

Effect of Plant extract of *Nigilla Sativa* on the effectiveness of the Parasite *Entamoeba histolytica* in Patients from Al-Hammar Marsh Iraq .Al-Nasiriyah.

Hussain Ahmed Shariff *, Reyam Najj Ajmi **and Abd-Aljabar Jasim Jaryan***

*University of Al-Mustansiriya – Collage of Pharmacy .

** – Collage of Science .

الخلاصة:

تم اختيار اشخاص يعانون حالات الاسهال الحاد من مجتمع هور الحمار والمراجعين لمستوصف الجبايش في مدينة الناصرية خلال شهر حزيران 2009 فحصت عينات البراز وأظهرت نتائج الدراسة أن نسبة الإصابة الكلية بطفيلي بلغت 49.5% *Entamoeba histolytica*. وكانت أعلى في الذكور حيث بلغت 54.8% مقابل 45.2% للإناث.

وللتحري عن امكانية تنمية هذا الطفيلي في وسط زرع مناسب والمدة التي يبقى فيها الطفيلي حياً في هذا الوسط تم معاملة إنزيم السستائين بروتينيز المقيد بالمستخلص الزيتي لنبات الحبة السوداء و المترونيدازول والتتيدازول فوجد من خلال النتائج تنشيط الفعالية الإنزيمية إلى (34، 62، 55 و 60) وحدة/مل على التوالي. واثبت ان الوسط (semi solid medium for parasitic amoebae) نجاحه في انماء الطفيلي

اثبت استخدام المستخلص الزيتي للحبة السوداء نجاحه في قتل الطفيلي المستتبت في الوسط الزرع في مدة قصيرة. ونجاحه كعلاج للزحار الاميبي مقارنة بعقار (Metronidazole) خاصة عند استخدامه بجرعة قدرها (0.04) مل درس تأثير المستخلص النباتي والعقاقير الطبية في الأطوار المتغذية لطفيلي *E. histolytica* في الوسط الزرع، فوجد إن المستخلص المائي للحبة السوداء وعقاري المترونيدازول والتتيدازول قد أثرا على الأطوار المتغذية لطفيلي *E. histolytica* نستنتج من هذه الدراسة استعمال مستخلص النبات كعقار لمعالجة حالات الإسهال الناتجة عن الإصابة بطفيلي *E. histolytica*

Abstract:

Stool samples from people which were suffering from acute diarrhea had been chosen At Al-Hammar Marsh and from reviewers of a clinic in the city of Al-Nasiriyah Chibaach during the month of June 2009 .Stool examination results showed that the proportion of total parasite infection was 49% *Entamoeba histolytica* Infections were higher in Males ,reaching 54,8 % compared with 54,2 % for females .To investigate the possibility of parasitic Amoeba growth Semi Solid Medium was very successful as a good media for

that purpose .Using the oily extract of the black seed had an a good effect on amoebic Parasite killing for a short period in compare with Metronidazole drug ,especially with the dose of (0.04) ml ,results from using a mixture of a black seed oil and the enzyme of Cystein proteinase, Metronidazole and Tinodazole showed a great inhibition for the parasite enzymic activity to (34,48,50)ml, respectively.

As a conclusion the results showed that black seed oil extract and these three drugs were very effective factors on the trophozoits phases of *Entamoeba histolytica* Parasite and the most effectiveness can be gaine from the black seed oil extract.

Key: Black seed oil .Metronidazole .Tinodazole .Cysteine proteinase

Introduction:

Entamoeba histolytica is a widely distributed all over the world. The infection with *E. histolytica* is increased in tropic and subtropic regions, particularly in poor sanitation areas (DiMiceli, 2004). It is responsible for at least 50 million cases of diarrhea and an estimated 100,000 deaths per annum and ranks second only to malaria as a cause of mortality. Infection with *E. histolytica* leads to amoebic colitis, colonic ulceration and amoebic liver abscess (Moncada *et al.* , 2005).

The trophozoite in its natural habitate in the luman of large intestine especially in cecal and sigmoidorectal region causes symptomatic amoebiasis including diarrhea, abdominal pain and cramping or amoeba may produce, but patient show no clinical symptoms called Asymptomatic carriers (Levinson & Jawetz, 2000).

Several means of transmitting *E. histolytica* are known, ingestion of the infective cyst occure in contaminated food and water and it may also be transferred via homosexual men (Tanyuksel & Petri, 2003).

Cysteine proteinase considerd to be a key virulence factor which lysis the target cell (Que *et al.*, 2003). other virulence factors such as hyaluronidase, phospholipase and hemolysine which are important in pathogenesis of amoebiasis (Nickel *et al.*, 2000). Infection with *E.histolytic* can controlled by inhibite the virulence factors especially cysteine proteinase (Que&Reed ,2000).

Nitroimidazole, particularly metronidazole are the mainstay of therapy for invasive amebiasis, also Tinidazole is better tolerated and it allows shorter periods of treatment. nitroimidazole treatment should be followed by paramomycin or the second line agent diloxanide furoate to cure luminal infection. Metronidazole and paramomycin should not be given at the same time, since the diarrhea that is a common side effect of paramomycin may make it difficult to assess the patients response to therapy (Haque *et al.* , 2003) .

Aim of the research:

- 1- Determine the effect of black seed oil extract of the plant on the pathogenesis *E.histolytica* cultured and compare their effectiveness with the effectiveness of drug Metronidazole.
- 2- Studying the effect of some plant extracts and drugs on *E. histolytica* and immobilized cysteine proteinase *In vitro*.
- 3- Immobilization of cysteine proteinase enzyme which produced by *E. histolytica* by using calcium alginate and determination the optimal condition to produce cysteine proteinase .

Materials and Methods:

During the present study a total of 24 stool sample were collected randomly from patients suffering from sever diarrhea who admitted into Al – Hammar marshes in Al- Nasiriyah government at the interval from june 2009 to. Each stool sample was placed in a clean plastic container and labeled with patient information such as sex, age and educational level of paterfamilias. The sample was examined within 30 minute to insure the presence of *E. histolytica* trophozoite.

Ensure the use of a number of laboratory equipment and laboratory tools and dyes, medicines, medicinal herbs have been prepared solutions according to common methods of *E. histolytica*: A pilot study of aqueous extracts of the plant for grain on the black center-grown by *E.histolytica*: Transplant center to record *E. histolytica* and after the end of the incubation period, took the pipe and divided into groups, as follows:

Group A: Were taken (4) tubes and add to it (500) mg / kg of the drug Metronidiazole daily for one week with a daily tests represented by microscopic examination and see the changes.

Group B: Were taken (4) tubes and add to it (0.03) ml of an aqueous extract of black bean plant daily for a week with a microscopic examination to observe the changes.

Group C: Were taken (4) tubes and add to it (0.04) ml of an aqueous extract of the plant to the grain of the black day for a week with a microscopic examination to observe the changes. This dose is equivalent to 0.4 g of bark powder learners.

Group D: Were taken (4) tubes and add to it (0,05) ml of an aqueous extract of black bean plant daily for a week with a microscopic examination to observe the changes. This dose is equivalent to 0.5 g of ginger powder.

Two type of direct wet film preparation were done for each sample at the same time, one by using normal saline 0.85% for detection the motility of trophozoite and other by lugol's iodine 5% for demonstrating structures of *E. histolytica* (Ziebig, 1997). After samples collectin the steps we are achieved that included:

Isolation of *E. histolytica* and Inoculation and maintenance of *E. histolytica* in culture media , Immobilization of cysteine proteinase produces y *E. histolytica*, Concentration with ammonium sulphate and Immobilization of enzyme with Ca-alginate and estimation of enzymatic activity .

Results and Discussion:

1: Detection of *E. histolytica*

Examined the effectiveness of the *E. histolytica* parasite cultured in the middle (Semi Solid medium for parasitic amoebae) and a dose of (0.03) ml of this oil, since for the death of the parasite was observed when examining a sample from the mid-microscopically, and after the third day of direct with adding the extract. changes in the parasite cultured in the center and the (0.04) ml of an aqueous extract of the *Nigella sativa* , Examined the effectiveness of the aqueous extract of the parasite *E. histolytica* cultured in the middle (Semi Solid medium for parasitic amoebae) and a dose of (0.04) ml of this oil because of the death of the parasite was observed when examining a sample from the mid-microscopically, and after the second day of direct with adding the extract. The changes in the parasite cultured in the center and the pea-genitive (0,05) ml of an aqueous extract of the plant *Nigella sativa*.

Examined the effectiveness extract of the plant parasite on the black seed oil cultivar *E. histolytica* in the middle (Semi Solid medium for parasitic amoebae) and a dose of (0,05) ml of this oil, since for the death of the parasite was observed when examining a sample from the mid-microscopically after the third day from direct with adding the extract..

Male	%	Female	%
18	58.0	13	41.9
15	34.0	36	67.2
20	57.1	15	42.8
33	58.9	20	41.0
31	61.2	19	36.7
23	60.2	15	39.4
16	64.6	11	33.3
9	61.5	5	34.4
8	38.3	14	63.6
12	63.1	9	32.8
8	54.3	5	41.4
6	75.0	4	29.0
16.5	54.8	12	45.2

Table-1: Distribution of injury between males and females

T (tabled) = 23.28 T (calculated) = 2.015

2-Changes in the Parasite *E.histolytica* cultured in the middle -drug and genitive (Mteronidazole):

Examined the effectiveness of the drug (Meteronidazole) on the parasite *E.histolytica* cultured in the center (Semi solid medium for parasitic amoebae) and genitive (500) mg / kg of the drug, it has been observed the death of the parasite when examining a sample from the mid-microscopically, and after the fourth day of direct to add real estate to the center as the work of the drug to kill the parasite in a few days and this result was an approach by the finding when the drug dose (500) mg / kg against infection with this parasite, and the approach to reach him (William and Sodeman, 2000) when used for a dose (750) mg / kg per day against parasite infection *E.histolytica* .

Showed the results of using doses different from the aqueous extract of the plant *Nigella sativa* effective at killing the parasite cultured in the middle(Semi solid medium for parasitic amoebae), especially when you use a dose (0.04) ml of the extract and in the second day of direct with adding the extract and return the effectiveness of the plant extract to this plant because it contains active substances of these materials earned extract oil shale properties and this is consistent with what he referred to (Cimanga, R, K, *etal* ,2006).

The incidence of amoebic dysentery was higher in males (54.8) than females (45.2) (Table 4-1). However, statistical analysis showed no statistically significant differences between the percentage of cases with male and female. May be due to socio-economic level the same, and this result was consistent with the study of (Younis, 2007) and(Ngrenngarmler, 2007).

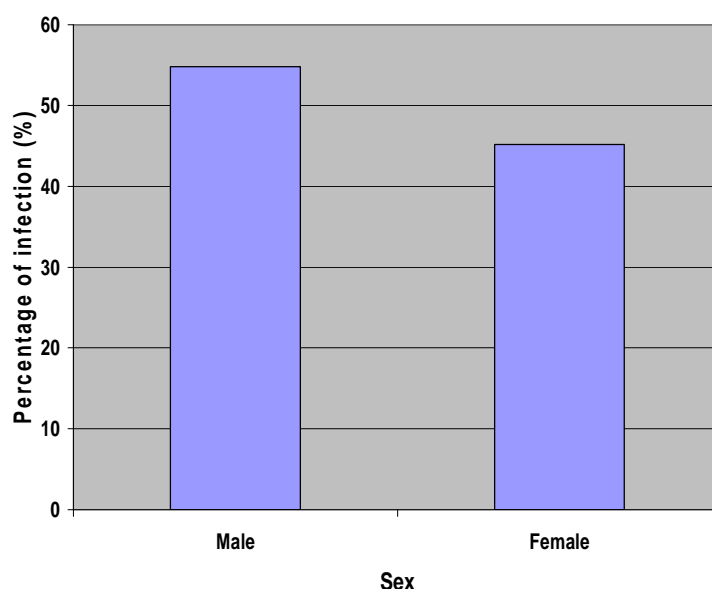


Figure-1:The difference in the level of injury between males and females.

Showed that the addition of D- mannose sugar plays an essential role in *E. histolytica* trophozoite adherence to target cells by allowing the identification of specific receptors implicated colonization adhesion of *E. histolytica* to human red blood cells which results mainly from the interaction of specific surface

proteins called adhesins with molecules containing specific carbohydrate residues that are responsible for the binding and that may be found on the surface of cells from infected organs as well as of human red blood cells (Lopez-Revilla & Cano-Mancera, 1982),(Huston *et al*, 2003).

The effect of *N. sativa* oil was tested on Ca-alginate immobilized cysteine proteinase activity. *N. sativa* oil has partial inhibitory effect on immobilized cysteine proteinase. this reduction was considered statistically significant ($p < 0.05$). the mechanism by which enzymatic activity of immobilized cysteine proteinase was reduced may be due to the possible presence of active components and minerals such as zinc and copper which affected the enzyme structure and its active site, the copper has inhibitory effect on cysteine proteinase through oxidative (-SH) groups in active site to form complex with protein, this effectively blocking the active site and preventing enzyme binding with substrate (Ahmad *et al.*, 2004).

Metronidazole decreased the enzymatic activity of immobilized cysteine proteinase, this decreasing may be due to the chemical compound of drug which interferes with proteine synthesis (Laurence *et al.* 1997). Metronidazole could be used as anti-amoebic drugs until the *E. histolytica* become resistance to metronidazole because it is effective on the cysteine proteinase which considered as an important virulence factors for *E. histolytica* (Que & Reed, 2000). We noted reducing in immobilized cysteine proteinase activity when used tinidazole, the enzymatic activity become 50 u/ml. this results is similar to that of metronidazole. Tinidazole used as anti-amoebic drugs and it is a useful alternative to metronidazole in the treatment of amoebiasis (Laurence *et al.*, 1997). Therefore tinidazole is still used as a good anti-amoebic drug inspite of presence resistant strain of *E. histolytica*; this may be due to its effect on cysteine proteinase.

The effect of *N. sativa* oil on *E. histolytica* trophozoite may due to the presence of active compounds containing different classes of alkaloids (Marikawa *et al.*, 2004) that block protein synthesis of *E. histolytica* trophozoite, beside *N. sativa* seeds also contain phenolic compounds (Riaz *et al.*, 1996) these molecules are well known for their diverse physiological properties including among others, anti-parasitic anti- inflammatory and anti- carcinogenic (Ma & Kinner, 2002).

N. sativa seed have been reported to exert anti-helminthic (Azza *et al.*, 2005)

The ability of *N. sativa* oil to kill the trophozoite of *E. histolytica* in culture media may be also due to their harmful effect on bacteria living in amoeba xenic culture, because *E. histolytica* is more virulent in association with suitable bacterial cells (Clark & Diamond, 2002),(Al-jubory ,2005).

The results of this study is in agreement with results of (Adagu *et al.* 2002) that metronidazole had an effect in killing the trophozoite of *E. histolytica* by alteration in the protoplasmic organelles of the amoeba and inhibitory protein

synthesis and inhibitory effect of Tinidazole on growth of *E. histolytica* (Al – banea, 2006).

Tinidazole has similar effect to metronidazole they retain to 5-Nitroimidazole groups which has anti-infective agent; it causes cell swelling and distorted cell shape, plasma membrane damage and formation of extensive empty areas in the cytoplasm of the amoeba (Chavez-Mungnia *et al.*, 2004).(Bansal *et al.*2005)

Materials	Enzymatic activity(u/ml)
<i>Nigella sativa</i>	34
Metronidazole	48
Tinidazole	50

T (tabled) = 17.077 T (calculated) = 19.127

Table- 2: Effect of drugs on *E. histolytica* In vitro

Conclusions

- 1- Ability of *E. histolytica* trophozoite to produce virulence factors such as colonization factors, hemolysin, phospholipase and cysteine proteinase *In vitro*.
- 2- Use proven plant extract of the plant (*Nigella sativa*) success as a treatment for amoebic dysentery compared with the drug (Meteronidazole) especially when used at a dose of (0.04) ml of it.
- 3- The (Semi solid medium for parasitic amoebae) proved to be successful to the development of parasite *E.histolytica* for eight days.

Recommendation:

Modern techniques must be used to get this kind of alternative therapy and further studies on other types of plant and medicinal herbs.

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