

Original paper

The Effect of Phoenix Dactylifera Extract on Giardiasis in Laboratory Mice: Case Control Study

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Abstract

Background: Giardiasis is a common parasitic infection in the tropical and subtropical areas. It is a common cause of gastroenteritis in humans and other domestic animals. This parasite cause great morbidity and ill health, like mal absorption, irritable bowel and loss of work days. It is usually treated by drug called metronidazole which has bad neurological and gastro intestinal side effects. Recently many plant extract are tried in treatment of some intestinal parasite to avoid the risk of chemical compound and one of these extract is the phoenix dactylifera.

Objectives: To study the effect of Phoenix dactylifera on intestinal giardiasis in mice and compare its effect with metronidazole. This extract of date palm is a natural herbal medicine with supposed negligible side effect.

Material and methods: This is a case-control study done in cage of al- Al- Habboby laboratory in corporation with the college of pharmacy, Karbala University. The study was done in the period from 1/11/2013 to 20/11/2013. Sufficient amount of the extract phoenix dactylifera had been prepared for treatment trial. 75 mice rendered infected with giardiasis by giving 2 ml of the dilute stool from infected animals. Then the animals are classified into 5 groups, each group composed of 15 mice. The first three groups were given, 1000mg/ kg, 2000 mg/ kg, and 3000mg/kg respectively. The fourth group was given metronidazole, 200mg/kg and lastly the fifth group was given distil water as a control group. The mice were sacrificed according to the protocols of animal rights and all biopsies sent to histological lab of our college.

Results: the group of mice given 3000mg/kg of the extract, showed negative stool examination for giardiasis in the 4th day which is similar to the result of metronidazole. It was also statically significant.

Conclusion and recommendation: Phoenix dactylifera has similar efficacy to metronidazole in laboratory animal. This natural treatment may be beneficial in treatment of human giardiasis.

Key words: Giardiasis, phoenix dactylifera, Date palm,

Introduction

Epidemiology and clinical features of giardiasis

Giardiasis (popularly known as **beaver fever**)⁽¹⁾ is a parasitic disease caused by the flagellate protozoan *giardia lambia* (also sometimes called *Giardia intestinalis* and *Giardia duodenalis*).⁽²⁾ The giardia organism inhabits the digestive tract of a wide variety of domestic and wild animal species, as well as humans (figure 1). It is a common cause of gastroenteritis in humans, infecting approximately 200 million people

worldwide. Infections occur through faeco-oral route. Figure 1.⁽²⁾

Symptoms include loss of appetite, diarrhea, hematuria (blood in urine), loose or watery stool, stomach cramps, upset stomach, projectile vomiting (uncommon), bloating, excessive gas, and burping (often sulfurous). Symptoms typically begin one to two weeks after infection and may wane and reappear cyclically. Symptoms are caused by *Giardia* organisms coating the inside of the small intestine and blocking nutrient absorption. Most people are asymptomatic; only about a third of

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infected people exhibit symptoms. Untreated, symptoms may last for six weeks or longer, Symptomatic infections are well recognized as causing lactose intolerance⁽³⁾ which, while usually temporary, may become permanent.⁽⁴⁾⁽⁵⁾ Although hydrogen breath tests indicate poorer rates of carbohydrate absorption in those asymptomatically infected, such tests are not diagnostic of infection.⁽⁶⁾ It has been suggested that these observations are explained by symptomatic giardia infection allowing for the overgrowth of other bacteria.^(6,7) Some studies have shown giardiasis should be considered as a cause of deficiency as a result of the problems caused within the intestinal absorption system.⁽⁸⁾ Giardiasis is passed via the fecal-oral route. Primary routes are personal contact and contaminated water and food. The more susceptible are institutional or day-care workers, travelers, those eating improperly treated food or drink, and people who have contact with individuals already infected, Giardia are flagellated protozoans that cause decreased expression of brush border enzymes, morphological changes to the microvillus, and apoptosis of small intestinal epithelial cells. There is no invasion of giardia trophozoites, and small intestinal morphology may appear normal in light microscopy.⁽⁸⁾ The attachment of trophozoites causes villus flattening and inhibition of disaccharidase activities. Ultimately, the enteric microbios of the intestine overgrow and may be the cause of further symptoms, though this idea has not been fully investigated. The alteration of the villi leads to an inability of nutrient and water absorption from the intestine, resulting in diarrhea, one of the predominant symptoms. In the case of asymptomatic giardiasis, there can be malabsorption with or without histological changes to the small intestine. The degree to which malabsorption occurs in symptomatic and asymptomatic cases is highly varied ,*Giardia* protects its own growth, reducing the formation of nitric oxide by consuming

all local arginine, which is the necessary substrate for the production of nitric oxide. Arginine starvation is known to be a cause of programmed cell death, and local removal is a strong apoptotic agent.⁽⁹⁾

Diagnosis

The mainstay of diagnosis of giardiasis is stool microscopy. This can be for motile trophozoites or for the distinctive oval *G.lambli*a cysts.

Immunologic test, enzyme-linked immunosorbent assay (ELISA), is now available. These tests are capable of a 90% detection rate or more.⁽¹⁰⁾

Because Giardia lambia is difficult to detect, often leading to misdiagnoses, several tests should be conducted over a one-week period.⁽¹¹⁾

In certain cases of malabsorption jejunal aspirate through gastrojejunoscopy (OGD) may be required to establish the diagnosis.⁽¹¹⁾

Treatment

Treatment is not always necessary as the infection usually resolves by itself. But in others, the illness is acute or symptoms persist and medications are needed to treat it. Usually

Metronidazole, albendazole, and tinidazole are used.

Both need a five to 10 day long course; albendazole is taken once a day, while metronidazole needs to be taken three times a day⁽¹²⁾

Mertionidazole is the standard treatment of intestinal giardiasis in human being, usually given in a dose of 400mg/kg tid for 10 days. Tinidaazole or metronidazole can be given in a single dose of 2gm for 3 days.

Metronidazole is an antibiotic used mainly for anaerobic bacterial infections; it is also used in the treatment of parasitic infections due to giardia and ameabia. Other indications of metronidazole is the irradiation of helicobacter pylori in peptic ulcer as part of triple therapy in addition to clarithromycin and omeprazole. Metronidazole is relatively safe dug when used in the recommended dosage and duration. Side effect include metallic taste,

gastric upset and peripheral neuropathy if used for long duration. ⁽¹²⁾

Phoenix Dactylifera

The extract of phoenix dactylifera is taken from the date palm which is a very famous plant in Iraq. This plant is famous in giving dates which have much nutritional and medical value. It is regarded as a good source of energy in addition it contain many mineral like iron and also it is used for treatment of many gastrointestinal disorders in many nations. Phoenix dactylifera (PD) is a refined, extremely, white powder that disperse quickly in the air. It has a smell similar to seminal fluid. Old Arab doctor mentioned the properties of PD and they said that it strengthen the stomach and make it dry, decrease viscosity of blood and remove fever. It is also beneficial for our viscera and shortness of breath. When it is taken with honey it is tonic for whole blood generally and for sexual function specially, and if this mixture placed in the uterus it prevent sterility and if women took it before intercourse it help for pregnancy, it is tonic foe body and ovaries and it regulate the menstrual cycle and help in the formation of ova. PD is composed of 17% sugar, 22% protein, and 54% calcium. It also contains vitamin B, C, and mineral like phosphate and iron. Also it contains the estrogen hormone. In modern medicine it is proved that its help in sexual function, increase lipido and errection in males. It also increases the number of ova in female and increase the chance of pregnancy. ⁽¹³⁾

Many papers had been published in Iraq and many other Arabian countries on the effect of plant extract on intestinal parasites.

Shnawa found that the extract of Artemisia was effective against giardia lambia when given to laboratory rats in a dose of 4 mg/kg. ⁽¹⁴⁾ Ankri et al in his study on entamebia histolytica found that allicin extract from garlic has a role in destructing the trophozotes of this parasite. He found that this extract inhibits the enzymes cystein protinase. A concentration of 500 mg

totally eliminates the trophozoite of this parasite. ⁽¹⁵⁾

Al masoody also demonstrated in her thesis that garlic and pomegranate cortex has a good effect in treating white mice infected with Trichomonas Muris and she found the cold, aqueous extract of garlic was the best extract which has significant effect, another study on garlic extract it appeared that it contain active substances like allylmercaptan and allyl alcohol which have inhibitory effect on giardia lambia. ⁽¹⁶⁾

In another study upon the effect of certain plants on pathological bacteria , they noticed that, paganum, camomile, fenugreek and sumac have inhibitory effect on the microbe e. coli and salmonella species but they didn't mentioned the inhibitory concentration. ⁽¹⁷⁾ In 2001 Echevarrial and Idavoy mentioned that garlic extract have an killing effect on Giardia lambia at 2.05 mg/l. ⁽¹⁸⁾

Lastly the study of alsultan found that the extract Plantago ovate was significantly effective in treating girdia lambia in comparism with metronidazole. ⁽¹⁹⁾

We design this study to find the role of phoenix dactylifera in treatment of giardia lambia and to compare this effect with the standard treatment which is metronidazole.

Material and Methods

This case control study was done in the college of pharmacy, Karbala University in corporation with the cage of Al- Habboby laboratory in the period from 1/11/2013 to 20/11/2013. It include 75 mice rendered infected by giardiasis. The study was done according to the protocols of animal rights, First we prepare the extract of phoenix dactylifera by placing 100mg of the seeds in a 500ml flask and contain 100 ml of boiled distilled water and then mixing the content for 15 minutes and then left the flask to be cold for 30 minutes. then we filters the solution by apiece of clean clothes and we took the filtrate and putting it in a small class bottles and lastly we put

the bottles in an oven at 70 degree to make it solid and dry and then we got the wanted concentration by weighing it to test its effect on healthy mice.

The second step was rendering the mice infected by feeding them giardia lambia which cause giardiasis. This done by, giving them 2 ml of the diluted, infected stool which contain the trophozoite and the cystic stages through nasogastric tubes. The infected faces have been prepared and were ready to use at the time of the experiment.

The third step is started after appearance of giardiasis symptoms on the mice and confirmed by examining their stool. Then classification of the total number of mice which was 75 into five groups, each group contains 15 mice. The first three groups were given, 1000mg/kg, 2000mg/kg, and 3000 mg /kg respectively twice daily. The fourth group was given metronidazole 200mg/kg twice daily and the last group was given distill water as a control group. Follow-up of treatment was done by daily examination of the stool under microscope looking for giardiasis infection both trophozoites and cystic stages.

Lastly after the finishing the experiments the group taking the extract phoenix were sacrificed according to the protocols of animal rights. Histological evaluation. For the assessment of pathological alterations, small and large intestine samples from each mouse were collected, fixed in 4% paraformaldehyde, and embedded in histowax. Tissues were cut into sections of 5 μ m and stained with hematoxylin-eosin for assaying the histological changes or May-Grünwald-Giemsa for eosinophilic cell detection. At least 4 to 5 random sections per intestinal portion (small intestine or colon) and per mouse were examined.

The preparation of histological sections

The important steps were:

1. Washing and fixation. Washing was done by Boin solution and alcohol 70% several times then followed by fixation by formaldehyde to prevent infection and to

keep tissue without change and also to make them taking the stain

2. Dehydration. Done by increasing concentration of ethyl alcohol: 85%, 95%, and 100%.

3. Clearing. Clearing was done by paraffin for 1.5 hours for two times.

4. Embedding. Embedding was done by paraffin block. For embedding the tissue in iron block, the temperature of the block was 58 C. We left these block for the second day for hardening. The blocks were cut in a diameter of 7 micrometer by using a special instrument. Then the tissue is transmitted to hot path for fattening and spreading. Then we prepared class sides which are painted by sticky substance which is prepared from egg albumin and glycerin. Then we remove excess paraffin by boiling. Then we put them in alcoholic solution of decreasing concentrations to return water to tissues. The last step was to add colors by eosin and haematoxylin. Then we put a cover slide and examined under microscope. Tissue biopsies from intestine were examined for all the samples which were taken from all mice that have been treated by the phoenix dactylifera and other agents

Preparation of Eosin.

We took 5 gm of Eosin powder and we add 95 ml of distill water and then we add thymol to prevent fermentation, then we filtered the solution and keep it until usage.

Preparation of haematoxylin.

Prepared by the way of standar Alum and it was as follow, haematoxyllin 0.5 gm, alcohol 100ml of 100% concentration (aluminium – Amonia sulfate) .03 gm. HGO and distill water.

Statistical analysis

Statistical analysis. Statistical differences between different types of treatments and for significant difference between the studied groups and the control group was done by using a one-tailed Student's test .P values under 0.05 were considered significant.

Results

After giving the extract in the three concentration, metronidazole and distill water to the study groups for 8 days the following results were obtained

Table (1) showed the following results:

When we used phoenix dactylifera extract at concentration 1000mg/kg, giardia lambia disappeared from the stool of infected mice at 7 day of giving the extract. Table 1

When we used phoenix dactylifera extract at concentration 2000mg/kg, giardia lambia disappeared from the stool of infected mice at 5 day of giving the extract. Table 2

When we used phoenix dactylifera extract at concentration 3000mg/kg, giardia lambia disappeared from the stool of infected mice at 4 day of giving the extract. Table 3

When we used metronidazole at concentration 200mg/kg, giardia lambia disappeared from the stool of infected mice at 4 day of giving the drug. Table 4

When we used distilled water the diarrhea continued and all the mice dead at the end of 8 days. Table 5

Intestinal sections stained with hematoxyline and eosin showed a spectrum of pathological changes with shortened fused villi, heavy lymphocytic infiltration

in the lamina propria and atrophy in mucosal and ganglion cells which showed decrease in number per base of villus. These changes encountered in mice treated with distill water and low doses of phoenix, while the intestinal mucosa were normal in mice treated with phoenix at 3000 mg /kg and also when treated with metronidazole at 200 mg/ kg.

We found significant difference between the concentrations of the extract of phoenix dactylifera and metronidazole. Also we found significant difference between the number of treatment days between the extract and metronidazole. Table (2, 3, 4, 5, 6)

Discussion

The plant extract are one of the important chemical material that have strong anti-parasitic and antibacterial activities. So these extract are used in the treatment and elimination of many of these pathogenic agents. Their anti-bacterial and anti-parasitic activities are due to the many powerful chemical that they contain like phenols, fatty acids and thousands of other chemical previously and currently used in medicine. ⁽²⁰⁾

Table 1. The effect of phoenix, metronidazole and distill water on giardiasis of infected mice according to duration of therapy

days	Phoenix 1000mg/kg	Phoenix 2000mg/kg	Phoenix3000mg/kg	Metronidazole200mg/kg	D.water	
1	3	3	3	3	3	
2	3	3	3	2	3	
3	3	2	1	1	3	
4	3	1	0	0	3	
5	2	0	0	0	3	
6	1	0	0	0	0	
7	0	0	0	0	0	
8	0	0	0	0	Dead	

Calculated F value for days= 33.4,

Table F 0.05= 3.605

Calculated F for concentration= 7.123

F 0.05=3.21

Numbers represent infected and dead mice

0: No infected mice

Phoinex at 3000mg/kg showed similar effect to flagyl (metronidazole)

Phinex at 1000mg/kg and at 2000mg /kg showed less effect tan flagyl

Table 2. The effect of phoenix 3000mg/kg and distill water on giardiasis of infected mice according to duration of therapy

Days	Distill water	Phinex 3000mg/kg
1	3	3
2	3	3
3	3	1
4	3	0
5	3	0
6	0	0
7	0	0
8	0	0

P value less than 0.05 (significant difference)

Numbers under the column of d. water and phoenix represent infected mice

Table 3. The effect of metronidazole 500mg/kg and distill water on giardiasis of infected mice according to duration of therapy

Days	d. water	Metronidazole
1	3	3
2	3	2
3	3	1
4	3	0
5	3	0
6	0	0
7	0	0
8	0	0

P value less than 0.05 (significant difference)

Numbers under the column of d. water and phoenix represent infected mice

Table 4. The effect of phoinex 1000mg/kg and distill water on giardiasis of infected mice according to duration of therapy

Days	d. water	Phoinex 1000mg/kg
1	3	3
2	3	3
3	3	3
4	3	3
5	3	2
6	0	1
7	0	0
8	0	0

P value more than 0.05 (no significant difference)

Numbers under the column of d. water and phoenix represent infected or dead mice

Table 5. The effect of metronidazole 500mg/kg bd and distill water on giardiasis of infected mice according to duration of therapy

Days	d. water	Phoinex 2000mg/kg
1	3	3
2	3	3
3	3	2
4	3	1
5	3	0
6	0	0
7	0	0
8	0	0

P value more than 0.05 (no significant difference)

Numbers under the column of d. water and phinex represent infected mice

Table 6. Mice mortality according to days of experiment (horizontal column) and type of treatment given (vertical column).

Days /treatments options	1	2	3	4	5	6	7	8	Mean
d.water	3.00 a	3.00 a	3.00 A	3.00 a	3.00 a	0.00 d	0.00 d	0.00 d	1.875 A
Phoinex 1000mg/kg BD	3.00 a	3.00 a	3.00 A	3.00 a	2.00 b	1.00 c	0.00 d	0.00 d	1.875 A
Phoinex 2000mg/kg BD	3.00 a	3.00 a	3.00 A	2.00 b	1.00 c	0.00 d	0.00 d	0.00 d	1.375 B
Phinex 3000mg/kg BD	3.00 a	3.00 a	1.00 C	0.00 d	0.00 d	0.00 d	0.00 d	0.00 d	0.875 C
Metronidazole 500mg/kg BD	3.00 a	2.00 b	1.00 C	0.00 d	0.00 d	0.00 d	0.00 d	0.00 d	0.750 C
Mean of days	3.00	2.80	2.20	1.60	1.00	0.20	0.00	0.00	

Note: a, b, c represent statistical difference

Dates have high tannin content and are used medicinally as a detergent (having cleansing power) and astringent in intestinal troubles. As a syrup, or paste, dates have been administered for sore throat, colds, bronchial catarrh, and taken to relieve fever and a number of other complaints. One traditional belief is that it can counteract alcohol intoxication. The seed powder also is used in some traditional medicines.⁽¹³⁾

A gum that exudes from the wounded trunk is employed in India for treating diarrhea and genito-urinary ailments. The roots are used against toothache. The pollen yields an estrogenic principle, estrone, and has a gonado-tropic effect on young rats.

In this study we select the plant extract phoenix dactylifera, because this substance is famous, available and used in treatment of diarrhea for long time. It is used by non-medical people as part of paramedical treatment. In this study we noticed that the concentration of 1000mg/g and 2000mg/kg have a little or insufficient effect on irradiation of giardia lambia. The animal still have diarrhea and the trophozoite still can be detected from the stool of these animal. While the concentration of 3000mg/dl was highly effective and it destroyed the parasite totally in the fourth day and this result was similar to the effect of metronidazole which also showed destruction of the giardia lambia in the fourth day. This is similar to one study which revealed that phoenix could have a

lethal effect on many intestinal parasite like giardiasis.⁽²¹⁾

The effect of high concentration 3000mg/dl on giardia lambia was so high and effective for many reasons. First a large amount of the extract reaches the intestine and so the parasite absorbed these chemical present in the extract so a lethal concentration reach the parasite. The second reason was the very high, continuous toxic concentration available to kill the parasite.⁽²¹⁾ The important chemical present in the extract which thought to play a role in killing the parasite are; fatty acids, bases, hydrocarbons, and many other non-polar substances which are soluble in water.⁽²²⁾

The seeds phoenix dactylifera are resistant to fermentation and destruction by strong acids and bases. So they can resist the strong acids present in the stomach and can also resist the strong bases present in the intestine. This high resistance makes them available in high concentration in the intestine where the parasite is present. So the atmosphere became unsuitable for the parasite making it to change into cystic forms and excreted in the stool prevent its growth.

The histological results which showed villus atrophy, heavy infiltration of small intestine of the vertebrate with lymphocytes and decreased numbers of glandulae cells were similar to many studies done on mice infected with giardiasis.⁽²¹⁾

Conclusion

Phoenix dactylifera has similar efficacy to metronidazole in laboratory animal. This natural treatment may be beneficial in treatment of human giardiasis.

Recommendation

We recommend further study and evaluation of this extract on other parasites and to use large samples for the study to be more significant

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