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Comparative study between alcoholic extract of *Allium sativus* and Levozan in effect on *Haemonchus contortus* larvae

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ABSTRACT

The current study show the prevalence of *Haemonchus contortus* in sheep in Tikrit city and its surroundings for the period from the beginning of July 2018 to August 2019. The study was carried out on 315 fecal samples from two genders, their ages between (6 months to 5 years). The results showed the eggs of the following:Nematodes: *Haemonchus contortus, Marshallagia marshalli , Ostertagia circumcincta , Lung worms , Trichostrongylus spp , Nematodirus spp , Chabertia ovina, Strongyloides papillosus, Cooperia spp.,* Trematoda: *Paramphistomum cervie*. Cestoda: *Moniezia spp..* The epidemiological study appeared that the rate of infection with *Haemonchus contortus* was 18.2%. The high rate of infection was among (1-2) years old. The distribution of infection was high in April (45%).Al-hamraa region recorded high rate of infection (32%) in comparative with other regions of the study. *H. contortus* recorded the highest severity of infection with average (1336 egg/gm of feces).

The aim of this study was to explore the potential activities of the alcoholic extracts of garlic, with different concentrations 50, 75, 100 mg/ml ,on *Haemonchus contortus* larvae in vitro in comparative with Levazan drug ,. The result showed an effect of alcoholic extract of two plants on the larva in comparative with control group. It was found that the alcoholic extract of garlic had greater inhibitory effect and killed all the larvae during the fourth day, while the alcoholic extract of cloves killed the larva on the tenth day. The Levazan drug had the highest effect on larva, all the larvae were killed during 8 hr. of using the drug compared to control group.

1.Introduction

Sheep represent an important aspect of livestock in Iraq and the Arab world, as the number of sheep in the Arab world is more than 105 million head, while the number of sheep in Iraq is estimated at about 9 million head [1]. It is a great wealth that requires attention, care and development. Sheep constitute a major source of meat in the Arab world, 33.3% of the total meat, as is the case in developed countries. In addition to being an important source of 16.74% milk, leather and wool [2]. The diseases caused by parasites have a negative role in limiting the prosperity and growth of livestock in most countries of the world, including Iraq. This is due to the economic losses resulting from the decimation of large numbers of animals, the obstruction to their normal growth and the decrease in their production, as well as the costs of control and treatment [3].

Parasitic infections spread among sheep flocks widely, and at the forefront of that infection with internal worms comes as open breeding of sheep flocks provides direct and indirect contact between them and other animals for the transmission of these infections between different animals, and epidemiological and environmental factors play an important role in this field [4]. Many epidemiological surveys indicated that sheep are exposed to many parasitic infections, especially those that parasitize the digestive system, as infection with these parasites in small animals is characterized by diarrhea with a severe watery texture, yellow or green color and dehydration in the body [5]. Parasites also affect animal health, represented by weight loss, lack of milk production, poor wool, poor births, and decreased reproductive efficiency, as well as economic damage from veterinary health services and exerted effort [6]. Among those infections caused by parasites is Haemonchosis, which is caused by the well-known gastro-intestinal worms *H. contortus*. Therefore, Haemonchosis is a disease of economic impact and sometimes causes severe anemia and rapid death [7].

H. contortus belongs to the nematode trichostrongylidae, which infects the rennet abomasum of goats, sheep, camels, and other ruminants in almost all subtropics and temperate regions worldwide. [8] in 2010 Noble and others observed that the main source of nutrition for these worms is blood, and thus infection with this parasite can cause severe anemia in infected animals, loss of appetite, weight loss, and a decrease in wool growth that results in some The reasons for the death of the animal. Young animals are more susceptible to infection while larger animals are more likely to be resistant to disease [9].

2.Materials and methods

2.1.Collection samples

This study was conducted for the period from the beginning of July 2018 until the end of August 2019, when 315 samples of blood and stool, of both sexes, of different ages ranging between (6 months - 5 years) were collected from sheep breeding fields in Tikrit and its suburbs.

a- Fecal Samples

Using the disposable paws, an appropriate amount of fecal matter was collected directly from the animal's rectum, placed in small plastic containers and transported in containers containing ice bags to the laboratory for the purpose of carrying out various tests on it.

b- Blood Sample

Blood samples were taken from the jugular vein using disposable plastic syringes after sterilizing the area with 70% ethyl alcohol, where 10 ml of blood were drawn

from each animal, and the sample was divided into two parts (2.5 ml and placed in tubes containing an EDTA anticoagulant for the purpose of Various blood tests and immunological tests were performed and 7.5 ml were placed in test tubes free of anticoagulant to separate the blood serum for the purpose of conducting biochemical tests on it), where blood tests were performed as soon as possible, during which the samples were kept in the refrigerator, while the serums were kept at a degree of 20 M o until the tests are carried out.

c-Clinical examination of sheep:

Special forms were prepared to record information about the cases included in the study, and included the case number, its date, the name and address of the animal's owner, information about the animals, the history of the disease, and the results of the clinical, microscopic and diagnostic examination.

2.2.Laboratory tests

A-stool tests:

a-Flotation Method

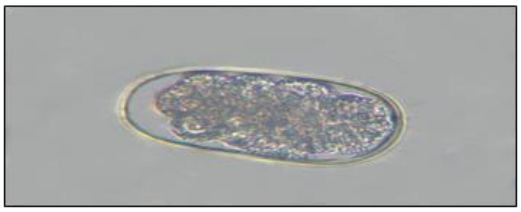
This method was used for the purpose of detecting Nematodes eggs and Cestodes. Two gm of feces was placed in a 250 ml beaker and 90 ml of saturated sugar solution with a density of 1.12 was added to it [10]. The mixture was mixed well, it was filtered with a colander and then transferred a quantity of it to test tubes of 15 ml until the height of the surface level of the solution was noticed above the edges of the tube, and then the cover of the glass slide was carefully placed on it, and after 30 minutes the slide cover was lifted and placed on a glass slide and examined under a microscope [11].

b- Sedimentaion Method

This method was used for the purpose of detecting Trematodes eggs, where 2 grams of feces were placed in a 250 ml beaker and 90 ml of water were added to it and the mixture was mixed well and filtered, then transferred to 15 ml test tubes and placed in a centrifuge for two minutes and then a portion was taken Of the precipitate was placed on a glass slide, the cover of the glass slide was placed over it and examined under the microscope [10].

c-Egg Measurement

After extracting the magnification factor of the microscope using only the measuring platform and the eyepiece [11], the length and width of the egg were measured.



Haemonchus contortus **egg (400X)**

d-Egg Count

The number of eggs per gram of feces was calculated using a Mac Master method, where 2 grams of stool was accurately weighed, then 28 ml of saturated sugar solution was added to it and the solution was filtered through the filter, then part of the solution was withdrawn with a medical syringe and transferred to the Mac Master calculation stone until it was filled and left to be The eggs float on the surface, and the final number of eggs was extracted [10] according to the following equation:

$$EPG = \frac{\text{The total number of eggs counted}}{\text{The total number of the counting chamber}} \times 200$$

e-Fecal culture to grow larvae

This method was used to distinguish the larvae of roundworms Strongylus. An appropriate amount of feces was placed in plastic containers and a small amount of fine charcoal was mixed with it

with drops of water to moisten the feces and incubated at a temperature of 26 ° C in the incubator for seven days, after which the larvae were separated by the Soulsby method [12].

f-The Baerman Method

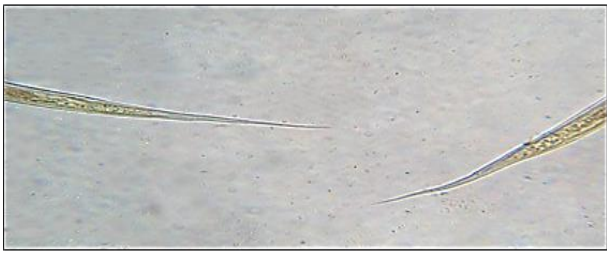
This method was used for the purpose of distinguishing nematodes larvae and diagnosing lung worms infection, where 2 grams of feces were placed on a piece of gauze and wrapped around it and then placed inside the glass funnel of the Berman device and submerged in warm water and left at room temperature for 24 hours, after which the three or four drops were collected The first was placed on a glass slide, the cover of the slide was placed over it and examined under the microscope. The liquid was collected, placed in test tubes and expelled in a centrifuge, then the sediment containing the larvae was taken [10].

g-Larvae Diagnosis

Nematode larvae were diagnosed with similar eggs by obtaining the third larva, L3, by implanting feces and diagnosing it by identifying the length and shape of the esophagus, the sheath, its length and the number of intestinal cells. The larva was measured using an Ocular micrometer and based on [12-13] who listed the morphological characteristics of larvae as in the following table:

Table (1): **morphological and diagnostic characteristics of the third stage larva of L3**

Total length (Micron)	**Tail shape**	**Sheath length (Micron)**	**The number of intestinal cells**	**Description of the body and anterior end**	**Genus of larva**
866 – 749	The sheath deviates from a straight body and the end of the tail is pointed	119 – 87	16 cells	The front end is tapered, long and conical in shape	***Haemonchus***



tail shape of *Haemonchus contortus* (400X)

B-Blood Tests

A digital coulter counter was used to measure the total number of red blood cells, hemoglobin concentration, the volume of compacted blood cells, the total number of blood platelets and the total number of white blood cells, and the Erythrocyte Sedimentation Rate (ESR) was measured using the inserted Westcreen tube [14].

C-Biochemical Tests

a- Estimate the amount of total protein in serum

The Biuret Method was used to estimate the total protein of the serum samples, which depends on the combination of protein present in blood serum with a solution of aqueous copper sulfate in the presence of a strong base such as sodium hydroxide and

produced a purple complex compound that absorbs light at a wavelength of 540 nm [15].

2.3 Preparation of solutions

1-Prepare a Biuret solution from dissolving 1.5 g of aqueous copper sulfate and 6 g of sodium and potassium tartrate in 500 ml of distilled water with constant stirring, then add with stirring 300 ml 10% of sodium hydroxide and complete the volume to a liter with distilled water.

2-Standard Protein Solution 1000 / 100ml

Dissolve 1000 mg of Bovine serum albumin (BSA) in 100 ml of distilled water.

a- The method of work

Put 0.1 ml of blood serum into a test tube, and prepare an efficient solution of Blank by taking 0.1 ml of distilled water into another test tube and add 0.9 ml of distilled water to each, then add 4 ml of Biuret solution with good shaking, and leave at a temperature The room for 30 minutes, and then the absorbance intensity was read by a spectrophotometer at a wavelength of 540 nm. The total protein concentration in the blood serum samples was determined using the standard curve for protein that was prepared from different concentrations ranging between (0-10) mg / mL of bovine serumalbumin.

b- Measuring the concentration of albumin, BUN, ALT and AST

The above-mentioned analyzes were measured in serum by adding ready-made standard solutions to the serum according to the manufacturer's instructions using a spectrophotometer. The globulin concentration was determined by subtracting the albumin concentration from the total protein concentration [16].

2.4 Plants under study

1-garlic plant

Garlic extract was obtained from White Fields Company

2- Preparation of alcoholic extracts

Gasparre *et. al.* In (2018)[17], modified from the researcher's basic method [18] was adopted in preparing alcoholic extracts by crushing (20g) of plant parts in (200 cm3) of ethyl alcohol at a concentration of (95%) inside Ice water bath, after which the mixture is shaken well with an electric motor device (stirrer), and left in the refrigerator for a period of (24hours), then the mixture is filtered through several layers of gauze.

In order to get rid of the alcohol, the mixture is placed in a rotary evaporator device that works on the basis of evaporation under vacuum pressure and a temperature of no more than (40%), and after the evaporation of the solvent (alcohol) from the mixture, a thick layer of the extract is formed that is dried by cooling under A vacuum pressure in a lyophilizer, and the samples are then preserved by freezing until they are used.

3-Make the required relief

Dilution was done for the above aqueous and alcoholic extracts according to the dilution law

$$N1 * V1 = N2 * V2$$

Extracting was prepared at a concentration of 50%, 75%, and 100% from plants.

Therapeutic part

The alcoholic extracts of the garlic under study and the drug Levosan were tested on *H. contortus* larvae, which were grown in the culture medium (faeces) and isolated according to the Berman method and kept in test tubes containing glucose and stored in the incubator at 26 ° C. Where the

tubes containing the larvae were treated with plant extracts at the different concentrations mentioned above and by repeating each concentration(50%,75%,100%), leaving a tube as a control group. The treated tubes are monitored daily for 18 days. The number of live and dead larvae are counted on a single-field glass slide using light microscopy.

2.5 Statistical analysis

The results were analyzed statistically using the Sigma Static program using the electronic calculator, to extract the mean and the standard error using the t-test and the One Way ANOVA-test, and the level of significant difference for the tests was at a probability level ($p < 0.05$).

3.Result and discussion

3.1.Epidemiology of *Haemonchus contortus*

The results showed that the rate of infection for this worm is 18.2% for the total percentage of infections with diagnosed stomach, intestinal and lung worms. The flotation method was the most effective method for diagnosing worm eggs while the eggs were not diagnosed using the sedimentation method. There are many factors that played a role in the worm epidemic, as follows:

3.1.1.Age of the animal

The results of the epidemiological study of the worm *(H. contortus*) showed that the highest rate of infection was in animals whose ages ranged between 1-2 years and by 38%, and the lowest rate at the age of 4-5 years, reaching 6%. It was noticed through the current study that the spread of worms according to the age group was the highest rate in ages ranging between 1-2 years, while the lowest rates of infection were in ages that exceeded the age of 4 years. Our results differed with what the researcher Jean (2016)[19] found in His study that young ages are less affected by Hemonchus worms and indicated that the reason for this is due to the activity of the immune system and the quality of metabolism and maximum benefit from the food provided to them, while older ages are more vulnerable to infection with this disease due to aging and the occurrence of oxidative stress for all cells and tissues of the body, including The immune system, which affects the health of the animal in general and makes it vulnerable to many diseases, including parasitic infections. As for the researcher, Ameen *et al.,* (2016)[20], his results are consistent with our results in the issue of infection of young ages without old, and that the reason for this is often due to the administrative nature of the breeding fields, as old animals are less likely to contract diseases, including parasitic diseases, due to the use of drugs and continuous vaccination By the breeders and the frequent care of them, which contributes to the elimination of worms and the strengthening of the immune system. As for young ages, they are often grazed in open areas and depend on the method of open grazing in the pastures, so they are vulnerable to infection with parasitic diseases.

3.2 Curative study

The therapeutic effect of the studied plants was measured on in vitro cultivated *H. contortus* larvae. In our current study, it was found that there is inhibitory efficacy of the alcoholic plant extracts of garlic with different concentrations (50,75,100) on the larvae of *H. contortus*, which indicates that they contain effective compounds that affect worms and can be used as a treatment, as follows:

3.2.1.The effect of garlic extract

It was noticed through the results that the aqueous and alcoholic extract of the garlic plant had an effective effect on the worm's larvae, as the results of the study showed that the effect of the alcoholic extract on the larvae

of the hemonchus worm was more effective than the aqueous extract as in Table (2). It was also found that the concentration of 50 mg / ml had the largest effect in killing the larvae, although all the concentrations of the aqueous extract affected all the larvae on the fifth day and killed all the larvae on the fourth day by using the alcohol extract.the effect of alcoholic extract more than aqueous extract.

The results obtained are in agreement with the findings of Niezen *et. al.*, (2012)[24]; Alawa *et. al.*, (2013)[25], who conducted a comprehensive study on the fertility of the Hemonchus parasite and its eggs and larvae and concluded that the aqueous garlic extracts inhibited the growth of larvae and delayed the hatching of eggs and died, and they explained that the use of garlic extracts caused an increase in the temperature of the culture media, which affected the growing larvae. Researchers Veerakumari and Chitra, (2014)[26] showed that the cause of larvae death when treated with garlic extract is due to the occurrence of oxidative stress that occurs due to hyperthermia in the culture medium, which in turn affects the vitality and efficacy of the larvae and then the death of those larvae due to the enzymatic disturbance caused by Formation of hydrogen peroxide H_2O_2, which causes damage to larval membranes due to its high oxidative activity, and thus the death of larvae. Our results are also in agreement with the findings of the researcher Duval, (2004)[27], who showed that garlic contains a high percentage of Tannin, which changes the natural physiological activities in living organisms, so this substance affects absorption, movement and reproduction, causing disturbance in activities. Larvae vitality and thus their death.

Among the studies that dealt with the effect of the alcoholic extract of garlic and its effect on the Hemonchus parasite was what was done by the researcher (Bastidas, 2009)[28], who conducted an extensive study on garlic extract and its effect on the parasite. The larvae and then their death. The results we obtained also agreed with the researcher Anthony *et.al.*, (2015)[29], who explained the cause of hemonchus larvae death when treated with garlic extract, to the garlic containing a high percentage of Allicin, which affects the outer layer of the larvae Cuticle, interfering with the fats and proteins in the membrane, causing the rupture of the membrane and the permeability of the material. To and from the larva randomly, it causes membrane degeneration and larval death.

Table (2): Average number of larvae in the cultivated medium and treated with garlic

average	Day5	Day4	Day 3	Day 2	Day1	Days / conc.	Type of extract
41 a		0	22	42	59	50	alcoholic extract
37 b		0	17	39	55	75	
34 c		0	12	37	53	100	
			17 c	39.33 b	55.66 a	Average days in alcohol extract	

-The different letters in the rows indicate the presence of significant differences at the level of probability (P ≥ 0.05)

-The different letters in the columns indicate the presence of significant differences at the probability level (P ≥ 0.05)

3.2.2.Levosan

The results of the study showed that the effect of the drug Levosan on the larvae of the Hemonchus worm, as the larvae were killed within eight hours of their development on the first day, at a rate of 100% .

4.Conclusion

In conclusion , the Levosan drug effect at a rate 100% on the larvae and killed it within eight hours on the first day. The effect of alcoholic extraction of Garlic more than aqueous extraction of garlic.

دراسة مقارنة في تاثير مستخلص الكحولي لنبات الثوم والقرنفل وعقار الليفوزان على يرقات دودة *Haemonchus contortus*

سناء سعود احمد

فرع الطب الاحياء المجهرية – كلية الطب البيطري- جامعة تكريت– تكريت - العراق

اظهرت نتائج الدراسة الحالية انتشار دودة *Haemonchus contortus* في الاغنام في مدينة تكريت وضواحيها للفترة من بداية شهر تموز 2018 ولغاية شهر آب 2019 ، اجريت الدراسة على 315 عينة من كلا الجنسين تراوحت اعمارها من ستة اشهر الى خمس سنوات. بينت النتائج وجود بيوض الديدان التالية : *Haemonchus contortus, Marshallagia marshalli , Ostertagia circumcincta , Lung worms , Trichostrongylus spp , Nematodirus spp , Chabertia ovina, Strongyloides papillosus, Cooperia spp.,* Trematoda: *Paramphistomum cervie*. Cestoda: *Moniezia spp.* اظهرت الدراسة الوبائية انتشار دودة *Haemonchus contortus* فكانت 18.2 % اعلى نسبة خمج في الاعمار بين 1-2 سنة و اعلى نسبة خمج في شهر نيسان، وسجلت منطقة الحمرة اعلى نسبة 32% مقارنة مع المناطق الاخرى.

كما هدفت الدراسة الى معرفة تأثير مستخلصات الثوم والقرنفل على يرقات دودة *Haemonchus contortus* مقارنة مع عقار الليفوزان، حيث بينت النتائج تأثير المستخلص الكحولي لكلا النباتين على اليرقات مع مجموعة السيطرة، وكان تأثير مستخلص الثوم الكحولي اعلى ، حيث ثبط نمو اليرقات في اليوم الرابع بينما كان تأثير مستخلص القرنفل على اليرقات في اليوم العاشر. اما عقار الليفوزان فكان له التأثير الاكبر حيث قتل جميع اليرقات خلال ثمان ساعات مقارنة مع مجموعة السيطرة