Antiparasitic Efficiency of *Artemisia absinthium* on *Toxocara cati* in Naturally Infected Cats

Doğal Enfekte Kedilerde *Toxocara cati*'ye *Artemisia absinthium*'un Etkisi

Kader Yıldız[1], Mehmet Başalan[2], Özkan Duru[3], Sami Gökpınar[1]

[1]Kırıkkale Üniversitesi Veteriner Fakültesi, Parazitoloji Anabilim Dalı, Kırıkkale, Turkey
[2]Kırıkkale Üniversitesi Veteriner Fakültesi, Hayvan Besleme ve Beslenme Hastalıkları Anabilim Dalı, Kırıkkale, Turkey
[3]Kırıkkale Üniversitesi Veteriner Fakültesi, Biyokimya Anabilim Dalı, Kırıkkale, Turkey

ABSTRACT

Objective: The first aim of the present study was to determine the efficiency of *A. absinthium* extract on cats naturally infected with *Toxocara cati*. The second aim was to determine the efficiency of the extract on the embryonic development of *T. cati* eggs *in vitro*.
Methods: *Artemisia absinthium* extract was orally administrated to cats at the doses of 300 mg/kg and 600 mg/kg body weight in Group 1 and 2, respectively. It was given only once a day and the treatment continued 7 consecutive days. The faeces of the cats were examined both macroscopically and microscopically by flotation procedure with saturated salt solution pre-, during and post- treatment period. The faecal analysis was maintained during 8 days after completing the extract administration. The alteration of faecal egg numbers was performed by using the McMaster technique.
Results: The faecal egg numbers per gram were decreased gradually in cats in the trial groups. In the treatment period, the activities of ALT, AST, ALP, urea and creatinine were located within the physiological ranges in cats. In *in vitro* trials with *A. absinthium* extract, the embryonic development of *T. cati* eggs was identical in all groups (treatment and control). *A. absinthium* extract did not inhibit larval development in eggs in in vitro trials.
Conclusion: This plant extract may be an alternative choice in the treatment of parasitic diseases in future.
(Turkiye Parazitol Derg 2011; 35: 10-4)

Key Words: Antiparasitic efficiency, *Artemisia absinthium*, cat, *Toxocara cati*

Received: 21.12.2010 **Accepted:** 15.02.2011

ÖZET

Amaç: Bu çalışmada *Toxocara cati* ile doğal enfekte kedilere *Artemisia absinthum* ekstresinin etkinliğini ile *in vitro* ortamda *T.cati* yumurtasındaki embriyonik gelişime etkisinin belirlenmesi amaçlanmıştır.
Yöntemler: *Artemisia absinthum* ekstresi kedilere 300 mg/kg (Grup 1) ve 600 mg/kg (Grup 2) dozda ağızdan verilmiştir. Ekstre günde bir kez olacak biçimde 7 gün süreyle uygulanmıştır. Kedilerin dışkıları tedavi öncesinde, tedavi esnasında ve tedavi sonrasında makroskobik ve doymuş tuzlu su flotasyon yöntemi ile mikroskobik olarak incelenmiştir. Ekstre verilmesi bittikten sonraki 8 gün süreyle dışkı incelenmiştir. Dışkı yumurta sayısındaki değişim McMaster tekniği kullanılarak belirlenmiştir.
Bulgular: Deneme gruplarındaki kedilerde gram dışkıdaki yumurta sayısının dereceli olarak azaldığı gözlenmiştir. Tedavi esnasında kedilerde ALT, AST, ALP, üre ve kreatinin aktivitelerinin fizyolojik değerler arasında olduğu belirlenmiştir. *A.abstinhum* ile yapılan *in vitro* denemelerde tüm gruplarda (tedavi ve kontrol) *T.cati* yumurtaları içindeki embriyonik gelişimin benzer olduğu gözlenmiştir. *A.absinthum* ekstresi in vitro ortamda larva gelişimini engellemediği belirlenmiştir.
Sonuç: Bu bitki ekstresi gelecekte parazit hastalıkalrının tedavisi için bir alternatif olabilir. *(Turkiye Parazitol Derg 2011; 35: 10-4)*

Anahtar Sözcükler: Antiparazitik etki, *Artemisia absinthum*, kedi, *Toxocara cati*

Geliş Tarihi: 21.12.2010 **Kabul Tarihi:** 15.02.2011

This study is presented in 16th National Congress on Parasitology which was held in Adana (1-7 November 2009)

Address for Correspondence / Yazışma Adresi: Dr. Kader Yıldız, Kırıkkale Üniversitesi Veteriner Fakültesi, Parazitoloji Anabilim Dalı, Kırıkkale, Turkey Phone: +90 318 357 33 01 Fax: +90 318 357 33 04 E-mail: kaderyildiz@hotmail.com
doi:10.5152/tpd.2011.03

INTRODUCTION

Toxocara cati lives in the intestines of cats and other Felidae species (1). This parasite is considered to be one of the most important helminth species of the cat and different rates of its distribution in cats have been reported both in Turkey (2-4) and in various regions of the world (5-7). Different infection routes, such as eating parasite eggs, carrying infectious stage larvae or various paratenic hosts and galactogenic infection have been reported in the life cycle of *T. cati* (1, 8, 9). Transplacental infection does not occur in the developmental period of *T. cati* (8). The prepatent period continues approximately 8 weeks with oral infections by eggs which developed infective stage larva (8). Catarrhal inflammation, dehydration, anaemia, weight loss and anorexia can be considered as the most common symptoms in cat with toxocariosis (8).

Many drugs have been developed for the therapy of parasitic infections in animals over the years. Nowadays, alternative therapy choices are increasingly tested for the treatment of parasitic diseases because of resistance development of parasites to antiparasitic drugs, residues in host tissues which was later consumed by humans, and harmful metabolites were excreted with animal faeces to free living arthropods in environment (10-12). Some of the plant extracts have been proved to be successful agents in the treatment of parasitic diseases (10, 13-15). One of them is *Artemisia absinthium* and also known as wormwood. The name "*Artemisia*" is derived from Goddess Artemis who was said to have discovered the plant's effects, whereas "*absinthium*" means undrinkable because of the very bitter taste of the plant (16). This plant has been used for therapeutic purpose since the antique Egyptians (16). The dried whole plant and essential oil of *A. absinthium* have traditionally been used as an anthelminthic, antiseptic, antispasmodic and sedative (16). Its essential oil with very bitter taste has also been instigated as a vermifuge which is a more acceptable form. Also, it was reported that wormwood increases the secretions of gastric and pancreatic enzymes and bile in humans (17).

Artemisia absinthium is a well known alternative therapeutic, with particular application in the treatment of nematode infection (16). However, data is absent regarding the impact of wormwood on toxocariosis of cats and additionally its effect on the liver and kidney of cats after administration. In the present study, it was aimed to determine the efficiency of *A. absinthium* extract on cats naturally infected with *T. cati* and on larvated eggs *in vitro*. The alteration of alanine aminotransferase (ALT), alkaline phosphatases (ALP) and aspartate aminotransferase (AST) in sera are significant for monitoring liver function (18). The alterations of urea and creatinine in sera are essential for monitoring kidney function (18). The second aim was to detect the alterations of ALT, ALP, AST, urea and creatinine in the sera of cats after *A.absinthium* extract had been administered

MATERIALS AND METHODS

Composing the trial groups

In present study, *A. absinthium* extract was administrated to domestic, shorthair, 2-3 year-old pet female cats naturally infected with *T. cati* in Kırıkkale University, Faculty of Veterinary Medicine Clinics. The study was approved by the Ethical Committee of Animal Research of Kırıkkale University (file number: 09/13-157). Infected cats were divided into three groups according to the number of *T. cati* eggs per gram (EPG) in faeces. EPG of cats in Group 1 (n:5) and Group 2 (n:3) follows as 150-750 and 1550-4300, respectively. EPG of cats in the control group (n:3) is 300-750.

Preparing A.absinthium extract

Artemisia absinthium procured from a local herbal market was identified and authenticated by a botanist. Flower parts of *A. absinthium* plants were granulated manually with a grinder. The oil part of the plants was extracted by the extraction method. The samples were placed in the Soxhlet apparatus. The plant oil extract was obtained by using di-ethyl ether at 30-35°C in a 7 hour period. To evaporate di-ethyl ether, the extract was left at room temperature overnight uncovered.

In vivo trial

The extract of *A. absinthium* diluted in 5 ml of distilled water was given orally to naturally infected cats with plastic syringes without the needle. The extract was administrated as 300 mg/kg and 600 mg/kg body weight (b.w.) in Group 1 and 2, respectively. It was given only once a day and the treatment continued for 7 consecutive days. The extracts were prepared fresh before each administration. The extract was given carefully to the cats. The control group was only administrated water. The cat faeces were daily examined both macroscopically and microscopically by using the flotation procedure with saturated salt solution. The alteration of faecal *T.cati* eggs numbers was detected in animals with the McMaster technique. The faecal analysis was maintained during 8 days after completing the extract administration. The cats were hospitalised during the research period and were fed with commercial dry cat food.

The blood samples were taken by punction of the vena cephalica antebrachi to the tubes with heparin at the 6^{th}, 12^{th}, 24^{th}, 48^{th}, 72^{th} hours of the treatment period and 21^{th} days after began the treatment period of cats (Group 1, 2 and control group). The activity of ALT, AST, ALP, urea and creatinine were measured with Shimadzu UV-1700 spectrophotometer using the manufacturer's kit (Teco® Diagnostics and DDS diagnostics kits) according to kinetics and endpoint spectrophotometric methods.

In vitro trial

Toxocara cati eggs were collected from faeces before the extract administrations to naturally infected cats. The collected egg concentration was diluted with PBS as 500 eggs per ml. *A. absinthium* extract was diluted with distilled water in concentrations of 0.3 mg/ml and 0.6 mg/ml. Three trial groups were constructed as follows: - G1=0.3 mg/ml, G2=0.6 mg/ml and G3=control. One ml of egg solution was added to the glass tubes of each group. Then, one ml of *A .absinthium* extract was added to egg solutions in tubes G1 and G2. Only PBS was added in the control group (G3). All groups were incubated in an incubator at 26°C. The egg samples were taken in the control and trial groups at 24^{th} hours, 7^{th}, 14^{th} and 21^{th} days, and examined under the light microscope to determine the larval development rate in *T. cati* eggs.

RESULTS

Artemisia absinthium extracts were well tolerated by cats in the present study. We have not seen any side effects in cats that used *A. absinthium* extract. Table 1 represent the efficiency of *A.abstinhum* extract on the *T. cati* infection of cats. The faecal eggs number per gram was decreased gradually in the cats of Group 1 (Table 1). In one of the cats (no: 5) in Group 1, the faecal egg output stopped at the 6th day after the treatment started and deformed parasite body was found in the faeces of this cat. The faecal egg output stopped 2 days after the end of the treatment period in another cat (no: 4) but no adult parasite body was found in faeces. The faecal egg output was decreased in other cats in Group 1.

In Group 2, *A. absinthium* extract dose 600 mg/kg b.w. generally decreased faecal egg number in all the cats examined (Table 1).

In *in vitro* trials, the embryonic development was identical in all groups. Developed larvae were observed in *T. cati* eggs at the 21th day. *A. absinthium* extract did not inhibit larval development in the eggs (Figure 1).

The activity of ALT, AST, ALP, urea and creatinine were observed to be at physiological levels in the sera of cats examined (Table 2).

DISCUSSION

Toxocara cati is one of the most prevalent nematode parasites found in the cat intestine (1, 8). *T. cati* has attracted great attention zoonotically because of its visceral larvae migrating potential to human (8, 9). Today, different medicament choices such as moxidectin, piperazine, fenbendazol, flubendazole, pyrantel, selamectin and milbemycin are available for the treatment of toxocariosis in cats (19-23). However, development of alternative treatment methods has been brought about by veterinarians and researchers.

Artemisia species are known to have antiparasitic efficiency (11,15, 24-26). *Haemonchus contortus* eggs decrease in faeces of sheep after administration of *A. absinthium* extract (26). The effect of extract is expressed as killing the parasite or causing paralysis (26). Larval rate of *Trichinella spiralis* is decreased in mice muscles following 20 consecutive days administration of 300 and 600 mg/kg b.w. doses of *A. absinthium* extract (25). In the present study, the doses of *A. absinthium* extract used in the cats were detected according to Caner et al. (25). *Artemisia absinthium* extract with the doses of 300 mg/kg and 600 mg/kg b.w. diminished faecal egg number in all cats naturally infected with *T. cati* in this study. Moreover, the extract completely stopped *T. cati* eggs in the faeces of two cats in Group 1. The ending of faecal egg output at the 6th day of treatment in one cat (no: 5) in Group 1 may be related to the observed deformed parasite body in faeces of this cat. The faecal egg number, however, was only diminished in Group 2. It was thought that the egg laying potential of *T. cati* may be decreased by *A. absinthium* extract in the present study. However, the larvae development was not inhibited by the extract in the *in vitro* study. No contraindicative effect was observed in the cats after extract administration.

Artemisia species were divided into two sub-groups with regard to oil composition; one group was characterised by the presence

Table 1. The faecal egg numbers of *A.absinthium* in the cats during and after treatment

			EPG* in treatment days														
			During treatment							Following treatment							
Groups	Cat no	A.absinthum extract dose	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Group 1	1	300mg/kg b.w.	350	150	200	200	400	200	200	200	200	200	150	150	150	100	100	100
	2		750	500	450	600	600	500	600	450	400	400	450	400	400	400	350	350
	3		750	750	600	600	450	450	400	400	350	400	250	200	200	200	150	150
	4		350	300	350	300	200	150	150	100	50	100	0	0	0	0	0	0
	5		150	150	100	100	50	50	0	0	0	0	0	0	0	0	0	0
Group 2	1	600mg/kg b.w.	1550	1500	1450	1500	1400	1500	1450	1250	750	600	400	350	100	100	100	100
	2		1900	1750	1050	700	700	700	1000	1000	650	500	250	200	200	200	150	100
	3		4300	3200	2400	2800	2050	2500	2750	3850	1600	4500	1400	1400	1300	1300	1250	1250
Control Group	1	none	750	600	600	400	450	400	550	650	650	500	600	700	450	600	650	550
	2		550	600	400	450	500	500	550	400	500	750	400	450	550	650	650	500
	3		300	350	350	400	450	400	350	350	300	450	350	450	350	500	350	300

*Eggs number of per gram in faeces

Table 2. Mean serum enzyme levels at various times after the treatment period started in the cats

Groups	Hours	ALT (U/L) mean (min-max)	AST (U/L) mean (min-max)	ALP ALP mean (min-max)	Urea (mg/dl) mean (min-max)	Creatinine Creatinine mean (min-max)
Group 1 (n:5)	0	18.5 (11-32)	19 (15-21)	51 (35-72)	51.2 (45.5-58.6)	1.2 (0.9-1.5)
	6	20.6 (14-28)	21.6 (14-30)	51.2 (38-74)	54.9 (46.8-58.8)	1.3 (1.2-1.4)
	24	20.2 (18-24)	20.8 (11-32)	51.8 (41-65)	56.1 (45.5-65.3)	1.3 (1.2-1.4)
	48	25.4 (14-36)	20.8 (14-25)	46 (32-62)	56.1 (48.0-61.1)	1.3 (1.2-1.5)
	72	20.2 (10-24)	20.4 (15-24)	47.2 (35-58)	55.9 (49.5-59.2)	1.2 (1.1-1.5)
	15th day	22.3 (20-24)	21.2 (18-24)	42.3 (35-54)	55.2 (55.0-58.5)	1.3 (1.1-1.4)
Group 2 (n:3)	0	11 (11)	23 (23)	48 (48)	51.3 (51.3)	1.5 (1.5)
	6	18.3 (15-24)	19.6 (14-24)	38 (35-42)	50 (44.0-55.0)	1.5 (1.4-1.6)
	24	20.3 (14-28)	19.6 (14-24)	38 (35-42)	50.3 (48.9-50.8)	1.4 (1.4)
	48	21 (15-30)	22.6 (19-26)	38.6 (33-45)	49.3 (45.2-52.0)	1.4 (1.3-1.4)
	72	22.5 (20-25)	19 (18-20)	34.5 (34-35)	34.3 (44.8-58.0)	1.5 (1.5)
	15th day	20.3 (19-22)	19.3 (18-21)	36.3 (30-40)	53.1 (48.2-56.5)	1.3 (1.2-1.4)
Control Group (n:3)	0	15 (14-16)	18 (16-20)	45 (35-65)	50.1 (46.4-55.4)	1.2 (1.2)
	6	19.6 (17-23)	19.6 (17-25)	52.3 (48-64)	50.5 (44.6-54.3)	1.3 (1.2-1.4)
	24	23.6 (21-26)	19 (19)	46.3 (35-58)	57.7 (52.4-60.4)	1.3 (1.3)
	48	24 (17-32)	23.6 (22-25)	51 (39-60)	54.2 (52.7-55.6)	1.2 (1.1-1.3)
	72	23 (16-29)	22 (19-24)	51.3 (34-62)	54.0 (48.2-57.2)	1.3 (1.2-1.4)
	15th day	22.6 (19-27)	21.3 (21-22)	48.6 (41-57)	52.9 (47.9-56.8)	1.2 (1.1-1.3)

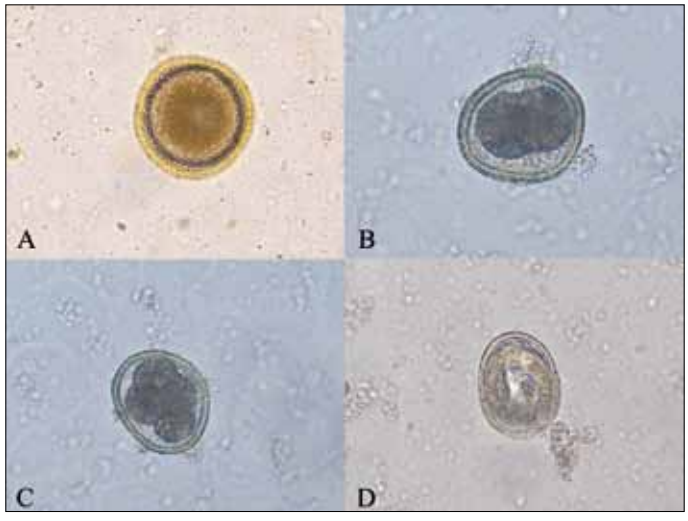

Figure 1. Embryonic development of *Toxocara cati* eggs in *in vitro* trial (A: *T. cati* egg at 24th hour. B: Two blastomeres forms of *T. cati* egg at 7th day. C: More than four blastomeres in egg at 14th day. D: Developed larvae in *T. cati* egg at 21th day).

of camphor and 1.8-cineole and the second group contained mostly a-thujone (16). Camphor and 1.8-cineole are frequent components of aromatic medicinal herbs, and are reported to have antimicrobial properties (30) and, additionally, these components are known to have antiparasitic efficiency (24). The composition of *A. absinthium* varies from country to country with respect to the soil composition on which it is grown (27, 31, 32).

The major components of essential oils in *Artemisia* species produced in Turkey was reported as camphor and 1.8-cineole (27). These essential oils possess an antiparasitic efficiency (28, 29). Cineole is reported to have high larvicidal activity on *Anisakis simplex* L3 (28). Camphor has a strong destructive effect on *Demodex* spp. *in vitro*. The main mechanism of camphor oil may be related to direct contact and neuromuscular toxicity (29). The effect of essential oils in *A. absinthium* extracts administrated in our study was mild toxocariosis in the cats.

The presence of liver disorders is often recognized on the basis of elevated serum activities of enzymes of hepatic origin. When hepatic necrosis is present, the serum enzyme activities such as ALT and AST are increased. The liver is exposed to a wide variety of toxins, drugs and drug metabolites that may influence the serum activity of enzymes from the liver (18). There are many possible drugs that stimulate some increase in liver ALP and ALT activities (18). Creatinine and urea are indicators of renal function (18). In our study, the activities of AST, ALT, ALP, creatinine and urea in the sera of cats after the administrated *A. absinthium* extract were located between physiological ranges.

In conclusion, the extract of *A. absinthium* was orally administrated in cats naturally infected with *T. cati* as a preliminary study. Further research on *A. absinthium* may be encouraged with some important results of this study, such as diminished faecal egg output in infected cats, no pathological effect observed on serum activities of the hepatic origin enzymes, creatinine and urea in cat sera after application. However, the difficulty of oral

administration of *A. absinthium* extracts due to its bitter taste should be overcome. In further studies, the extract of *A. absinthium* can be used at a higher dose than administrated in the present study and placed in capsules delivered to the intestine to facilitate application, or combine with other compounds to increase its effect on parasites. This plant extract may be an alternative choice in the treatment of parasitic diseases in future.

Conflict of Interest

No conflict of interest is declared by the authors.

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