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# PREVALENCE OF *HAEMONCHUS* WORMS IN SHEEP AT ASSIUT GOVERNORATE, AND PRELIMINARY EVALUATION OF THE ANTHELMENTIC ACTIVITY OF *FERULA HERMONIS* EXTRACTS AGAINST THEIR DIFFERENT STAGES

(With 6 Tables and One Plate)

By

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## مدى انتشار ديدان الهيمونكس فى الاغنام بمحافظة أسيوط وتقييم أولى لفعالية مستخلص نبات شرش الزلوع على اطوارها المختلفة

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لدراسة مدى إنتشار ديدان الهيمونكس فى الاغنام بمحافظة أسيوط تم فحص عدد ١٥٠ عينة براز من الأغنام وكانت إجمالي نسبة الإصابة الكلية بديدان الهيمونكس ١٨,٠% وقد كانت نسبة الإصابة في الحيوانات البالغة ٣٠,٠% بينما كانت الحيوانات الصغيرة خالية من الإصابة. ولتحديد أنواع ديدان الهيمونكس الموجودة بالأغنام تم فحص ٥٣ معدة من الأغنام المذبوحة بمجزر أسيوط حيث بلغت نسبة الإصابة فيها٤٩,٠٦%. وتم تصنيف نوعين من ديدان الهيمونكس في تلك الحيوانات هما: هيمونكس كونتورتس وهيمونكس بلاساى وقد تم وصف الخصائص المورفولوجية للديدان البالغة لكل منهما. واشتمل الجزء الثاني من البحث على دراسة معملية لتقييم فاعلية نبات شرش الزلوع ضد ديدان الهيمونكس. فقد تم دراسة تأثير خمسة خلاصات لنبات شرش الزلوع على الأطوار البالغة والطور المعدي لديدان الهيمونكس. أوضحت الدراسة أن هناك فاعلية قوية لجميع الخلاصات المستخدمة على حيوية كل من الأطوار البالغة ويرقات تلك الديدان حيث بلغ معدل نفوق اليرقات في بعض منها ١٠٠% وقد وجد أن خلاصة خلات الأيثيل لنبات شرش الزلوع عند تركيز ٥ مج/ مل ، ٢,٥ مج/ مل يعطى أعلى تأثير مثبط لهذه للديدان ويرقاتها معمليا. ويعتبر هذا البحث أول دراسة لتقييم فاعلية الخلاصات المختلفة لنبات شرش الزلوع على ديدان الهيمونكس.

## SUMMARY

In a survey on *Heamonchus* infection in sheep at Assiut Governorate, the overall infection rate was 18% out of 150 living animals that diagnosed by faecal samples examination and 49.06 % out of 53 slaughtered sheep

that diagnosed by abomusum examination. In adult sheep the infection rate was 30 %, while *Heamonchus* eggs were not detected in lambs. Two species of *Heamonchus* were detected in the present work: *H. contortus* and *H. placei*. The morphological characters of each species were described. The second part of the present study was carried out to evaluate *in-vitro* the anthelmintic effect of different extracts of *Ferula hermonis*. The obtained results showed that all extracts of *F. hermonis* have variable degree of anthelmintic action against both adult worms of *Haemonchus* and their 3rd stage larvae *in- vitro*. Ethyl acetate extract of *F. hermonis* at 5 mg / ml and 2.5 mg / ml has the greatest inhibitory action against both adult worms and 3rd stage larvae of *Haemonchus*. Our present investigation is the first study to evaluate the anthelmintic activity of different extracts of *Ferula hermonis against Haemonchus*.

***Key words:*** *Haemonchus worms, sheep parasites, Anthelmentic activity Of Ferula hermonis extracts*

## INTRODUCTION

Genus *Haemonchus* is a common parasite of domesticated ruminants (sheep, goats and cattle) and it is considered as the most pathogenic nematodes of them (Gibbs and Herd, 1986). It is a blood sucking parasite inducing reduction on feed conversion with slow- rate of weight gain, poor production and reproduction efficiencies (Jakhsi *et al.*, 2006).

Three species of genus *Haemonchus* are known to occur in domestic and wild ruminants (*H. contortus, H. placei and H. similis*) and they have great deal of morphological variations (Dunn, 1978). The importance of *H. contortus* as a blood sucking parasite of sheep has been widely recognized, as the adult stages of the worm causing sufficient hemorrhage in the host abomasum, with subsequent severe and sometimes fatal anaemia (Ali, 1981). Human infection with *Haemonchus* worms has been reported previously by Faust and Roussell (1959) and Jefferey and Leach (1984).

Resistance of nematodes to different anthelmintics has been reported world wide, whilst parasite control can no longer be achieved in some regions (Waller, 1994). Alternative strategies for parasite control are urgently needed; these include the use of forages or plants with anthelmintic properties (Athanasiadou *et al.*, 2000).

*Ferula* (Umbelliferae) is a large genus of about 130 species distributed throughout the Mediterranean area and Central Asia (Frensh,

1971). Several species have been used in folk medicine. The roots and seeds of *Ferula hermonis* Boiss, locally named 'zallouh root', are endemic species of Mount Hermon in Lebanon and Syria and have been traditionally used in Middle East as potential aphrodisiac for both men and women (Lev & Amar 2002).

Although several *Ferula* species have been used in folk medicine, some *Ferula* species have toxic effects to grazing animals, the toxicity is due to two quite rare groups of coumarin-C-prenylated derivatives (Appendino, 1997 and Rubiolo *et al.*, 2006). The effect of the extracts and essential oils on root-knot nematode was also studied by Al-Banna *et al.* (2003).

The roots of *F. hermonis* has been subjected to chemical studies. Many compounds belonging to daucane sesquiterpes of jaeschkeanadiol and its derivatives were isolated (Diab *et al.*, 2001a, Diab *et al.*, 2001b and Galal *et al.*, 2001).

The objective of the present work was conducted to evaluate the prevalence of *Haemonchus* infection in sheep at Assiut Governorate in addition to *in vitro* evaluation of the anthelmintic properties of different *Ferula hermonis* extracts against their different stages of *H. contortus* which were isolated from the examined sheep.

## MATERIALS and METHODS

**1- Collection and examination of faecal samples:**

- One hundred and fifty rectal faecal samples were collected from sheep (60 less than six months and 90 more than one year old). These samples were collected from Bany sanad farm (104) and other private sporadic samples (46). Each sample was collected in clean plastic cup and delivered directly to the laboratory for examination.
- Faecal samples were examined for the presence of *Haemonchus* eggs by the centrifugation floatation technique using saturated sodium chloride solution ( Abdel- Gawad, 1972).
- Faecal culture was performed for collection of *Haemonchus* larvae according to Eckert (1960).

**2 - Collection of adult worms:**

Abomasums of 53 slaughtered sheep (more than 8 months) were collected and examined grossly for detection and counting of *Haemonchus* worms. Adult worms were collected, processed and mounted in jelly according to Pritchard and Gunther (1982). The mounted worms were identified according to Lichtenfel *et al.* (1995).

**3 - Experimental work:**

In vitro experiments that were undertaken to determine the effect of different *F. hermonis* extracts on the motility and mortality of the adult worms, as well as the third stage larvae of *Hemonchus contortus*, including :-

**3-1 Plant materials:** The dried roots of *F. hermonis* Boiss were purchased from herbal store of Syria. The plant was kindly authenticated by Department of Botany, Faculty of Science, Assiut University, Assiut, Egypt. The roots were crushed to fine powder and stored in dark glass till used.

**3- 2 Extraction:** [ Ibraheim and Boulatova ( 2002)]

The roots of *F. hermonis* Boiss 2.0 Kg were extracted successively in a soxhlet apparatus using hexane, chloroform, ethyl acetate and finally with methanol till exhaustion in each case. After evaporation the solvent under reduced pressure at 50 °C till the residue became solvent free in each case (Ibraheim and Boulatova, 2002) and finally obtained 180 g of hexane extract (FHH), 78 g of chloroform extract (FHC), 32 g of ethyl acetate extract (FHE) and 54 g of methanol extract (FHM). The percentage yield of each extract was listed in Table (1).

The aqueous extract was prepared by extraction of 500 g of the dried roots of the plant with distilled water at 50°C for three hours, and the process was tried twice (Kaville and Kom,1966). The aqueous extract was collected and the water was removed by lypholization to afford 90 g of the aqueous extract (FHA). The aqueous extract was subjected to preliminary phytochemical screening and the obtained results were also recorded in Table (1).

**Table 1:** Different *F. hermonis* roots extracts with their yield percentage and preliminary phytochemical screening results.

| Extract (Symbol) | Amount obtained (Plant Material) | % yield | Results of preliminary phytochemical screening (Aqueous ex.) |
|---|---|---|---|
| Hexane extract (FHH) | 180 (2.0 Kg) | 9.0 | Sterols and/or triterpens (+)<br>Coumarins (+)<br>Alkaloids (+)<br>Tannins (+)<br>Saponins (+)<br>Lactones and/or esters (+)<br>Flavonoids (-)<br>Anthraquinones (-) |
| Chloroform extract (FHC) | 78 (2.0 Kg) | 3.9 | |
| Ethyl acetate extract (FHE) | 35 (2.0 Kg) | 1.75 | |
| Methanol extract (FHM) | 54 (2.0 Kg) | 2.7 | |
| Total aqueous extract (FHA) | 90 (0.5 Kg) | 18.0 | |

(+) Present (-) Absent

**3- 3 Preparation of the extracts for biological study:**

The residue of the aqueous (5 g) was dissolved in 80 ml of distilled water using magnetic stirrer. The solution was transferred to 100 ml volumetric flask and the volume was completed to 100 ml with distilled water. From each of other extracts 5 g residue was dissolved in distilled water using 2% Tween 80 as solubilizing agent. The solution was then transferred to 100 ml volumetric flask and the volume is completed to 100 ml. The same amount of Tween 80 was used as negative control. The final concentration of each extract was 50 mg /m l (Baraka, 2001).

**3- 4 Studied activity of different *F. hermonis* extracts:**

**A- On adult worms *H. contortus*:**

One hundred adult worms of *H. contortus* were collected and divided into ten groups (ten worms in each) in separate Petri - dishes and then exposed to one of the following treatments at 37°C:

1- Hexane extract [FHH] (at 50mg / ml.).
2- Chloroform extract [FHC] (at 50mg / ml.).
3- Ethyl acetate extract [FHE] (at 50mg / ml.)
4- Methanol extract [FHM] (at 50mg / ml.)
5- Total aqueous extract [FHA] (at 50mg / ml.)
6- Avimec (Ivermectin) 10mg /ml(positive control).
7- Avimec 5 mg/ml. (positive control).
8- Avimec 2.5 mg /ml. (positive control)
9- Tween 80 [2%] (negative control). 10- Phosphate buffer PBS.

The mortality or inhibition of motility of the worms was observed after 1, 2, 3 and 6 hours intervals. Finally, the treated worms were kept for 30 minutes in lukewarm fresh PBS to observe the revival of their motility.

**B- On 3$^{rd}$ stage larvae of *H. contortus*:**

In the same manner, 300 of *H. contortus* third stage larvae were exposed to one of previous treatments after dividing them into ten groups (30 larvae in each) in separate small Petri- dishes. The viability of the larvae was observed microscopically after 1, 2 and 24 hours intervals. Finally, the treated larvae were kept for 30 minutes in lukewarm fresh PBS to observe the revival of their motility.

**3- 5 Determine minimal inhibitory concentration (MIC) of ethyl acetate extract;**

Dried ethyl acetate extract (FHE) was dissolved as previously mentioned in 2% tween 80 and serially diluted with PBS to obtain (5.0, 2.5, 1.25 and 0.625 %) concentrations immediately prior to use. The

effect of each concentration on adult worms of *H. contortus* was studied as mentioned before in (3-4 A).

## RESULTS

Out of 150 faecal samples of sheep examined in the present work, 27 (18.0%) were harboring *Haemonchus* eggs. Most infected cases were detected in private sporadic animals (56.5%), and only one sheep (0.96%) was infected in Bany- sanad farm.

The infection rate of *Haemonchus* in adult sheep samples was (30.0%), while *Haemonchus* eggs were not detected in examined lambs (Table 2).

**Table 2:** Prevalence of *Haemonchus* infection in examined sheep at Assiut Governorate.

| Samples | Young N=60 | | | Adult N=90 | | | Total N=150 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Ex. | Inf. | % | Ex. | Inf. | % | Ex. | Inf. | % |
| Bany -sanad samples | 49 | 0 | 0 | 55 | 1 | 1.8 | 104 | 1 | 0.96 |
| Private sporadic samples | 11 | 0 | 0 | 35 | 26 | 74. 3 | 46 | 26 | 56.5 |
| Total | 60 | 0 | 0 | 90 | 27 | 30.0 | 150 | 27 | 18.0 |

Adult worms of *Haemonchus* were detected in 26 (49.06%) of examined 53 abomasums of slaughtered sheep. The worm burden of *Haemonchus* in infected animals ranged from 1- 275 worms with average 20 worms / host. Two species of *Haemonchus* were identified in the present work:

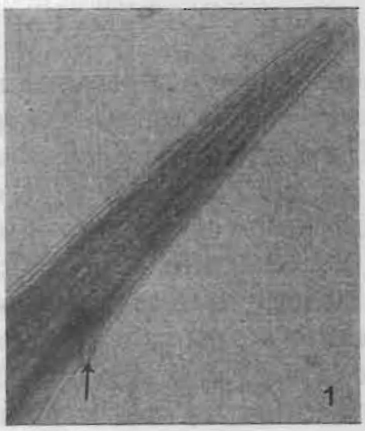
- *Haemonchus contortus* and *Haemonchus placei.*

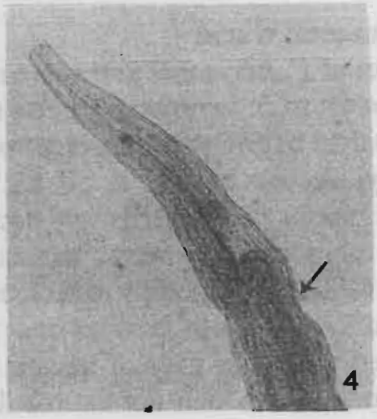
The morphological characters of adult worms of each species are presented at Table (3) & Plate 1.

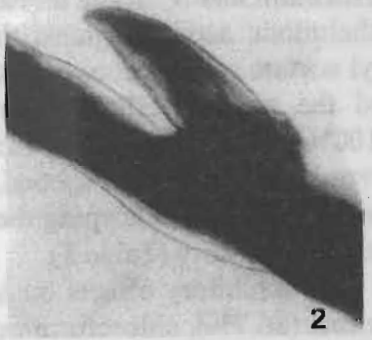
**Table 3:** Morphological characters of detected *Haemonchus* species.

| ***Characteristics*** | ***Haemonchus contortus*** | ***Haemonchus placei*** |
|---|---|---|
| **Male** | | |
| Body length(mm) | 17-19 | 14-16 |
| Cervical papillae | 280- 365.5 | 340- 390 |
| Esophageal length | 1530- 1720 | 1470- 1590 |
| Spicule length | 485 -498 | 475- 485 |
| Spicule character | United | Bifurcated posteriorly |
| **Female** | | |
| Body length(mm) | 14- 29 | 12- 31 |
| Cervical papillae | 350- 480 | 355- 490 |
| Esophageal length | 1690- 1780 | 1710- 1780 |
| Ovejectors character | Large lobe over vulva | Protruded knob over vulva |
| Tail | 520- 600 | 480- 630 |

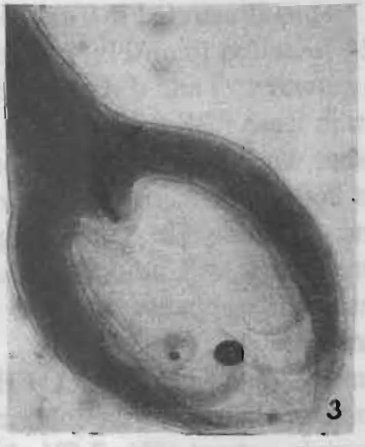
- All measurements in microns except total length in millimeter







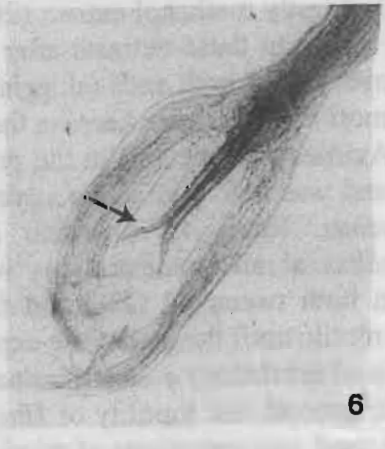




**Plate I:** Morphological characters of adult worms of *Haemonchus* species:
(1) Anterior end of *H. contortus* showing lateral cervical papillae (arrow).
(2) Female vulvar flap of *H. contortus*.
(3) Posterior end of male *H. contortus* showing adherent of spicule tips.
(4) Anterior end of *H. placei* showing lateral cervical papillae (arrow).
(5) Female vulvar flap of *H. placei*.
(6) Posterior end of male *H. placei* showing separation of spicule tips.

**Experimental work:**

**A-Effect of *F. hermonis* extracts on adult worms of *Haemonchus*:**

Most of *F. hermonis* extracts showed anthelmintic activity against adult worms of *Haemonchus in vitro*. The obtained results indicated that the ethyl acetate extract at 50 mg / ml inhibited the motility of all isolated worms (100%) after two hours. The rates of inhibition of worms in the other extracts were: aqueous extract (40%), and chloroform extract (20%). Temporally effect on motility of *Haemonchus* worms were noticed in both hexane and methanol extracts, where most of examined worms in case of hexane extract and all worms in methanol extract retained to their normal motility after keeping them in fresh PBS for 30 min. (Table 4).

**B- Effect of *F. hermonis* extracts on *Haemonchus* 3rd stage larvae:**

All *F. hermonis* extracts showed anthelmintic activity against the 3rd stage larvae of *Haemonchus*. Both ethyl acetate and aqueous extracts of *F. hermonis* at 50 mg/ ml produced the greatest effect where the mortality rate of isolated larvae was (100%). The movement of isolated larvae had stopped at once and all larvae became crescent shaped, non refractive after 24 h. Also, they remained the same after keeping them in fresh PBS for 30 min. that indicated their non viability (Table 5).

Other extracts of *Ferula* had only inhibitory effects on larval motility and their rates were: hexane extract (86.7%), chloroform extract (70 %) and lastly methanol extract (26.7%) as illustrated in Table 5. The isolated larvae in these extracts after 24 h. coiled themselves with very slow movement at both ends (sluggish movement) and did not return to normal motility again after keeping them in fresh PBS for 30 min.

Avimec (Ivermectin) in the present work was used as a positive control and was highly effective against both adult worms and larvae of *Haemonchus*. Rates of inhibition and mortality in each stage of *Haemonchus* at all concentrations were 100%. All adult worms and larvae in both tween 80 (2%) and fresh PBS (negative control) were actively motile until the end of the experiment.

**C- Minimal inhibitory concentration of ethyl acetate extract (FHE):**

In general, the motility of *Haemonchus* worms were inhibited at different used concentrations of ethyl acetate extract (5%, 2.5%, 1.25% and 0.625%), but when checked in fresh PBS for 30 minutes only the worms at both 1.25% and 0.625% of Ethyl acetate extract had returned to normal motility (Table 6).

**Table 4:** *In vitro* effect of *F. hermonis* different extracts on adult worms of *Haemonchus*.

| Reagents | No. of adult worms showing motility at different time factors (h.) post exposure. 0 | 1h | 2 h | 3h | 6h | fresh PBS for 30 mint.* | % of inhibited worms |
|---|---|---|---|---|---|---|---|
| Ferula aqueous 5% | 10 | 10 | 8 | 8 | 6 | 6 | 40% |
| Ethyl acetate ext. 5% | 10 | 10 | 0 | 0 | 0 | 0 | 100% |
| Chloroform ext. 5% | 10 | 10 | 10 | 10 | 8 | 8 | 20% |
| Hexane ext. 5% | 10 | 10 | 10 | 10 | 3 | 6 | 40% |
| Methanol ext. 5% | 10 | 10 | 10 | 10 | 8 | 10 | 0 |
| Avimec 10 mg. | 10 | 0 | 0 | 0 | 0 | 0 | 100% |
| 5mg. | 10 | 0 | 0 | 0 | 0 | 0 | 100% |
| 2.5mg | 10 | 10 | 0 | 0 | 0 | 0 | 100% |
| Tween 80 2% | 10 | 10 | 10 | 10 | 10 | 10 | 0 |
| PBS. | 10 | 10 | 10 | 10 | 10 | 10 | 0 |

*Indicated that all examined worms were checked in PBS for 30 mint after exposure to the different treatments to confirm the inhibitory effect on their mortality.

**Table 5:** In vitro effect of *F. hermonis* different extracts on *Haemonchus* larvae.

| Reagents | No.of 3rd stage larvae showing motility at different time factors (h.) post exposure. 0 | 1h | 2 h | 24 h | fresh PBS. For 30 mint. * | % of inhibited larvae |
|---|---|---|---|---|---|---|
| Aqueous extract 5% | 30 | 18 | 18 | 0 | 0 | 100%** |
| Ethyl acetate ext. 5% | 30 | 12 | 5 | 0 | 0 | 100%** |
| Chloroform ext. 5% | 30 | 20 | 15 | 5 | 9 | 70% |
| Hexane ext. 5% | 30 | 22 | 19 | 2 | 4 | 86.7% |
| Methanol ext. 5% | 30 | 16 | 16 | 16 | 22 | 26.7% |
| Avimec 10.0 mg. | 30 | 0 | 0 | 0 | 0 | 100%** |
| 5.0 mg. | 30 | 0 | 0 | 0 | 0 | 100%** |
| 2.5 mg | 30 | 0 | 0 | 0 | 0 | 100%** |
| Tween 80 2% | 30 | 30 | 30 | 30 | 30 | 0 |
| PBS. | 30 | 30 | 30 | 30 | 30 | 0 |

*Indicated that all examined larvae were checked in PBS for 30 mint after exposure to the different treatments to confirm the inhibitory effect on their mortality.

** Indicated non viable larvae.

**Table 6:** Effect of different concentrations of *F. hermonis* ethyl acetate extract on adult worms of *Haemonchus*.

| concentrations | No. of worms showing motility at different time factors (h.) post exposure. | | | | | | % of inhabited worm |
|---|---|---|---|---|---|---|---|
| | 0 | 1h | 2 h | 3h | 6h | fresh PBS for 30 mint. * | |
| Ethyl acetate ext. 5 % | 10 | 10 | 0 | 0 | 0 | 0 | 100% |
| 2.5 % | 10 | 10 | 10 | 10 | 0 | 0 | 100% |
| 1.25 % | 10 | 10 | 10 | 10 | 0 | 10 | 0 |
| 0.625 % | 10 | 10 | 10 | 10 | 0 | 10 | 0 |

*Indicated that examined worms were checked in PBS for 30 mint. after exposure to the different treatments to confirm the inhibitory effect on their mortality.

## DISCUSSION

*Haemonchus* worms are considered one of the major internal parasites of economic importance, where they have direct and indirect effect on health and production of animals especially the subclinical infection (Sykes, 1978).

In the present work the infection rate of *Haemonchus* was 18.0 % in examined faecal samples. In private sporadic cases the infection rate was considered very high (56.5%) than that recorded in Bany-sanad farm samples (0.96%). The obtained result might be related to periodical using of antihelmintic drugs in addition to improvement of the sanitary conditions in the farm.

The present study confirmed that lambs have a certain immunity against *Heamonchus* infection, where the infection detected only in adults. Gill *et al.* (1993) and Jakhsi *et al.* (2006) mentioned that experimentally infected lambs with *Haemonchus* showed ability to resist and limit the infection as a result of self- cure phenomenon.

Concerning the examination of abomasums of 53 slaughtered sheep, the infection rate of *Haemonchus* worms was 49.06%. The result was considered higher than that recorded by Monib (1977), Hassona (1979) and Hashem (1997) who detected *Haemonchus* in 32.2 %, 38.7% and 30.17 of examined sheep respectively. Both detected species of *Haemonchus* (*H. contortus & H. placei*) in the present work were recorded also in sheep by Monib (1977) and El-Akabawy (1987).

Parasitic resistance to anthelmintics is a growing problem in the control of gastrointestinal nematodiasis in small ruminants (Smith and

Sherman 1994). Recently, there is increasing interest in non – chemical method for controlling parasites.

The results of the present experimental work showed that different extracts of *Ferula hermonis* had variable degree of anthelmintic action against adult worms of *Haemonchus* and their 3rd stage larvae *in vitro*. Their inhibitory effect ranged from 20 – 100 % on adult worms and from 26.7- 86.7 % on their 3rd stage larvae of *Haemonchus*. While the mortality rates were 100% against 3rd stage larvae for both ethyl acetate and aqueous extracts at 50 mg / ml.

Although the mechanisms by which the *F. hermonis* extracts inhibit adult worms and kill 3rd stage larvae of *Haemonchus* are still unknown, but when the viability of the treated larvae was checked in fresh PBS for 30 min., most of inhibited larvae did not retrain again to their normal motility. We suggested that *Ferula* extracts may penetrate the wall of the larvae and affect the muscular activity.

Ethyl acetate extract was more effective than other extracts of *Ferula,* where the inhibition rate against both stages of *Haemonchus* was 100%. This effect was similar to that we had tested with the different concentrations of Avimec.

The differences in the activity between the experimented *F. hermonis* extracts may be attributed to differences in the polarities of each solvent and hence in the constituents of each extract. The ethyl acetate extract which contained the polar compounds (as tannins, saponins and other glycosides) showed the highest effect on both the worms and the larvae. The hexane extract which contained the lowest polar compounds (as sesquiterpens, some coumarins and triterpens) showed moderate effects. While the chloroform fraction which is considered as non-polar solvent that may contained less polar compounds (as ducanes and coumarins) showed the less activity. The methanol extract after ethyl acetate extraction which contained mainly the highly polar constituents (as sugars, carbohydrates, amino-acids..etc) had no activity.

The total aqueous extract which contained the polar compounds and saturated with the non-polar compounds showed moderate activities on both *Haemonchus* worms and their larvae. Further studies should be done to isolate the compounds responsible for such activities and to confirm their anthelmintic effect *in vivo* as well as to elucidate the mechanism of action.

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