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Effect of aqueous extract of the plant *Mentha longifola* in viability of *Leishmania donovani in vivo*

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Abstract

This study included the using aqueous extract of plant *Mentha longifola* with the concentration (25 , 50 , 75) mg /ml to study its effect on the vitality of the parasite *Leishmania donovani in vivo* in male laboratory mice *Mus musculus* . The animals were divided into three groups injected with *Leishmania donovani* and the next day treated with aqueous extract of the plant *M. longifola* ,the first group treated with plant extract and concentration 25 mg /ml , second group treated with plant extract and concentration 50 mg /ml and the third group treated with plant extract and concentration 75 mg /ml . In addition to the positive control group which injected with parasite and untreated with plant extract and negative control group which non-injected with parasite and untreated with plant extract .

After 60 day of infection with the parasite and treated with plant extract were dissected animals and taking the weights of each the animal's body , liver and spleen to a statement rates of infection in the organs. The current study showed that groups of animals which infected with parasite and treated with plant extract showed a significant decrease ($p<0.01$) in the weights of the liver , length of spleen , ratio of spleen enlarged , number of parasites in spleen and delayed hypersensitivity compared with positive control group ,and this a decrease was increases with the concentration of the plant extract used where it was noted that the group treated by concentration 75mg/ml of the plant extract does not differ significantly from the negative control group .

Key word: *Leishmania donovani*, treatment, Mentha *longifola*, Leishmaniasis.

تاثير المستخلص المائي لنبات البطنج *Mentha longifola* في حيوية طفيلي *Leishmania donovani* داخل الجسم الحي

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الخلاصة:

تضمنت الدراسة الحالية استخدام المستخلص المائي لنبات البطنج وبتراكيز (25 ,50, 75) ملغم / مليليتر لدراسة تاثيره في حيوية طفيلي اللشمانيا الاحشائية داخل الجسم الحي في ذكور الفئران المختبرية . قسمت الحيوانات الى ثلاث مجاميع حقنت بطفيلي اللشمانيا الاحشائية وفي اليوم التالي جرعت بالمستخلص المائي لنبات البطنج , المجموعة الاولى جرعت بتركيز 25 ملغم / مليليتر من المستخلص , المجموعة الثانية جرعت بتركيز 50 ملغم / مليليتر من المستخلص والمجموعة الثالثة جرعت بتركيز 75 ملغم / مليليتر من المستخلص , بالاضافة الى مجموعة السيطرة الموجبة التي حقنت بالطفيلي وغير معاملة بالمستخلص ومجموعة السيطرة السالبة غير محقونة بالطفيلي وغير معاملة بالمستخلص .

وبعد 60 يوم من الخمج بالطفيلي والمعاملة بالمستخلص شرحت الحيوانات واخذ وزن كل من الحيوان والكبد والطحال لبيان معدل الاصابة في هذه الاعضاء . اوضحت الدراسة الحالية ان مجاميع الحيوانات المخمجة بطفيلي اللشمانيا الاحشائية والمعاملة بالمستخلص المائي لنبات البطنج اظهرت انخفاضا معنويا ($p<0.01$) في وزن الكبد وطول الطحال ومعامل تضخمه وعدد الطفيليات في الطحال واختبار فرط الحساسية المتاخرة مقارنة بالسيطرة الموجبة وهذا الانخفاض يزداد بزيادة تركيز المستخلص النباتي حيث لوحظ ان المجموعة الحيوانية المعاملة بالتركيز 75 ملغم / مليليتر من المستخلص النباتي لم تظهر فروق معنوية مقارنة مع مجموعة السيطرة السالبة .

1.Introduction

Leishmaniasis are a wide distribution of parasitic disease caused by dimorphic protozoan flagellates of the genus *Leishmania* . These obligatory parasites of host macrophages are transmitted by different species of phlebotomine sand fly (Pearson and Sousa , 1996) .

There are over 20 species and subspecies of the genus *Leishmania* that infect humans via the bite of sand flies . Sand flies of the species *Lutzomyia* serve as the vector in the New World , while the *phlebotomus* species transmit infection in Old World . They tiny sand colored the female is blood – feeding that breed in forest areas caves and burrows in tropical and sub tropical region (Markel and Makhoul , 2004) .

Leishmaniasis infection spread on 98 countries ranging number of people infected with the disease about 12 million person ,while infection rates of 2 million new cases annually , with an estimated 75% of the case represent cutaneous leishmaniasis and 25 % represent visceral leishmaniasis (Alvar *et al*.,2012).

The saliva of the insect vectors of the genus *Phelbotomus* also plays an important role at the beginning of the infection , as the insect saliva contains Salivary Immunosuppressive Protein (SIP) that works to restrain the first attempts of the immune system to resist the parasite (Martin *et al* ., 2007) .

Pentavalent antimonial are the standard first- line treatment for leishmaniasis (Aggarwal *et al* .,1999 ; Berman ,1996) ,although resistance is a growing problem (Khalil *et al* ., 1998) .

So let the need to reach an anti- parasitic safe for human use of natural sources , whether plant or animal , several plant products have been tested and found to possess some anti-leishmanial activity . *Mentha* species of the family labiatae are well known in traditional medicine (Lewis and Elvin – Lewis , 1977) .

Mentha longifola know herbal has elongated oval or spear leaves arranged on the stem of the plant in smooth cross pairs or wrinkled to fluff regularly serrated, upper leaves sitting either lower with short stem (PDR for herbal medicine , 1998) . *M. longifola* has many therapeutic uses it was repelling gas , anti-colic , sweating helps , anti-vomiting , treats indigestion accompanied by gas and dysmenorrhoea , as for the external uses sterile and anti –itch (AL-Zuobidy *et al* ., 1996) . It is an anti- flat worm (Sharathcharel *et al* ., 1995) ,anti- microbial and anti –fungal (Mucciarell et al ., 2001)

The present study aims to study the effect of aqueous extract of the plant *Mentha longifola* in viabilityof *Leishmania donovani* in vivo .

2. Material and methods

2.1. Source of the parasite

It was obtained pure isolation and diagnosed of *Leishmania donovani* from the Department of Biology – College of Science – University of Thi-Qar .

2.2. Prepare cold aqueous extract of the plant

It was obtained the plant *Mentha longifola* from local markets in AL-Nassiriya province. Aqueous extract was prepared according to the method of Harbone (1984) , where taking 20 gm of dry powdered of plant and put in a glass beaker 1000 ml capacity contains 100 ml of cold distilled water at room temperature . Then mixed the plant material by magnetic starrier for 15 minutes ,then it was nominated by a piece of muslin cloth to separate large pieces then transfer the filtrate into the centrifuge speed of 3000 rpm for 15 minutes in order to precipitate the plant smaller pieces to get a clear plant extract ,then put the filtrate in Petri dishes to dry at room temperature , after obtaining the dry powder remember at 20 c^0 until use .

2.3. Experimental design

25 male of mice *Mus musculus* aged (8- 10) weeks by 5 animals for each group . Four groups were injected intraperitoneal with *Leishmania donovani* parasite and dose $1.2\times 1\ 0^6$ parasite /0.2ml ,then the fifth group was not injected with the parasite and injected with normal saline to be a negative control group ,in the next day the previous groups treated with aqueous extract of plant *Mentha longifola* and dose 0.2 ml as follow :

1-The first group were treated with plant extract and concentration of 25 mg / ml for five days .
2- The second group were treated with plant extract and concentration of 50 mg / ml for five days .
3- The third group were treated with plant extract and concentration of 75 mg / ml for five days .
4- The fourth group injected with parasite and non treated with plant extract to be a positive control group .
5-The fifth group non-injected with parasite and untreated with plant extract to be a negative control group .
After 60 day of injection parasite and treated with plant extract were dissected animals and then taking weight of spleen and liver and measured length of spleen and sectioning each liver and spleen into small pieces to work impression on the slide and stained with Giemsa dye ,other pieces have been cultivated on the NNN media of the parasite

2.4. Evaluate the effectiveness of the aqueous extract of plant *Mentha longifola* in vitality of parasite *Leishmania donovani* .

It was use the following standards to compare the progress of infection in the infected animals which treated with plant extract:
1-Changes in the liver weight increase and changes in the length of the spleen increase (Stauber , 1966)
2- Ratio of spleen enlarged = weight of spleen (mg) / weight of body (gm) (Stauber ,1953)
3- Parasites density (amastigote) and the number in spleen (Stauber , 1958)
and it adopted the standard way Stauber ,(1958) to determining the density of parasites
number of parasites in spleen =weight of spleen (mg) × mean number of parasites in cell of animal × Stauber factor (200000) .
4- Result of hypersensitivity test, Guirges (1971) method is used in the preparation and conduct of this test
5- Appearance of promastigote form in liver and spleen culture

2.5. Statistical analysis

Statistical Package for Social Science (SPSS) system was using to analyzed data using ANOVA analysis and extraction of the least significant difference (LSD) below the level of probability (p=0.01)

3.Result

3.1. Effect of aqueous extract of the plant *Mentha longifola* in liver weight of laboratory male mice infected with *Leishmania donovani.*

The results of the current study , as shown in Table (1) that animal groups which infected with parasites and treated with aqueous extract of the plant *Mentha longifola* by three different concentrations showed significant($p < 0.01$) a decrease in liver weight compared with the positive control group .It is noted that the third group which treated with concentration 75mg/ ml of extract recorded less in the liver weight showed no significant difference ($p > 0.01$) compared with the negative control group. .

Table (1) Effect of aqueous extract of the plant *Mentha longifola* in liver weight of laboratory male mice infected with *Leishmania donovani*

Groups	Weight of liver / gm
Group 1	2.0± 2 0.042 *
Group 2	1.92± 0.036
Group3	1.44 ± 0.019
Positive control	2.23 ± 0.046
Negative control	1.31± 0.013

*Values are means ± S. D.

3.2. Effect of aqueous extract of the plant *Mentha longifola* in the length of spleen of laboratory male mice infected with *Leishmania donovani* .

In the table (2)the present study show that animals groups which infected with parasite and treated with aqueous extract of the plant showed decrease in the length of spleen compared with positive control group and were significant differences($p<0.01$) between these groups and the positive control group . It is noted that the third group which treated with concentration 75mg /ml of extract recorded less the length of the spleen showed no significant difference($p>0.01$) compared with the negative control group .

Table (2) Effect of aqueous extract of the plant *Mentha longifola* in the length of spleen of laboratory male mice infected with *Leishmania donovani* .

Groups	length of spleen / cm
Group 1	2.29 ± 0.074*
Group 2	1.97± 0.025
Group3	1.87 ±0.019
Positive control	2.29 ± 0.074
Negative control	1.73± 0.042

* Values are means ± S. D.

3.3. Effect of aqueous extract of the plant *Mentha longifola* in ratio of spleen enlarged of laboratory male mice infected with *Leishmania donovani* .

Animals groups infected with parasite and treated with plant extract shows a reduction in ratio of spleen enlarged compared with positive control group , the differences were significant (p <0.01) . This reduction increases with concentration of plant extract where the third group which treated with concentration 75 mg /ml of plant extract showed lowest rate in the enlarged spleen and showed no significant difference(p>0.01) compared with the negative control group .Table (3) .

Table(3) Effect of aqueous extract of the plant *Mentha longifola* in ratio of spleen enlarged of laboratory male mice infected with *Leishmania donovani* .

Groups	Ratio of spleen enlarged mg/ gm
Group 1	3.20± 0.071 *
Group 2	2.46± 0.061
Group3	1.95 ± 0.074
Positive control	3.90± 0,027
Negative control	1.86± 0.040

* Values are means ± S. D.

3.4. Effect of aqueous extract of the plant *Mentha longifola* in the number of parasites in the spleen of laboratory male mice infected with *Leishmania donovani* .

In Table (4) note that the number of parasites in the spleen of animal groups infected *leishmania donovani* parasites and treated with plant extract decreased significantly (p<0.01) when compared with the positive control group and the numbers of the parasites in the spleen decreased with increasing concentration of the plant extract Where it was the third group treated with highest concentration of plant extract did not show significant difference when compared with the negative control.

Table (4) Effect of aqueous extract of the plant *Mentha longifola* in the number of parasites in the spleen of laboratory male mice infected with *Leishmania donovani*

Groups	Number of parasites in the spleen × 10^6
Group 1	12.00 ± 0.15*
Group 2	6.46 ± 0.39
Group3	0.00 ± 0.00
Positive control	18.12 ± 0.92
Negative control	0.00 ± 0.00

*Values are means ± S. D.

3.5. Effect of aqueous extract of the plant *Mentha longifola* in the difference in the thickness of the foot pad of laboratory male mice infected with *Leishmania donovani*.

Animal groups infected with *leishmania donovani* parasite and treated with plant extract showed significant(p <0.01) decrease in delayed hypersensitivity , represented by the difference in the thickness of the foot pad , compared with the positive control and were significant differences between all groups .Table (5) ..

Table (5) Effect of aqueous extract of the plant *Mentha longifola* in the difference in the thickness of the foot pad of laboratory male mice infected with *Leishmania donovani*

Groups	Difference in the thickness of the foot pad(cm)
Group 1	0.39 ± 0.014 *
Group 2	0.27 ± 0.019
Group3	0.20 ± 0.015
Positive control	0.60 ± 0.23
Negative control	0.16 ± 0.011

*Values are means ± S. D.

3.6. The results of the examination impression, smear and culturing of parasites

Third group and a negative control group showed negative results for each impression, smear and culturing. Positive control group showed positive results for both impression and culturing to the liver and spleen and negative to the smear and culturing of blood. The second and third group showed positive results for the impression of liver and spleen only .Table (6) .

Table (6) The results of the examination impression, smear and culturing of parasites

Groups	Liver		Spleen		Blood	
	Impression	Culture	Impression	Culture	Smear	Culture
Group 1	+	-	+	-	-	-
Group 2	+	-	+	-	-	-
Group 3	-	-	-	-	-	-
Positive control	+	+	+	+	-	-
Negative control	-	-	-	-	-	-

4.Discussion

By the results of the present study showed that the infected groups with *leishmania donovani* parasites and treated with aqueous extract of the plant *M. Longifola* and different concentrations showed a significant decrease in liver weight, length of the spleen and ratio of spleen enlarged compared with the positive control , It was noted that the rate of decrease in liver weight , length of the spleen and ratio of spleen enlarged increases with the concentration of the plant extract which used, where it was noted that the third group of treated animals with concentration 75 mg / ml of the plant extract does not differ significantly from the negative group. This is an indication not get infection in this groups and not to increase the length of the spleen and it enlarged in the third group treated with concentration 75 mg / ml as well as in other treated groups that indication of the non-arrival of the parasites to these organ, where it helped aqueous extract of the plant *M.longifola* in reducing the large number of these parasites, as is the spleen of the most important organs of that can be to give an early and clear results agreement with many of the researches that also showed that many positive results giving the spleen where the liver is negative for the presence of parasites(Ott *et al* ., 1967) .

The increase in the size of liver and spleen in the non -treated group with plant extract this evidence of the infection and that the results of the multiplication and accumulation of the parasites as well as changes in the number of defensive cells increase in these organs (Stauber, 1966).

This is agreement with AL- GazI(2014) where he noted an increase in the weight of liver and spleen in animals infected with *leishmania donovani* parasites and non – treated with camel milk . As for the number of the parasites in the spleen was noted that number in the spleen of the group animales treated with plant extract that decrease with increase the concentration of extract Where it was noted that the third group treated with concentration 75 mg / ml of plant extract did not get the infection this is agreement with Ott *et al* ., (1967) and Hanson and Stauber (1964) That the spleen be more sensitive to infection than the liver and the number of parasites in the spleen is more than the number in the liver in the same period if taken the difference in size between the two organs into consideration . As for the results of the impression , smear and culturing third group treated with concentration75mg / ml of the extract showed negative results for both impression and culturing , the positive control group showed positive results for both impression and culturing while the animal groups which treated with concentration 25mg/ ml and 50mg /ml of the plant extract showed positive results for both the liver and spleen impression but they showed negative results for culturing all groups showed negative results of the smear and blood culturing, Hill (1986) noted the difficulty of obtaining the cells in the

blood, especially that taken from the heart .

The results of the delayed hyper sensitivity showed decrease with increase the concentration of the plant extract and this is evidence that delayed sensitivity increases with infection Where it was noted that the third group treated with concentration 75mg/ ml of the plant extract showed the lowest value in the delayed hypersensitivity compared with the other groups and did not significantly differ from the negative control group.

The reason in the decrease of infection and not even occur in the treated animals groups treated with aqueous extract of plant *M .longifola* its contains many important compounds of the most important its volatile oil that exists by 0.5 % (AL- Zoubidy *et al.,* 1996) . Studies have shown the plant of the genus *Mentha* possess important antimicrobial activities(Mimica *et al.*, 2003) , mainly due to the presence of oxygenated monoterpenes in their chemical composition (Kitic *et al.*, 2002 ; Karaman *et al* ., 2003 ;Sahin *et al* ., 2003 ; and Hussain *et al*., 2010) . The effect of ethanolic extract of *M. Longifola* was evaluated against *Entamoeba histolytica* and *Giardia duodenalis* by EL-Badry *et al* ., (2010) , Nthaisi *et al*., (2012) observed Inhibition in the hatching eggs and mortality rate in larvae of some intestinal nematodes using aqueous and acetonic extract of plant *Mentha* and at different concentrations.

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