ISSN 1991- 8690 الترقيم الدولي ٨٦٩٠ - ١٩٩١

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Effect of alcoholic extract of the plant Nigella Sativa on viability of eggs and adults of the liver giant worm Fasciola gigantica In vitro.

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Abstract

The study aimed to examine the effect of *Nigella sativa* extract on viability of eggs and adults of *Fasciola gigantica In vitro*. *N.sativa* brought from local markt of Al-Najaf city while eggs and adults worm of the parasite collected from cows and buffaloes infected and in the abattoir of Al-Najaf city.Result showed a significant effect for extract in reducing of the percentage of egg hatching and increasing of the mortality percentage of adult worms compar with control groups.

تأثيرالمستخلص الكحولي لنبات الحبة السوداء في حيوية بيوض وبالغات دودة الكبد العملاقة *Fasciola gigantica* في الزجاج

الخلاصة

هدفت الدراسة الحالية،لاختبارالتأثيرالحيوي مستخلص الكحول الاثيلي لبذور الحبة السوداء*Nigella sativa* في بيوض وبالغات دودة الكبد العملاقة *Fasciola gigantica* في الزجاج. جلبت بذورالحبة السوداء من الاسواق المحلية لمدينة النجف بينما البيوض والبالغات تم جمعها من الأبقار والجاموس المصابة من مجزرة مدينة النجف.اظهرت النتائج ان لمستخلص الكحول الاثيلي لبذور الحبة السوداء تأثيراً معنوياً في خفض النسب المئوية لفقس البيوض وزيادة النسب المئوية لهلاك الديدان البالغة كلما زاد تركيزالمستخلص مقارنة بمعاملة السيطرة.

Introduction

Fascioliasis disease is one of the most dangerous health problems because of its infection that cause economic losses by worms belonging to the genus *Fasciola* which generally affects animals as cows, sheep and rabbits (Andrews,1999;Souza *et al.*,2002;Zhang *et al.*,2005;Soliman, 2008).While humans infection are incidental so this Fascioliasis is animaldiseaseprimarily WHO,1995;Okewole et al.,2000;Hurtrez-Bousses et al .,2001). These worms belongto thePhylum:Platyhelminthes,Class:Trematodes, Sub class:Digenea and genus: Fasciola,Which includes two Species F.hepatica and F.gigantica, which are responsible for the Morbidity and Mortality in sheep and cattle (Soulsby,1982;Ahmed,2000;Tolan,2002; Mas-Coma et al.,2005).The disease is distributed in areas where there is Intermediate host which is one of the types of snails of the genus: Lymnaea that return to the Phylum:Mollusca the Miracidum stage of F.gigantica when penetrated this snail needed about 5-7weeks to development to Cercariae which encysted on vegetation as Metacercariae and transmitted to the final host from vertebrates which infected with Acute and Chronic phase characterized by weight loss in animals as well as the lack of milk production and cachaxia in severe infection (Saba et al.,2004). As well as the poor quality of the meat and damaged livers infected with the parasite and its effect on the fertility of animals in addition to the delay in the growth of small animals (Abunna et al.,2010;Hossain et al.,2011). Domestic and wild animals infected by parasite result from eating aquatic plants that infected by Metacercariae and causing Fascioliasis, which is a essential problem threaten the livestock,Wild animals infected consider as reservoir hosts of these worms or drinking water that contains on Metacercariae that swallowed to give Juvenile worms (Piqueras,1992; Roberts&Janovy,2000). The resistance of these worms against of these drugs is one of the most important problem to control distribution of this disease which requires continued research for more effective treatments and less toxic than previous treatments (Al-

Beitawi and El-Ghousein,2008).So researchers producing another of alternative treatments, such as plant extracts because of their effectiveness against the parasite and without any side effect (Ahmed,2000;Sunita&Singh,2011). Recent scientific studies note to the importance use of Medical Herbs and plant extracts in control of many different pathogens parasite that affects on humans and animals, From numerous studies of Nigella sativa extract proven the pharmacokinetics against many parasites because there are many active materials which have Medical importance (Al-Tamimy, 2001;Al-Zubaidy,2002).

Materials and Methods

1- Preparation of alcohol extract of *Nigella sativa* seeds & stock solutions

Nigella sativa seeds brought from local markets of AL-Najaf city, purified from impurities and kept at laboratory temperature in a dry place and grind by blender to get on fine powder.20gm of dry powder material of Nigella sativa seeds washed well and put in Thimbles of Soxhlet Apparatus according of the method of (Qureshi et al.,1992), added 200 ml of ethanol alcohol with concentration of 96% and for 24 hours, After that the extracted material concentrated by the rotary evaporator with temperature at 40-45°C and after evaporation of all ethanol mixed and observed thick material textures (Gelatinous formed), which was prepared the stoke solution from it, by dissolving 60gm of the extract in 100ml of distill water, and thus became the basic concentration of the solution (stoke solution) 60% or equivalent 600mg /ml. Then prepared three different concentration 20%,40%,60% of Nigella sativa extract to know its effect in the vitality of eggs and worms of F.gigantica which are prepared by mixing certain size of stoke solution according to the law $C1V1=C2V2$.

2- Eggs and Adult worms of parasite collection

Eggs isolated from the feces according to the method (Hillyer et al., 1996) 5gm of animal infected feces placed in a glass beaker 500ml and added tap water with mixed by the glass rod, then filtration by using a piece of gauze and collected the filtrate in other glass beaker,Then leaved for 10 minutes, to precipitated pouring the upper part of the solution and remained 10ml of sediment, added tap water and leaved for another 10 minutes to precipitated and poured the filtrate and stayed the sediment, repeated this process several times until they change the color of the liquid to the transparent color which contains only eggs of parasite, then used in subsequent experiments, The eggs diagnosed by depending on (Soulsby,1982). F.gigantica worms collected from the livers and bile ducts which isolated from cows and buffaloes infected and slaughtered in the abattoir of Al-Najaf livers and bile ducts placed in plastic containers and brought directly to the lab. Bile ducts dissecting by slicing blade and the worms isolated and washed several times with Normal saline solution to remove impurities, Then placed in glass bottles containing normal saline solution. Adult worms of the parasite F.gigantica diagnosed by depending on (Lotfy&Hiller,2003),Then used in subsequent experiments.

3- Effect of ethanol extract of Nigella sativa plant in the vitality of eggs and worms of F.gigantica In vitro

3-1:Effect of ethanol extract of Nigella sativa plant in the vitality of eggs In vitro: -

400eggs were divided into 4 groups, each group contain (100) eggs, which in turn were divided into 4 replicates (25 eggs/repeater) each repeater of eggs placed in a small glass bowl with 100ml of distill water and then added the three doses of Nigella sativa extract to the three groups of eggs, while the fourth group leave as a control were treated with only distill water, Eggs were exposed to concentrations of extracts for 24 hours, then the eggs transferred to the distill water without extracts and changed the distilled water every 48 hours until end the period of the experiment, groups of eggs leave in the dark inside the incubator at 25°C for a period of 17days, then calculated the percentage of hatched of parasite eggs (Al-Mayah,2002).

3-2: Effect of ethanol extract of Nigella sativa plant in the vitality of the worms In vitro:-

120 adult worms were divided into 4 groups, each group contain 30 worm which also divided into 3 replicates (10 worm/repeater) each repeater of worms placed in a small glass a bowl with 100ml of normal saline solution then added three concentrations of Nigella sativa extract to the three groups of worms and then recorded while the fourth group leaves as control the Mortality after 10minutes of exposure to different doses of the extract, The worm promised dead when their did not ability to move the body or oral sucker or anterior cone (Al-Mayah,2002).

4- Statistics analysis

Completely Randomized Design(CRD) is using with the experience of two factors (concentrations and time periods) and the results were tested by use LeastSignificance Differences(LSD) at the level of probability of (P < 0.05)(Al-Rawi and Khalafallah,2000).

Results

Has been test effectiveness Nigella Sativa extract in percentage for eggs hatch F. gigantica results shown in Table (1),Figure(1) that for extract there are significant effect in reducing percentage of hatching eggs, whenever greater dose of extract compared to control group rate was of hatching eggs 60.2% when treated with extract concentration 20% while 20.5% when treated with extract concentration 40% and 0% when treated with extract concentration 60% compared with control is 96.3 .May proved Statistical analysis (LSD) that concentration Highest effect is 60% .Has been test effectiveness of Nigella Sativa extract in percentage for mortality of adult worms, results showed in Table(2),Figure(2) that for extract there are significant effect in increase percentages for mortality of adult worms, whenever greater extract dose compared to control group was 65.4 % mortality of adult worms for extract concentration 20% While the mortality of adult worms 80.3% for extract concentration 40% and the mortality of adult worms 100% when treated with extract concentration 60% compared with control is 9.2. May proved Statistical analysis(LSD) that concentration Highest effect is 60% .

Table (1): Effect of the ethanol extract of Nigella Sativa plant in eggs hatch of F.gigantica in vitro

Extract Concentration %	Duration of the exposure/ hours	Number of eggs tested	Percentage rate for eggs hatch
Control	24	100	96.3
20	24	100	60.2
40	24	100	20.5
60	24	100	0

L.S.D=6.4

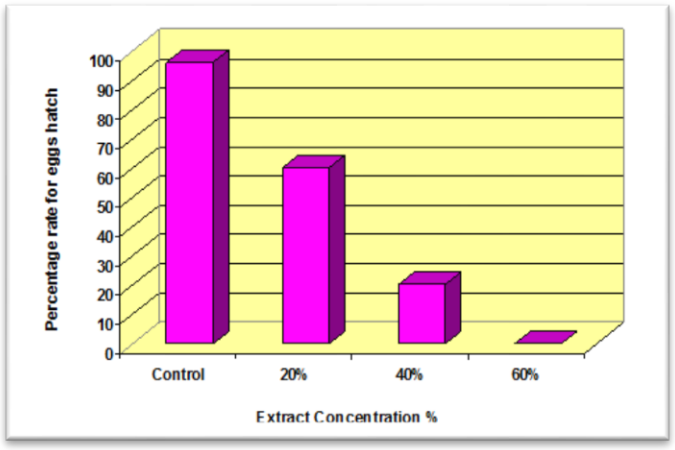

Figure (1): Effect of ethanol extract of Nigella sativa plant in eggs hatch of F.gigantica in vitro

L.S.D=6.4

Table (2): Effect of ethanol extract of Nigella Sativa plant in Mortality of adult worms F.gigantica In vitro

Extract Concentration %	Duration of the exposure / minutes	Number of worms tested	Percentage rate of decimation worms
Control	10	30	9.2
20	10	30	65.4
40	10	30	80.3
60	10	30	100

L.S.D=10.3

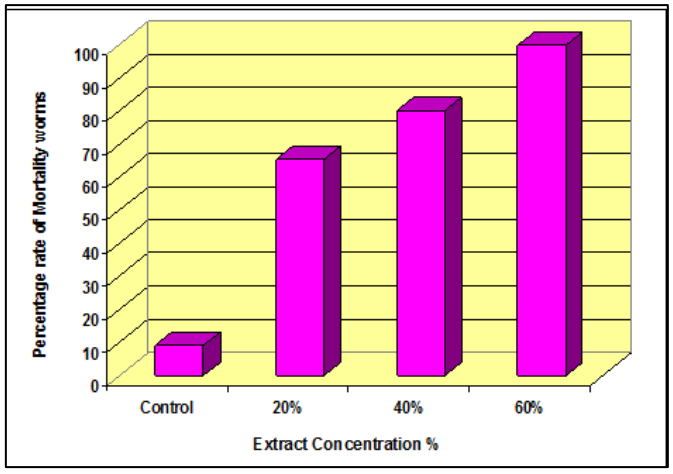

Figure (2):Effect of ethanol extract of *Nigella sativa* plant in Mortality adult worms F.gigantica In vitro

L.S.D=10.3

Discussion

The results showed that ethanol extract for Nigella Sativa seeds highly effective in mortality Miracidia inside eggs and therefore did not hatch as well as efficiency in mortality adult worms probably lead to extract contains on large proportion from compounds such as Tannins, Phenols, Alkaloids, Saponins and Thymoquinone.Where that inhibitory action mechanism to these compounds may due to found Tannins compounds which have ability on correlation with proteins found in cytoplasm cell which prevents analyzed because of occur obstruction to metabolic processes related with Nitrogen and amino acids which based in building mitochondria membrane , nuclei and Golgi particle important in continuing vitality of the parasite.May be inhibition mechanism Tannins for parasite on basis ability on sedimentation proteins found in cell wall because of capacity on union with proteins leading to changes in chemical characteristics for cellular wall or cell shape change entirety and this lead to death (Mukundan,1979;De-Tommasi et al.,2000;Ahmad et al.,2004). Nigella Sativa seeds contain on Tannins which have ability on union with damage tissue protein and is responsible for sterile and gripper action and this can be occur union between Tannins existing in extract with constituents proteins for structures and cellular organelles to parasite cause in mortality(Al-Shamaa,1989).May be cause this mechanism presence Phenols compounds which act on Denature proteins and stop action enzymes responsible for series from metabolic reactions basis and thereby lose ability parasite on life (Musa et al.,2004;Sogut et al.,2008). Inhibitory mechanism action to parasite may lead to Alkaloids compounds, which due to ability on break down cellular wall and content from proteins, fats and therefore mortality of the parasite(Anthony,1976; Hadjzadeh et al.,2007). Also may interpreted on based inhibition carbohydrate metabolism through influence on mitochondria and therefore obstruction breathing mechanism and then death of the parasite (Delorenzi et al.,2001;Ahmad et al.,2004;Sharma et al.,2009). Observed that Alkaloids have potent inhibitors for Monoamine oxidase enzyme which may cause physiological effects such as Hypothermia and Hypotensive (Codding,1983;Ismail et al.,2009). Mentioned (Ansari et al.,1988;Al-Akabi,2005;Al-Nuaimi et al.,2007) Saponins compounds in Nigella Sativa anthelmintic action and laxative for intestine contents, some researchers likely that inhibitory effective -ness for extracts of Nigella Sativa seeds to overlap in respiratory chain enzymes containing on Thyol group (sulfur - hydrogen SH) and through replacements and alternatives in group (C = O) associated with Thymoquinone compounds which conversion to Thymohydroquinone by gain hydrogen molecule, and consider latter compound is more toxic than original compound, and this according (Houghton *et al.*,1995;Khan *et al.*,2003;Kanter *et al.*,2005) where founds be Thymoquinone compounds working on close receptors enzyme especially respiratory enzymes container on Thyol group and this study satisfying with (Al-Hussainy,2010) where noted that alcohol extract for Nigella Sativa seeds good therapeutic efficiency.

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