Effect of *Artemisia herba-alba* Extracts on the Dwarf Tape Worm *Hymenolepis nana* in the White Mouse

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Abstract

Swiss albino mice (BALB/ C) infected with the adult dwarf tape worm *Hymenolepis nana* were orally administrated alcoholic extract (1400 mg/ kg) and hot water extract (4000 mg/ kg) of the desert wormwood *Artemisia herba-alba* .A third group of mice were administrated a vermifuge drug mebendazo1le (40 mg/ kg) for comparison.

Tape worm eggs disappeared with the mebendazole after 13 days of the treatment. Eggs disappearance took place after 15 days for mice treated with the alcoholic extract. Tape worms in mice group treated with the water extract continued to shed eggs for nine days. Howerer, death of some infected mice of this group began from the ninth day of treatment due to the effectiveness of such extract. Significant differences were noticed between different treatments and the period of administration. In vitro observation indicated that movement of the adult tape worm treated with the alcoholic extract of *A. herba – alba* (3.5%) was ceased after 11 minutes and such worms died after 20 minutes of exposure. In 10% water extract, worms movement was inhibited after 0.5 minute and they died after 35 minutes. *H. nana* treated with normal saline were still active for 400 minutes but died after 540 minutes . Significant differences were noted between control group and other mentioned groups.

Introduction

The treatment with plants and medical herbs is increasing nowadays. Each pharmacy now has herbal drugs. Many studies proved the pharmacological activity of many plant extracts against some pathogenic bacteria and fungi (El - Kady *et al*., 1993; Hernándes - Pérez *et al*., 1994) as well as against some parasites (Elisha *et al*., 1987). *Artemisia herba - alba* is a common herb species of medical importance in Iraq (Chakravarty, 1976). This plant is used in medicine as vermifuge especially against *Ascaris lumbricoides* (Al–Rawi & Chakravarty, 1988). Artemisinin is the antimalarial principle isolated from *Artemisia annua* (Luo & Shen, 1987).

Shnawa (1995) indicated that the aqueous extract of *Artemisia* spp. was active against the intestinal flagelate *Giardia lamblia* in the laboratory rats . Malagón *et al.* (1997) showed that the alcoholic extract of *A. ludoviciana mexicana* was affective against *Plasmodium yoelii yoelii* in laboratory mice. Al–Rubaie (1998) detected the activity of chloroformic extract of *A. herba-alba* in killing *Toxocara cati* larvae *in vivo* in laboratory mice viscera as well as *in vitro* killing of adults of the same species from the intestine of cats.

Due to the economic blockade imposed against Iraq since 1990 which resulted in a severe shortage of many drugs needed and as the Iraqi environment is rich with many medical herbs the present study was aimed to demonstrate the effect of *A. herba-alba* extracts on one of the common human tape worm *Hymenolepis nana* which also infects rodents.

Materials and Methods

A. herba-alba was bought from local market in Hilla city as dry flowers which were crushed. Alcoholic extract was prepared by adding 10 gm of the plant powder in an extract container of soxhlet extractor equipment according to Harborne (1984). An amount of 200 ml of ethanol as polar solvent was placed in container and the extraction process was done for 24 hrs. Dryed extract sample was obtained, dissolved in small amount of solvent and diluted with distilled water according to the requirable concentrations.Hot water extract was prepared by boiling 100 gm of the plant powder in 200 ml of distilled water, mixed up well and shaked for 15 min. The mixture was filtered through a Whatman No. 1 filter paper (Harborne, 1982). The filtrates was then placed in an oven (40 Cº) for complete drying. The dried extract was collected as a powde and diluted with distilled water according to required concentrations (Okanla *et al*., 1994).

In order to estimate the optimal concentration of alcoholic and hot water extracts that can be tolerated by the laboratory mice, a total of 18 mice infected with the dwarf tape worm were divided into six groups. The first group was given a water extract (2000 mg/ kg of body weight), the second one was given a water extract (4000 mg/ kg of body weight), while the third group was given an alcoholic extract (1000 mg/ kg of body weight), The fourth group was given an alcoholic extract (1400 mg/ kg of body weight), the fifth group was given mebendazole (40 mg/ kg) and the sixth group was given normal saline solution (0.85 %). The dosage was orally given in two doses (0.3 ml each). The first dose was given at the morning and the second one at the evening for 15 days. The general stool examination was done during the administration period for each mouse to search for worm eggs.

The effect of alcoholic and water extracts as well as the mebendazole in adults worms movement was studied *in vitro*. Worms were obtained from mice intestine, and distributed in four clean and sterile watch glasses. four worms were put in each glass. Water extract (10%) was added to the first glass, alcoholic extract (3.5%) was added to the second glass, mebendazole (6.6%) was added to

the third glass and normal saline was added to the fourth glass. The time of worm movement cessation and worm death was estimated.

Chi square test and analysis of variance (Campbell, 1967) were employed for the statistical analysis.

Results

Table (1) illustrated that when mice were administered high water extract (2000 and 4000 mg/ kg), for is days no effect was detected on the presence of worms in mice intestineas worm eggs were noticed in the faeces till the seventh day of treatment. However, no eggs were detected during the eleventh day of treatment. Mice administered alcoholic extract (1000and 1400 mg/ kg) showed partial disappearance of worm eggs after 15 and 11 days of treatment, respectively. Some adult worms were seen with the faeces. Mebendazole administration (40 mg/ kg) caused worm eggs disappearance after 15 days of treatment. Worm eggs were continuously found in mice faeces, which were given normal saline (control group). The statistical analysis revealed the presence of significant differences between each treatment above and dosage time.

Table (2) showed that mebendazole (6.6 %) caused worms movement paralysis after 15 minutes and caused their death during 40 minutes. The water extract (10 %) caused worms movement paralysis in half a minute and worms death in 35 minutes. Alcohol extract (3.5 %) caused worms movement paralysis in 11 minutes and death after 20 minutes. Significant differences between the treatments and control groups were detected.

Discussion

The water extract of *A. herba-alba* (2000 and 4000 mg/ kg) was inactive in expulsion of *H. nana* from the infected mice during the first nine days (Table 1) due to the insufficient amount of the extract which arrived to worms bodies inside the mice intestine as a result of the rapid absorption of the extract in the intestine. Luo & Shen (1987) pronounced that artemsinin, extracted from *A. annua* is absorbed rapidly in mice intestine when given orally, while in human intestine it needs about 0.75 hour to be absorbed when given orally (Titulaer *et al*., 1990).

The crude water extract contains several compounds such as alkaloids, glycosides, soaponins, tannins and other nonpolar water-soluble compounds (Al–Khazraji, 1991). The concentration of this water extract was higher enough to cause death of some mice in ninth day of administration (Table 1).Shnawa (1995) proved that the water extract of *Artemisia* sp. (4 gm/ kgm) was active against the intestinal flagellate *Giardia lamblia* inside the body of rats. Al – Rubaie (1999) showed that that water extract of *A. herba – alba* (20%) decreased the viability of protoscolices of *Echinococcus granulosus in vitro* and prevented the infection with hydatid cysts in mice bodies.

The alcoholic extract of *A. herba–alba* (1400 mg/ kgm) was active in expulsion of *H. nana* within 15 days of administration to infected mice (Table 1). This activation was caused by the presence of more active compounds in comparison

with the water extract (Al–Khazraji, 1991). The ethanolic extract of *A. ludoviciana mexicana* (450 mg/ kgm) affected 99.8 % of *Plasmodium yoelii yoelii* in mice bodies (Malagón *et al*., 1997). It seems that chloroformic extract of *A. herba-alba* (3200 mg/ kgm) has a killing effect for cats ascarids *Toxocara cati* in mice muscles and for adult worms in the intestine of infected cats (Al–Rubaie, 1998).

The mebendazole (40mg/ kgm) was active in *H. nana* expulsion from the intestine of infected mice within 13 days of dosing the drug (Table 1). This drug acts in the inhibition of microtubules formation in worms and prevents glucose absorption leading to sinking stored glycogen from the parasite hereby decreasing the energy in ATP form and causing the death of worm (Mosby-Year Book, 1996; Laurence *et al*., 1997).

Table (2) illustrated that the time needed for *H. nana* movement paralysis in 10% *A. herba-alba* water extract was 0.5 minute due to the presence of the above mentioned compounds (Al–Khazraji, 1991) as well as easy reach of the water extract to worm bodies *in vitro* by penetration of body wall and consequently worm killing. In the 3.5% , alcoholic extract movement was inhibited during 11 minutes and worms died within 20 minutes due to presence of more active compounds in this extract in comparison with the water extract. Mebendazole (6.6 %) caused the paralysis of worms within 15 minutes and killed them within 40 minutes (Table 2). This drug prevents sucking of glucose by the worm and subsequently stopping the release of energy in ATP form, leading to impossible movement and survival (Mosby-Year Book, 1996; Laurence *et al*., 1997). The exposing of *T. cati* larvae *in vitro* to 20% mebendazole showed their death within 30 minutes (Al–Rubaie, 1998).

Both the alcoholic extract of *A. herba–alba* and mebendazole were active in expulsion of *H. nana* because this worms lacks the digestive system, so it absorps its food through body integument. This facilitates penetration and arriving of large amounts of drug and plant extract to worm bodies (Zeibig, 1997).

Table (1): Effect of *A. herba-alba* water and alcoholic extracts and mebendazole on adult *H. nana* through the presence of worm eggs in faeces smears of infected mice.

Treatment type and concentration	Number of mice infected with *H. nana* after:							
	one day	three days	five days	seven days	nine days	11 days	13 days	15 days
Water extract (2000 mg/ kg)	3	3	3	3	2 D	0	0	0
Water extract (4000 mg/ kg)	3	3	3	3	2 D	0	0	0
Alcoholic extract (1000 mg/ kg)	3	3	3	3	3	3	3	2
Alcoholic extract (1400 mg/ kg)	3	3	3	3	3	2	1	0
Mebendazole (40 mg/ kg)	3	3	3	3	2	1	0	0
Normal saline (0.85 %)	3	3	3	3	3	3	3	3

D = Death of mice.
Calculated (F) for days = 10.2*, Tabulated (0.05) F = 3.605
Calculated (F) for concentrations = 5*, Tabulated (0.05) F = 3.21
* Significant differences ($P \leq 0.05$).

Table (2): Effect of *A. herba-alba* water and alcoholic extracts and mebendazole on movement paralysis and death of adult *H. nana in vitro* .

Treatment type and concentration	**Time needed for worms movement paralysis (minutes)**	**Time for worms death (minutes)**
Water extract (10 %)	0.5	35
Alcoholic extract (3.5 %)	11	20
Mebendazole (6.6 %)	15	40
Normal saline (0.85 %)	400	540
Calculated χ^2 Tabulated χ^2 (0.01)	12.5 * 5.99	6.5 * 5.99

* Significant differences ($P \leq 0.05$).

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تأثير مستخلصات نبات الشيح *Artemisia herba-alba* في الدودة الشريطية القزمة *Hymenolepis nana* في الفأر الأبيض

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الخلاصة

تم تجريع الفئران البيض Balb/C المصابة بالدودة الشـريطية القزمـة *Hymenolepis nana* المستخلص الكحولي (1400 ملغم/ كغم) والمستخلص المائي ألحار(4000 ملغم / كغم) من نبات الشـيح *Artemisia herba-alba* كما أعطيت مجموعة ثالثة من الفئران عقار الميبنـدازول Mebendazole الطارد للديدان (40 ملغم/ كغم) للمقارنة .

اختفت بيوض الديدان الشريطية من مسحات براز الفئران المصابة المعالجة بعقار الميبندازول في اليوم الثالث عشر من المعالجة واختفت في اليوم الخامس عشر من المعالجة بالمستخلص الكحولي. أمـا مجموعة الفئران المعالجة بالمستخلص المائي فقد استمر ظهور البيوض في مسحات برازها لمدة تسـعة أيام ولكن بعض تلك الفئران حصل موتها في اليوم التاسع من العلاج لعدم تحملهـا لهـذا المسـتخلص. وظهرت فروق معنوية بين مختلف المعاملات والفترة الزمنية للتجريع بينت المشـاهدات فـي الزجـاج *In vitro* أن الوقت اللازم لتثبيط حركة الديدان في المستخلص الكحولي بتركيز 3.5% هو 11 دقيقة مع حصول موت الديدان بعد مرور 20 دقيقة وفي المستخلص المائي بتركيز10% كان وقت تثبيط الحركـة هو نصف دقيقة ووقت موت الديدان كان 35 دقيقة. أما مجموعة السيطرة في المحلول الملحي الفسـلجي فقد كان تثبيط حركة الديدان بعد مرور 400 دقيقة ووقت موت الديدان هو 540 دقيقة. لقد ظهرت فروق معنوية بين مجموعة السيطرة والمعاملات الأخرى المذكورة.