

Investigation the level of IL-10 and IFN- γ in mice infected with *Entamoeba histolytica* and treated with earthworm powder

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Abstract

This study was carried out to detect the effect of earthworm powder on the *Entamoeba histolytica* in white mice and to measure the serum of two cytokines (IFN- γ and IL-10) of infected and treated mice with earthworm powder in comparing with metronidazole drug after 3, 7 and 9 days. Result showed that the infected mice which treated with earthworm powder in a concentration of 200 mg/ml for 10 days the level of IFN- γ in serum was (170.67, 150.33 and 145.67 pg/ml) respectively compared with control group, in the earthworm powder group (none infected) level of IFN- γ after 3, 7, 9 days was (105.50, 121.50, 127.00 pg/ml) respectively, also in the metronidazole treatment group IFN- γ level after 3, 7, 9 days was (188.33, 263.50, 253.33 pg/ml) respectively compared to control group. In the treatment earthworm powder group level IL-10 after 3, 7, 9 days was (108.67, 105.67 and 103.17 pg/ml) respectively compared with control group, but in the earthworm powder group (none infected) level IL-10 after 3, 7, 9 days was (95.67, 95.83, 95.67 pg/ml) respectively, also in the metronidazole treatment group IL-10 level after 3, 7, 9 days was (109.83, 107.67, 103.33 pg/ml) respectively compared with control groups.

Keywords: Earthworm powder, *Entamoeba histolytica*, IFN- γ , IL-10.

الخلاصة

أجريت هذه الدراسة للكشف عن تأثير مسحوق دودة الديدان على متحولة حالة للنسج في الفئران البيضاء وقياس مصل اثنين من الساييتوكينات (IL-10 و IFN- γ) في الفئران المصابة والمعالجة بمسحوق دودة الأرض مقارنة مع علاج الميترونيدازول بعد 3، 7، 9 أيام. وأظهرت النتائج أن الفئران المصابة التي تمت معالجتها بمسحوق دودة الأرض بتركيز 200 ملغم / مل لمدة 10 أيام كان مستوى IFN- γ في مصل الدم (170.67، 150.33، 145.67 بيكوغرام / مل) على التوالي مقارنة مع مجموعة السيطرة. في مجموعة دودة الأرض (الغير مصابة) كان مستوى IFN- γ بعد 3، 7، 9 أيام (105.50، 121.50، 127.00 بيكوغرام / مل) على التوالي. أيضا في مجموعة العلاج بالميترونيدازول كان مستوى IFN- γ بعد 3، 7، 9 أيام (188.33، 263.50، 253.33 بيكوغرام / مل) على التوالي مقارنة مع مجموعة السيطرة. بينما كان مستوى IL-10 في مجموعة العلاج بمسحوق دودة الأرض بعد 3، 7، 9 أيام (108.67، 105.67، 103.17 بيكوغرام / مل) على التوالي مقارنة مع مجموعة السيطرة، ولكن في مجموعة دودة الأرض (الغير مصابة) كان مستوى IL-10 بعد 3، 7، 9 أيام (95.67، 95.83، 95.67 بيكوغرام / مل) على التوالي، وكذلك في مجموعة العلاج بالميترونيدازول كان مستوى IL-10 بعد 3، 7، 9 أيام (109.83، 107.67، 103.33 بيكوغرام / مل) على التوالي مقارنة مع مجموعة السيطرة.

Introduction

Entamoeba histolytica is the most common parasites in the world cause amoebiasis, amoebic dysentery and hepatitis. The number of infections estimated with this is (50) million people worldwide and causing (110.000) deaths per year [1-3]. The patient suffers from several symptoms including diarrhea, low

weight, fatigue, headache, fever, abdominal pain, dysentery and liver abscess [4]. *Entamoeba histolytica* is a unicellular and eukaryotic, life cycle is simple as it is divided into two main stages: the active mobile stage called the trophozoite and the resistor stage called cyst [5]. Because of the serious and medical importance of this parasite in the last

four decades a determined effort to know more about the parasite, disease, how to treat, and the growing interest in using the numerous extracts in treatment of parasite infection as it contains some of the components hinder the growth of the parasite, as well as help eliminate gastrointestinal ulcers and healing [6-9]. Many drugs are used for the treatment of amoebiasis; the most use of them is metronidazole [10]. Metronidazole (MTZ) an antimicrobial agent that has been used in clinical medicine for more 45 years, it was effective against protozoal infections, such as amoebiasis, it damages the DNA and the sites of protein synthesis [11], but many reported side effects like gastrointestinal disorders, nausea and metallic taste [12]. Earthworm is considered as major invertebrate group that plays an important role in soil formation, transport and nutrient recycling. Earthworms are important components of soil system, mainly because of their positive effects on soil structure and function as well as earthworm helps to increase soil fertility so they referred a farmer's friend and they considered source of protein [13]. Earthworms have been used in medicine for various remedies since 1340 AD [14]. Earthworms working as a bio medicine, there are no side effects, safe for all ages (very good for children and adults), safe to consume in the long period and continuously. [15]. powder of earthworm is one extracts that can be given orally in the case of thrombus and contributes to being a factor curbing particularly on the accumulation of platelets and has impact on prevent blood clotting [16]. The powder of earthworm contains 65% protein, 19% carbohydrate, 16% lipid, minerals and various types of vitamins [17][18] and play a good role in pharmaceutical as antibiotic, anticancer, antihyperglycemia and antihypotension [19]. The earthworm extract contains a group of enzymes called the lumbrokinase, which are similar to the Omega-3 molecules in fish oil, the polyphenols in green tea, and curcumin and turmeric, these groups of enzymes consider as a valuable characteristic of earthworms [20]. Also [21] noticed that when earthworm powder

giving to infected mice with Leishmanial donovani after the days the numbers of the parasite in the liver tissue minimized significantly compared with mice treated with drug pentostam. Moreover, he noted that the earthworm powder causes repair in the liver tissue and that there was no damage noticed of the intestinal tissue, these results suggest that the indigenous earthworm powder could afford a significant hepatoprotective and anti-parasitic activity. Therefore, this study may explain the role of earthworm powder in the treatment of *Entamoeba histolytica* in laboratory animals.

Materials and Methods

Earth worm collection

Earthworm samples were collected from an orchard located near the Diyala River east of Baghdad governorate from the period (1/9/2016 to 1/11/2016). The samples were collected by digging the soil of orchard at different locations by field shovel (spade) and a depth of 0.5m, isolating worms by forceps, placed in a clean glass case containing moist soil, and then taken to the Mustansiriyah University laboratories for realize the experiment.

Powder extraction

Approximately 500 earthworms were collected, washed in running water to remove dirt from the surface of the body, after that it was put in distilled water for 6-8 hours flooding the earthworms with to allow the soil in the tract to the exit. Later earthworms were washed with distilled water and placed in a sterile beaker private, kept in the incubator for one day at a temperature of 55 °C. After that it has been taken out of the incubator and crushed, then turned into powder, powder stored in the refrigerator (-6°C). [22][23], Then prepared 200 mg/ml was prepared from the powder.

Feces samples

One hundred and thirty stool samples were collected from adults and Children suffering from diarrhea and not subject to treatment who attending to Ibn Al-balady Maternity &

Children's Hospital and Baghdad teaching hospital for the period from November 2016 to January 2017 in sterile plastic containers, then slides made from were microscopically examined table 1.

Table 1: Number of stool samples used in present study.

Total stool samples	130
Positive	30
Trophozoite	12
cyst	10
Trophozoite and cyst (neglected)	8

Microscopic examination

Slides are made for examining by light microscope to examine feces to ensure that it contains the parasite (trophozoite or cyst) by Wet preparation [24].

Purification of *E. histolytica*

According to Robert et al., 1976 method was utilized to isolate the parasite then cyst was suspended in phosphate buffer saline (PBS-7.2) and the final concentration was attended by rate 1×10^3 cells /0.1 ml.

Culturing of *E. histolytica*

Small amount of positive stool sample contain trophozoite was cultured on the Locke- egg (LE) medium according to [25], Culture tube incubated vertically at 37 °C for 48h. For experimental inoculation, actively growing trophozoite were sediment after chilling the culture tubes for (5 min) in an ice-water bath, and were finally suspended in PBS to final concentration of 1×10^6 trophozoite /ml.

Animals

Ninety male albino mice aged 6-10 week, weighing 25-35 gm were obtained from National Control Center for Drugs and Researches, the mice were housed under standard conditions and were fed with a conventional diet and water, stool of them was examined before beginning of the experiment to make sure that the mice are free from any intestinal parasites.

Experimental design

Animals were divided into five groups each group contains (18) mice, all groups were

immunosuppressed by dexamethasone phosphate (4 mg/ml) daily intramuscular dose of (0.1ml/mouse) for 5 days, then inoculated with (0.1ml) contain (1×10^6 trophozoite), after (24hr) all mice feces were examined, while the other two groups non infected, one group inoculated with earthworm powder, the last one give normal saline and consider as control negative, then each group was inoculated as follows:

- 1) Group one (none infected): inoculated orally by stomach tube with (0.1ml/day) of normal saline consider as control negative.
- 2) Group two (infected): inoculated orally by stomach tube with (0.1ml/day) of normal saline considered as control positive.
- 3) Group three (none infected): inoculated orally by stomach tube with (0.1ml/day) Earthworm powder in a dose of (200mg/ml).
- 4) Group four (infected): inoculated orally by stomach tube with (0.1ml/day) from Earthworm powder (200mg/ml). (EWP)
- 5) Group five (infected): given orally by stomach tube with (0.1ml/day) from Metronidazole at (40mg/ml). (M)

Measurement of *INF-γ* in mice serum

At the (3, 7, 9) day post-infection and treatment, six mice from each group for each period were sacrificed and the blood and were subjected for separation of sera. *INF-γ* Concentrations were determined by commercially available ELIS Kit of mouse *INF-γ* (K0331138) Komabiotech.

Measurement of *IL-10* in mice serum

At the (3, 7, 9) day post-infection and treatment, six mice from each group for each period were sacrificed and the blood and were subjected for separation of sera. *IL-10* Concentrations were determined by commercially available ELIS Kit of mouse *IL-10* (K0331213) Komabiotech.

Statistical analysis

The Statistical Analysis System- SAS 2012 program was used in this study. Least significant difference –LSD test (ANOVA) was used to significant compare between means [26].

Results and discussion

Interferon-gamma (IFN- γ) level

The level of IFN- γ appeared significantly ($P \leq 0.05$) increased in all treated groups compared with control positive except in the first day as shown in table 1. In the treatment earthworm powder group, the IFN- γ level after 3, 7, 9 days was (170.67 ± 4.78 , 150.33 ± 9.62 and 145.67 ± 4.82 pg/ml) respectively compared with control group. Also in the metronidazole treatment group IFN- γ level after 3, 7, 9 days was (188.33 ± 4.85 , 263.50 ± 7.20 , 253.33 ± 5.76 pg/ml) respectively compared with control group. In the earthworm powder group (none infected) the level of IFN- γ after 3, 7, 9 days was (105.50 ± 5.06 , 121.50 ± 6.13 , 127.00 ± 5.62 pg/ml) respectively compared with control group. In the control positive group noticed that the level of IFN- γ after 3, 7, 9 days was (183.00 ± 2.74 , 235.25 ± 42.20 , 294.25 ± 6.49 pg/ml) respectively compared with control group. In the third day, there were no significant differences between the metronidazole and earthworm powder. While in the seventh day and ninth day, there were significant differences between the (metronidazole and earthworm powder) groups comparing with control positive group, also it observed significant differences when comparing of the metronidazole group and earthworm powder group.

Interlukine-10 (IL-10) level

Table 2 shows the level of IL-10 in all treated groups, in the earthworm powder group, the results after 3, 7, 9 days were (108.67 ± 1.24 , 105.67 ± 1.11 and 103.17 ± 0.68 pg/ml) respectively compared with control group. As well as in the metronidazole treatment group IL-10 level after 3, 7, 9 days was (109.83 ± 1.34 , 107.67 ± 1.69 , 103.33 ± 1.10 pg/ml) respectively compared with control group, but the level of IL-10 after 3, 7, 9 days was (95.67 ± 2.35 , 95.83 ± 1.95 , 95.67 ± 1.79 pg/ml) in the earthworm powder group (none infected) respectively compared with control group. In control positive group, IL-10 level after 3, 7, 9 days was (109.83 ± 1.34 , 113.00 ± 2.23 , 115.67

± 1.69 pg/ml) respectively. In the third and seventh day, no significant differences were noted between the treatment groups (metronidazole and earthworm powder) when compared with the positive group, and it observed no significant differences between metronidazole group and the earthworm powder group. In the ninth day, there were significant differences between metronidazole group and control positive group, while it no significant differences between the earthworm powder group and control positive was observed, also there were significant differences between metronidazole group and the earthworm powder group. In current study, the rate of IFN- γ and IL-10 were increases in the control positive group and continues to elevate due to the immune response against the infection [27], the trophozoite stage penetration the host's intestinal tissue stimulates the formation of a cellular immune response that has a large role for the host against the parasite, the parasite's attempt to adhere with the epithelial cells in large intestine, that leads to stimulate inflammation and stimulate these cells to produce and release cytokines such as interferon gamma and interleukin 10, IFN- γ that act as agents pro-inflammatory of inflammatory cells such as macrophages, neutrophils and monocyte as these cells attack, swallow and digest the parasite [28] [29] when earthworm powder was given orally to the mice that leads to stimulate immune response against parasite (Fang, 1999), and that leads to increase the level of IFN- γ and IL-10 in mice serum after seventh day then decreased in the ninth day, earthworm powder important in repairing damaged cells in the intestine, it was a dietary supplement because it contains protein, carbohydrate and lipid [18]. So the powder of earthworm used to treat digestive tract infections such as dysentery, diarrhea and other stomach disorders such as an ulcer [15] In the earthworm powder group (none infected), the results showed no changes in the values of IFN- γ and IL-10 as they were approximated to the results of the control negative group. In metronidazole group, the

results showed after ninth day decreased IFN- γ and IL-10 due to the important role of metronidazole as antimicrobial agent that

shown to be effective against other protozoal infections and causes damage to DNA and proteins in the cell [30-31].

Table 2: level of IFN- γ in serum of treated and control groups. (mean \pm LSD) pg/ml.

Treatment groups	Time (day)			LSD value
	3	7	9	
Control negative	111.25 \pm 11.26	116.50 \pm 16.16	117.00 \pm 20.90	15.802 NS
Control positive	183.00 \pm 2.74	235.25 \pm 42.20	294.25 \pm 6.49	23.966 *
Metronidazole	188.33 \pm 4.85	263.50 \pm 7.20	253.33 \pm 5.76	27.831 *
Earthworm Powder: ve ⁻	105.50 \pm 5.06	121.50 \pm 6.13	127.00 \pm 5.62	20.072 *
Earthworm Powder: ve ⁺	170.67 \pm 4.78	150.33 \pm 9.62	145.67 \pm 4.82	18.446 *
LSD value	18.503 **	25.945 **	29.631 **	---

* Significant (P<0.05), NS: Non-Significant.

Table 3: The level of IL-10 in serum in treated and control groups (mean \pm SD) pg/ml

Treatment groups	Time (day)			LSD value
	3	7	9	
Control negative	95.50 \pm 1.71	95.33 \pm 1.88	94.83 \pm 1.95	5.315 NS
Control positive	109.83 \pm 1.34	113.00 \pm 2.23	115.67 \pm 1.69	6.751 NS
Metronidazole	109.83 \pm 1.34	107.67 \pm 1.69	103.33 \pm 1.10	6.892 NS
Earthworm Powder: ve ⁻	95.67 \pm 2.35	95.83 \pm 1.95	95.67 \pm 1.79	4.371 NS
Earthworm Powder: ve ⁺	108.67 \pm 1.24	105.67 \pm 1.11	103.17 \pm 0.68	6.041 NS
LSD value	6.249 *	8.036 *	6.179 *	---

* Significant (P<0.05), NS: Non-Significant.

Conclusions

In the present study it has been determined that earthworm powder acts as the strong material against *Entamoeba histolytica*, this study may thus lead to formulation of new natural anti-parasitic agent.

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