in test tubes test tubes free of anticoagulant and left 15 minutes at 37 °C, and then serum was obtained by centrifuge At a speed of 3000 r/min and for 15 minutes, it was kept at -20 °C in clean plastic tubes until biochemical tests were performed (Cholesterol, Triglyceride, Total protein, AST, ALT).

7 -Preparation of water extract of Eruca sativa: Prepared doses of water extract leaves and dry flowers of Eruca sativa L. by bringing the flowers and leaves of plants from local markets, where dried by bringing flowers and leaves of plants in oven at (25 °C) powder was then grinded in water, then solution was filtered, the filtrate was taken and residue was left according to (Gasior et al., 1999) and plant suspension was placed on rotary evaporator for 2 hours and then filtered.

8-Biochemical tests:
A: Measurement of serum cholesterol level: BIOLABO SA-France used special analysis kit.

B-Determination of serum triglycerides concentration:

The concentration of triglycerides in serum was estimated using BIOLABO SA, France.

C-Determination of serum total protein concentration:

Serum total protein concentration was estimated using BIOLABO SA, France.

D-Determination of glutamic oxaloacetate transaminase (GOT) concentration in serum

GOT enzyme was measured in laboratory serum mice using several special analyzes processed by the English company Randox (NO.147).

E-Determination of glutamic pyruvate transaminase (GPT) concentration in serum

Estimation of serum GPT enzyme concentration in laboratory mice using several special analyzes from Randox (NO.146).

9-Preparation of histological sections: The tissue sections were prepared from control group and infected, and treated mice with plant extracts of *Eruca sativa* (Banchroft & Stevence, 1982).

10-Statistical Analysis: Statistical analysis system-SAS (2010) was used in the statistical analysis of the studied data to study the effect of different factors on the studied traits, using LSD and Chi-squar

**Results and Discussion:** Fecal samples were collected from laboratories of Salahuddin Teaching Hospital for all age groups and both sexes for period from October 2018 to March 2019. *E. histolytica* was diagnosed by microscopic examination in only 63 samples out of 471 fecal samples with a percentage of 13.375%.

This result is comparable to Ibraheem (2018) in Baghdad 19.5% and Kadir *et al.* (2018) in Tikrit with an injury rate of 9.3%, while our results did not agree with those recorded (ALYassaree, 2004). Babil governorate recorded an infection rate of 29.5% (Ibraheem, 2008) in the city of Kirkuk, where the incidence rate was 41.1% when examining 1250 samples for children under 5 years, and the findings (Al-masoudi, 2009) in Babil Injury amounted to 34.3%.

The difference in incidence of parasite may be due to difference in level of sanitation and population density and personal hygiene and climatic conditions, geographical location, number of samples examined, extent of study and age groups
in population of study, while the similarity in infection rates may be due to similarity in level Cultural, Social and Health) (Kurt et al., 2007).

The highest incidence of parasite infection was recorded in December at 16.091%, followed by January at 15.306%, then November at 14.141%, and the lowest infection rate in October was 8.823% (Table 1). We note that infection rate is higher in winter months and was explained that cold and humid months of year help to vitality and sustainability of parasite cysts causing infection and for a longer period and this is due to seasonal changes that have a role in difference in this rate as well as factors of humidity and heat.

The highest infection rate was recorded in males at 18.627%, while in females it was 9.363%. When the results were analyzed statistically, significant differences were found between males and females in the susceptibility of parasite infection (Table 1).

This result is consistent with the findings (Ibraheem, 2008) in Kirkuk, where the rate of infection in males 49.1% and females 34.6%, and (Jasim, 2011) in Baghdad, while the rate of infection in males 54.6%, while in females was recorded 45%. 4%.

Table 1: Percentage of infection by sex and months of year under study

| months        | female          | male            |
|---------------|-----------------|-----------------|
|               | No. of samples  | Infected no.    | %   | No. of samples | Infected no. | %   |
| October 2018  | 37              | 2               | 5.405| 31            | 4            | 12.903| 8.823|
| November      | 62              | 7               | 11.290| 37            | 7            | 18.918| 14.141|
| December      | 48              | 4               | 8.333| 39            | 10           | 25.641| 16.091|
| January 2019  | 51              | 6               | 11.764| 47            | 9            | 19.148| 15.306|
| February      | 36              | 2               | 5.555| 22            | 5            | 22.727| 12.068|
| March         | 33              | 4               | 12.121| 28            | 3            | 10.714| 11.475|
| Total         | 267             | 25              | 9.363| 204           | 38           | 18.627| 13.375|
| Statistical analysis | **X²=18.937 | **X²= 13.505 | P-Value = 0.002 | P-Value= 0.019 |

- The sign ** denotes significant differences between the two groups at probability level P≤0.05.

The results showed the highest incidence of males in age group 1-10 years was 40.476%, followed by the age group 40-50 years and above by 30%, then category 11-20 years and 15.384%, and category 31-40 where it reached 10.638%, While lowest incidence in category 21-30 years was 7.142% (Table 2).

The highest infection rate for females was in the age group 1-10 years by 16.393%, followed by age group 11-20 years 13.461%, then category 41-50 years was
8.333%, and category 31-40 recorded 5%, and lowest rate of parasite infection was in age group 12-30 years, a percentage of 3.448% (Table 2). No significant differences were found at P≤0.05 probability level among all age groups and for both sexes, and significant differences were observed between age groups for each sex (Table 2).

It was found that the reason for high rate in this category is due to several reasons, including the incomplete immune system and therefore they are less resistant to infection as well as increased contact with the environment as well as non-adherence to rules of hygiene such as washing hands, trimming nails after playing, as well as to contact with pets.

Table (2): Percentage of Infection by age groups of samples under study

|   |  | male          | female         |   |    |
|---|---|---------------|----------------|---|----|
|   | No. of samples | Infected no. | % No. of samples | Infected no. | % |
|---|----------------|--------------|-----------------|--------------|----|
| 10-11 | 61 | 10 | 16.393 | 42 | 17 | 40.476 | 26.213 |
| 20-11 | 52 | 7 | 13.461 | 39 | 6 | 15.384 | 12.087 |
| 30-21 | 58 | 2 | 3.448 | 56 | 4 | 7.142 | 5.263 |
| 40-31 | 60 | 3 | 5 | 47 | 5 | 10.638 | 7.476 |
| 50-41 | 36 | 3 | 13.888 | 20 | 6 | 30 | 16.071 |
|    | 276 | 25 | 8.333 | 204 | 38 | 18.672 |

The results showed a variation in concentration of total lipids and proteins in laboratory mice infected with amoebic dysentery and treated with different concentrations of water extract of *Eruca sativa* and using three concentrations (0.5, 1, 2) ml, where it was noted that cholesterol decreased concentration when using the extract by 0.5 ml where it was 111.01 mg / dL, while highest concentration was 2 ml at 178.31 mg / dL compared to control group of 189.80 mg / dL (Table 3).

Triglycerides were also affected by the use of aqueous extract with a minimum concentration of 32.40 mg / dL when dose was used at a concentration of 0.5 ml, while highest concentration of triglycerides was 37.60 mg / dL at dose of 2 ml of plant extract, compared to control group of 39.80 mg / dL (Table 3).

The total protein was highest rate of 5.6 mg / dL when using 2 ml while lowest rate was 3.410 mg / dL when dose was 0.5 ml, compared to control group of 5.360 mg / dL. Statistical analysis showed that there were high significant differences between the three concentrations. The water effect of *Eruca sativa* was highest when using 2 ml of 5.102 mg / dL (Table 3).

Table (3): Cholesterol, Triglycerides and Total Protein Concentration Rate in mice Serum infected with amoebic dysentery and Treatment with *Eruca sativa* Extract

| concentration | Total Protein Mg/dl | Triglycerides Mg/dl | Cholesterol Mg/dl |
|---------------|---------------------|---------------------|-------------------|
|   |                     |                     |                   |
We note a decrease in the level of fat in general when infected with the amoeba parasite status of tissue, as results of case study came to confirm findings of (Bansal et al., 2005) which recorded a decrease in level of fat in general, and pointed out that parasites use fat during growth In host body, the cause of change in level of fat occurs in cases of acute infection, which contains host of large numbers of parasite amoebic dysentery where proteins envelope parasite to metabolize fat and cholesterol in cases of acute injury where fat is absorbed in intestine (Bansel et al., 2005), while this study was inconsistent With his (Faucher,2000) who referred to high cholesterol for people with parasites E.histolytica and G.lambia.

The reason for high cholesterol, triglycerides and total protein at 2 ml of plant extract is that many studies have shown that Eruca sativa is used in treatment of various infections and parasitic and bacterial. Or because it contains antioxidants and anti-inflammatory substances (Al-Snafi., 2013).

The results showed that liver enzymes were affected by use of different doses of Eruca sativa extract. The highest concentration of AST was 2 ml at 35.12 IU / L, while lowest enzyme concentration at 0.5 ml was 25.01 IU / L. 30 indicating a decrease in liver enzymes AST was affected by Eruca sativa extract of watercress plant (Table 4).

The liver enzyme ALT was also affected by the aqueous extract with the highest dose at 2 ml at 23.90 IU / L and the lowest at 0.5 ml at 14.23 IU / L compared to the control group which was 28.20 IU / L, indicating a decrease in enzymes. The liver AST was affected by watercress extract of watercress plant. Statistical analysis
showed that there were significant differences between three concentrations of plant water aqueous extract (Table 4).

Table (4): ALT and AST concentrations in laboratory mice infected with amoebic dysentery and treated with different concentrations of *Eruca sativa* extract

| concentration | ALT/GPT U/L | AST/GOT U/L |
|---------------|-------------|-------------|
|               | Mean        | St.D.       | Mean        | St.D.       |
| 0.5 ml        | 14.23       | 4.26        | 25.01       | 3.79        |
| 1 ml          | 24.10       | 3.98        | 31.40       | 4.02        |
| 2 ml          | 23.90       | 3.01        | 35.12       | 5.13        |
| Posative      | 13.44       | 4.30        | 16.97       | 6.27        |
| Control       | 28.20       | 4.73        | 30.00       | 2.906       |

- The sign ** denotes significant differences between the two groups at probability level P≤0.05.

The important role of *Eruca sativa* is to try to repair liver cells and thus treat them, as the synergistic action of the aqueous extract has an effect on revitalization of liver cells and because of chemical content of watercress led to effect on parasite and reduce its activity in impact on liver cells (Leo *et al.*, 2010).

The microscopic examination of tissue sections showed presence of renal glomeruli in cortical tissue in kidney that filled each Bowman purse and on surface of each glomerulus of lymphocytes, which were found around Bowman's portfolio as well. All the proximal and distal tubules had no satisfactory, The presence of renal leukocytes is surrounded by a number of lymphocytes (Fig. 1)

In mucous layer of intestine, villi are lined with simple vertical epithelial cells arranged regularly with emergence of capillary cells among these cells, pulp of villi contains several lymphocytes that are continuously leaking from underlying page under those vesicles with mucous glands (Figure 2).

The hepatocellular of liver contains multiple forms and sizes of hepatocytes, where near capsule appear enlarged and free of cytoplasm with other hepatocytes, which are small in size with presence of nuclei in some cells, The central vein in middle liver is a blood clot with leakage some lymphocytes (Figure 3).
Figure 1: Kidney cortex core: renal tubules (H & E, 200x)

Figure (2) infected intestines: intestinal villi (A), clotting in some vertical cells (B), lymphocytes in core of vesicles (C) (H & E, 200x)

Figure (3) Liver Circumference: central vein has a blood clot in hepatocyte (A), Cofer cells (B), lymphocytes (C) (H & E, 200x)

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