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Evaluation of the anthelmintic activity of pumpkin seeds (*Cucurbita maxima*) in mice naturally infected with *Aspiculuris tetraptera*

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The aim of this study was to investigate the anthelmintic effect of pumpkin seeds (*Cucurbita maxima*) against *Aspiculuris tetraptera* in naturally infected mice. For this purpose, the natural infection was determined by the cellophane tape (on the anal region), and the centrifugal flotation methods of stool samples in approximately 150 male Swiss albino mice (27 to 35 g). The infected mice (29 animals) were divided into four groups. The animals in the first (G_1) and second groups (G_2) received water and ethanol extracts of pumpkin seeds orally at the dose suggested for human for 7 days, respectively. The mice in the positive control group (G_3) were treated with ivermectin intramuscularly at a dose of 0.2 mg/kg body weight. The mice in fourth groups (G_4) received the same amount of serum physiologic orally. The mice were housed in clean polypropylene cages and maintained under standard laboratory conditions at an ambient temperature of 20±2°C with 45% relative humidity and a 12 h light dark cycle. At the eighth day of the study, all animals were killed humanly following inhalation anaesthesia. After euthanasia, the number of parasites in the intestine was counted. Data obtained from the treatment groups were compared using one-way ANOVA. The percentage efficacy of the drugs was calculated. The results of the study showed that the efficacies for water (G_1) or ethanol (G_2) extracts of pumpkin seed and ivermectin were 81, 85 and 91% compared with the negative control, respectively. These results revealed that pumpkin seed has high anthelmintic activity against nematodes as well as its continued use in traditional medicine for the treatment of helminthiasis.

Key words: *Aspiculuris tetraptera*, mice, anthelmintic effect, efficacy, *Cucurbita maxima*.

INTRODUCTION

The traditional medicinal plant use in all cultures is well documented. Plants have been the main component of

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traditional pharmacopoeias for generations, and continue to supply new remedies for the treatment of various maladies. According to the data of the World Health Organisation, 80% of people use these treatment methods in developing countries and 3.3 billion people benefit from medical plants as treatment. In recent years, their usage in developed countries has increased dramatically (Baytop et al., 1999; Saraçoğlu et al., 2005; Mindell, 2003; Saraç, 2006). The medicinal plants such as garlic, black cumin, stinger and pumpkin seeds have been used extensively in the treatments of various maladies as traditional medicine (Beech et al., 2011).

Squash is a member of Cucurbitaceae family that includes plants such as melon, watermelon, cucumber and gherkin. There are two kinds of squash as pumpkin and vegetable marrow. *Cucurbita maxima* (Lam.) (Vegetable marrow, Pumpkin), *Cucurbita moschata* (Lam.) Poiret (Winter squash) and *Cucurbita pepo* L. (Summer squash) are cultivated in Turkey and generally, *C.maxima* is used as an anthelmintic for medical purpose (Baytop, 1999; Saraçoğlu, 2005). Pumpkin seed is very rich in terms of amino acids that are building blocks of proteins, especially the essential amino acids such as phenylalanine, tryptophan and methionine which are not produced in the human body (Saraçoğlu, 2005).

It has a very rich composition of vitamin E, especially gamma tocopherol, unsaturated fatty acids which carry importance in terms of human health and phytosterol (Baytop, 1999; Saraçoğlu, 2005). It is known that not only the fruit but also the pumpkin seed has many benefits, so it has been used for a long time in traditional medicine in many countries as well as in Turkey. Pumpkin seed has been used in different parts of the world as traditional medicine for treatments of gastrointestinal parasites as anthelmintic, urinary dysfunctions, hyperplasia of prostate as a supporter, dysuria, cardiovascular disease, enuresis nocturna in children and lowering blood glucose (Saraçoğlu, 2005; Srivastava and Singh, 1967; Lans et al., 2007; Weiss, 1988; Dreikorn et al., 2002; Winkler et al., 2005). Moreover, it has been reported that compounds in pumpkin seed have an immune potential role (Winkler et al., 2005).

Despite the increase in scientific studies about pumpkin, there is a paucity of data available in the literature on its antiparasitic property. Especially the seeds of *C. maxima*, known as winter squash, are widely used to treatment of gastrointestinal parasites as an anthelmintic. It has been reported that a handful of pumpkin seed kernel was effective especially for the treatment of Taeniasis (Baytop, 1999; Cappelletti, 1985; Gonzales et al., 1974). It is known that the anthelmintic effect of pumpkin seed comes from cucurbitine, and is effective on turbellaria by paralyzing them (Asımgil, 2004; Baytop, 1999; Gonzales et al., 1974; Guarrera, 1999; Pieroni et al., 2005; Saraçoğlu, 2005). Díaz et al. (2004), reported that 1g meshed pumpkin seed displayed a high efficacy against turbellarias in dogs (Lans et al., 2007).

The purpose of this study was to evaluate the anthelmintic efficacy of pumpkin seeds (*Cucurbita maxima*) in mice naturally infected with *Aspiculuris tetraptera.* Hence, the results obtained from this trial could be extrapolated to human and other animal species.

MATERIALS AND METHODS

Experimental animals

One hundred and fifty (150) five-week-old male Swiss albino mice (27 to 35 g) were used in this study. They were housed in clean polypropylene cages and maintained under standard laboratory conditions at an ambient temperature of 20±2°C with 45% relative humidity and a 12 h light dark cycle. They were allowed free access to a standard pellet diet and water *ad libitum*. All animals were fed equally by 1/10 of their approximate body weights. Parasitological examinations of animals were made by centrifugal flotation techniques that were prepared by salt water and cellophane band method. The rats naturally infected by *A. tetraptera* were identified and used in the study. Before starting the study, the investigation was approved by the Experimental Animal Ethics Committee of the Abant İzzet Baysal University, Bolu, Turkey.

The mice (29 mice) naturally infected with *A. tetraptera* were divided into four groups (G_1, G_2, G_3 and G_4) according to the egg burden of the animals, and each group comprises 6 to 10 animals. The animals in G1 and G2 were given water and ethanol extracts of pumpkin seeds orally at a dose suggested for a human for 7 days. The animals in G_3 (positive control) were administered ivermectin intramuscularly at a dose of 0.2 mg/kg bodyweight. The rats in the control group (G_4) were given the same amount of water orally.

Preparation of pumpkin seed extract

The pumpkin seeds were bought from the local market in Bolu and taxonomically identified and authenticated by a taxonomist at the Department of Biology, Faculty of Art and Science, Abbant İzzet Baysal University, Bolu, Turkey. Approximately 200 g of pumpkin seed was milled fine and then water and ethanol extracts were prepared as follows.

Preparation of water extract

Pumpkin seed kernel (100 g) was milled and mixed with the addition of 300 ml distilled water at 40°C for 18 to 24 h. The obtained mixture was filtered and the liquid part (water) was evaporated by using a freeze dryer and pumpkin seed extract was obtained as pulverized.

Preparation of ethanol extract

Pumpkin seed kernel (100 g) was milled and extracted by adding 300 ml ethanol with a soxhlet extractor at 75°C for 24 h. At the end of this period, the mixture was filtered and the liquid part was evaporated by using a rotary evaporator. The remaining part of the pumpkin seed kernel extract was dissolved in 20 to 30 ml distilled water and the ethanol extraction of pumpkin seed was obtained as pulverized by evaporating the liquid part (water) by using freeze dryer. All the obtained extracts were dissolved by using sterile distilled water and the concentration to be given to rats, was prepared as 100 mg/ml. The water and ethanol extracts given orally

were prepared every day for a week with a dose of 100 mg/kg by using an injector.

The treatment of rats with ivermectin in the positive control group

Ivermectin was administered to each rat in the positive control group at a dose of 0.2 mg/kg by diluting ethanol and distilled water. For this, 0.5 ml of mixture which was obtained by mixing 100 ml ethanol and 400 ml distilled water with 0.5 ml of ivermectin, was given to each rat by single i.m. injection.

Treatment

All the rats were examined parasitiologically by saturated saltwater floatation method. On the same day, the water and ethanol extracts of pumpkin seeds were given to rats every day for a week with a dose of 100 mg/kg. On the first day of the study, the rats infected with *A. tetraptera* in the positive control group was treated with a single dose of 0.2 mg/kg of ivermectin following i.m. injection.

Autopsy

The next day of starting and giving the last pumpkin seed, all the rats were killed by inhalation anaesthesia. The intestines of rats in all groups were opened and washed with a saline solution. The parasites were collected and identified under a stereomicroscope. The worm burden were compared between groups.

Statistical analysis

The results were evaluated statistically by using the statistical package for social sciences (SPSS) statistical programme. The significance of difference between groups was calculated statistically by using One-Way Analysis of Variance (ANOVA) and these differences were compared with Benferonni test. The percentage treatment efficacy (%) was calculated using the formula:

Efficacy (%) = [(Cg – Tg) / Cg] × 100

Where Cg is the geometric mean of parasites counted from the control group, Tg is the geometric mean of parasites counted from the treatment group.

RESULTS

Clinically no adverse reactions were observed in any of the rats treated with extracts given during the study. The number of parasites collected from the intestines of rats in control and the experiment groups, min-max values, geometrical means and percentages of efficacy were given in Table 1. The total parasite numbers in control, ivermectin and ethanol and water extract groups of pumpkin seed were 249, 29, 60 and 107 respectively. The geometric means of parasite numbers obtained in the negative control group were significantly higher than those obtained in the experimental groups. The efficacies for ivermectin (G_3), water (G_1) and ethanol (G_2) extracts were 91, 85 and 81% compared to the negative control group (G_4), respectively.

It was obtained that extra treatment had an important effect on the number of parasites, $F(3, 25) = 9.89$, $p = 0.0001$, $\eta^2 = 0.54$. This treatment or try had an effect on explaining 54% of the change (variance) in parasite numbers. According to Benferonni test which kept the type I error constant, when compared to control group (M= 10.70, S.D. = 4.15), the number of parasites statistically low between ivermectin (M= 6.33, S.D.=3.88), ethanol (M= 8.57, S.D. = 4.96) and water extract (M= 10.70, S.D. = 4.15) groups with these last groups being not significantly different from each other. As a result, when compared to control group, it was obtained that ivermectin with pumpkin seed water and ethanol extracts importantly decrease parasite numbers (Table 1).

DISCUSSION

Medicinal plants such as onion, thyme, sweet balm, black cumin and stinger have been used widely as a traditional treatment. Pumpkin seeds have been used for traditional treatment worldwide especially in the Far East. According to the data transferred from generation to generation and scientific researches, pumpkin seed has been used in traditional treatment for especially urinary diseases, diabetes, prostate and parasitic infections. It has been reported that in the last ten years, pumpkin seeds has been used for antitumor, antidiabetics, antihypertensive, antibacterial, anti-inflammatory, immunostimulant and analgesic purposes (Jafarian et al., 2012).

Díaz et al. (2004), reported that the minimal effective amount of the seeds from *C. maxima* was 23 g (approximately 73 pumpkin seeds) in rats. Moreover, they observed that the pumpkin seeds displayed proteolytic effect and destructions on tegument including base membrane and cause reduction in the number of eggs of reproduction of parasite rings. It was also obtained that pumpkin seeds caused erosive gastritis in rats at 9 g/kg pumpkin seed. In another study, (Díaz et al., 2004), 23 g pumpkin seed in 100 ml distilled water showed antihelmintic activity such as changes in the parasite movements and destruction in tegumentine and eggs in reproduction rings of parasites. In Italy, it was reported that an anthelmintic activity was observed against tenias in dogs when 1 g of meshed pumpkin seed was mixed and given with 250 ml distilled water (Lans et al., 2007). In the present study, it was found that the anthelmintic activities of water and ethanol extracts on *A. tetraptera* were considerably high (81 and 85%, respectively).

Moreover, Mahmoud et al. (2002) reported that pumpkin seed extract prepared with boiled water caused destruction on parasite eggs after administration orally to the puppies infected with *Heterophyes heterophyes* for two weeks (Mahmoud et al., 2002). Similarly, *C. maxima* was given to winged animals at 50, 100 and 200 mg/kg doses for 10 days for investigation of its anthelmintic activity, but no activity was observed (Lahon et al., 1978). They only reported that 2 ml ethanol extraction (300

Table 1. Control and experimental groups, the number of parasites collected from the intestines of rats, min-max values and influence the geometric mean of the percentages.

Groups	No	Number of parasite after the autopsy	Total	Min-max	Geo-mean±SD	% Efficacy
Control group (G_4) (n:6)	1	17	249	17-84	35.84±25.40	-
	2	26				
	3	34				
	4	84				
	5	60				
	6	28				
Ivermectin (G_3) group (n:6)	1	5	29	1-10	3.32±3.88	91
	2	1				
	3	3				
	4	10				
	5	9				
	6	1				
Ethanol extract (G_2) (n:7)	1	16	60	1-16	5.44±6.34	85
	2	1				
	3	14				
	4	9				
	5	1				
	6	5				
	7	14				
Water extract (G_1) (n:10)	1	1	107	1-27	6.78±9.27	81
	2	23				
	3	4				
	4	27				
	5	15				
	6	2				
	7	6				
	8	9				
	9	17				
	10	3				

mg/ml) causes an 8.69% reduction in *Raillietina cesticillus* in the animals. In this previous study, it was obtained that water and ethanol extractions of pumpkin seed is effective as 81 and 85% respectively against *A. tetrapteraya* in the intestines of rats. It was observed that its effect is more when ethanol extract is compared to water extract. These obtained results have a quality that supports the general usage of pumpkin seed in treatment of parasite infections.

It has been reported that the types of extraction play an important role at the level of antiparasitic activity. In a previous study, Amorim et al. (1991) investigated that the antimalarial effect of ethanol extracts of *C. maxima* seeds against *Plasmodium berghei* ans 50% reduction in the blood parasite amount was observed in the infected rats given the extracts at a dose of 250/500 mg/kg. Antigiardial effects of petroleum ether and methanol extract of *C. maxima* and *C. pepo* seeds at doses of 250, 500 and 1000 ppm were investigated by Elhadi et al. (2013). The extract dose of *C. maxima* obtained after petroleum ether extraction at 500 and 1000 ppm have 48 h, whereas 250 ppm concentration has 100% activity against giardia in 72 h. Moreover, *C. pepo* has 83.7% activity against giardia in 96 h at a concentration of 500 ppm. It was reported that *C. maxima* can be used as a good anti giardial agent. They thought the anti giardial effect of *C. maxima* depends on the existence of triterpens (Cucurbitacin E and Cucurbitacin L 2-0-β glucoseids). In addition, Marie-Magdeleine et al. (2009) studied three different extractions (water, methanol and

dichloromethane) of *C. moschata in vitro* against *Haemoncus contortus*. They found that only water extract inhibited the larval developments of the parasite. It was reported that the pigs experimentally infected with *Oesophagostomum* sp. larvas were treated with pumpkin seeds following administration three times in a week at a dose of 5 g/kg and the results were compared with ivermectin treatment (Magi et al., 2005). The anthelmintic activity of pumpkin seeds (96.1%) was found similar to ivermectin treatment (97.5%).

With this study both in our country and in different parts of the world the anthelmintic effect of pumpkin seed (C. Maxima Lam) which is used in treatments of many parasitic diseases such as *Taenia saginata, Enterobius vermicularis ve Ascaris lumbricoides* was searched on *Enterobius vermicularis* and *A. tetraptera* in human as well as in natural infected rats.

Conclusion

The present study indicated that both water and ethanol extracts of pumpkin seeds were effective and displayed high anthelmintic efficacy (81 and 85%, respectively) against *A. tetraptera* in rats. Hence, the results obtained from this trial could be extrapolated to other animal species and human for the treatment of helminthiasis.

Conflicts of interest

The authors have none to declare.

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