YYU Veteriner Fakultesi Dergisi, 2013, 24 (1), 15 - 17
ISSN: 1017-8422; e-ISSN: 1308-3651

ORIGINAL ARTICLE

Anthelmintic Effect of *Plantago major* L. in Mice Infected With *Aspiculuris tetraptera*

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Received: 02.01.2013 Accepted: 09.01.2013

SUMMARY

The aim of the present study was to investigate the anthelmintic activity of *Plantago major* L. (plantain) in Swiss albino mice naturally infected with *Aspiculuris tetraptera.* Methanolic and aqueous extracts of *P. major* leaves were evaluated for their in vivo anthelmintic activity. The results showed that methanolic extract of *P. major* possessed only a slight anthelmintic activity (27.62%). In contrast, aqueous extract exhibited more potent anthelmintic activity (39.25%).

Key Words *Plantago major L., Plantain, Anthelmintic activity, Aspiculuris tetraptera*

Aspiculuris tetraptera ile İnfekte Farelerde *Plantago major* L.' un Antelmentik Etkisi

ÖZET

Bu çalışmada *Aspiculuris tetraptera* ile doğal infekte farelerde *Plantago major* L'un (sinirotu) antelmentik etkisinin incelenmesi amaçlandı. *P. major* yapraklarının metanolik ve sulu ekstraktlarının in vivo antelmentik aktiviteleri değerlendirildi. Sonuçlar metanolik ekstaktın %27.62 gibi az bir antelmentik aktiviteye sahip olmasına karşın, sulu ekstraktın daha güçlü (%39.25) bir etkiye sahip olduğunu göstermiştir.

Anahtar Kelimeler *Plantago major L., Sinir otu, Antelmentik etki, Aspiculuris tetraptera*

INTRODUCTION

Parasitic infections are common worldwide problems. Several drugs have been used for treatment of infections; as a consequence, some problems such as resistance and residue also increased (Coles, 1997). It is, therefore, necessary to find new compounds. Plants have been the most attractive sources. In recent years, the use of herbal medicines against different diseases has increased in developing countries.

Plantago major L. is a perennial plant that is known as "sinir otu" in Turkey. It's leaves grow in rosttes, and they are ovate to elliptical with paralel venation. *P. major* contains biologically active compounds such as polysaccharides, lipids, caffeic acid derivatives, flavonoids, iridoid glycosides, isomartynoside and terpens (Samuelsen, 2000; Kolak et al, 2011). *P. major* is used in the treatment of a number of diseases related to the skin, respiratory organs, immunsystem, digestive organs, reproduction, circulation, against cancer, for pain relief and against infections (Ravn and Brimer, 1998; Samuelsen, 2000; Rezaeipoor et al., 2000; Chaing et al., 2002; Galvez et al., 2003).

The pharmacological properties of *Plantago spp* such as antimicrobial (Karakas et al., 2012; Metiner et al., 2012; Stanisavljevic et al., 2008), wound healing (Zubair et al., 2012), antioxidative (Kolak et al., 2011; Stanisavljevic et al., 2008), hepatoprotective (Turel et al., 2009), antiinflammatory (Beara et al., 2010; Turel et al., 2009), immunomodulatory (Huang et al., 2009), anticholinesterase (Kolak et al., 2011) and antitumoral (Karakas et al., 2012; Ozaslan et al., 2007) effects have been detected. Bingol et al., 2010 reported that addition of Plantago major extract at differing levels into broiler diet did not affected animal performance and carcass parameters.

Aspiculuris tetreptera classified under Oxyuroid group is a natural and common intestinal parasite of mice and important since, it has been extensively used in determination of efficacy of several chemotherapeutic agents (Theodorides, 1976; Soulsby, 1982; Moulia et al., 1993)

The present study was performed to investigate the anthelmintic activity of leaves of *Plantago major* in Swiss albino mice naturally infected with *A. tetraptera*.

MATERIALS and METHODS

Plant materials: *Plantago major* L. leaves were collected from Van Province, East of Turkey in the spring of 2008. The voucher specimen was authenticated by Prof. Dr. Lütfü Behçet from Department of Biology, Faculty of Science and Art, Yuzuncu Yil University. The samples of *P.major* L. leaves were deposited at the herbarium unit. The herbarium number of *P.major* L. leaves is B-25.

Preparation of plant extracts: The air-dried plant material was pulverised and stored in dark bottles for further use.

Methanolic extract was prepared by mixing twenty gram powdered plant material with 250 ml methanol (99.5%) at 50°C in a soxhlet apparatus for 24 h. The methanolic extract was evaporated to dryness in vacuum to provide crude methanolic extract (CME). The yield of extract was approximately 31%. The CME was freshly suspended in

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distilled water/2% Tween-80 to obtain a suspension with a final concentration of 100 mg mL^{-1} .

To prepare aqueous extract, 10 g powdered plant material was infused with 100 ml distilled water at 50 °C for 2 h and filtered to avoid from particulate matter. The infusion was complemented to 100 ml with distilled water.

Pharmacological procedures: Swiss albino mice (23-25 g) were obtained from the animal house facility of the Faculty of Medicine, Yuzuncu Yil University, Van, Turkey. The mice were housed in the standard cages with pellet food (Van Animal Feed Factory, Van-Turkey) and water ad libitum, in the regulated light and temperature conditioned room (22±2 °C, 12 h of dark/light cycle). The approval of Animal Ethics Committee was obtained from Ethic Committee of Yuzuncu Yil University Faculty of Veterinary Medicine (number is 2005/003). The stool samples of 100 mice were examined for detecting naturally infected animals using centrifugal flotation technique in saturated zinc sulphate solution. Thirty nine infected mice (both sexes) were randomly divided into four groups. The animals were fasted for 4 h before treatment. The mice received 250 µl of 2% Tween 80 orally every day during 7 days in Group I (control). Ivermectin as reference drug was administered by intramuscular injection at a dose of 0.2 mg/kg in Group II. Mice were orally received 250 µl of aqueous extract (100 µl /10g mouse) in Group III and 250 µl of methanol extract (5 mg/10 g mouse) in Group IV daily for 7 days.

The mice fecal samples from the mice were examined on day 1 (pre-treatment), day of the treatment and for 7 days post-treatment on a daily basis using centrifugal flotation technique in saturated zinc sulphate. The mice were euthanised on the 8th day pos-treatment. Gastrointestinal tract was removed and washed with sterile saline solution. The contents were examined under a stereomicroscope to count and identify *A. tetraptera*. The efficacies of the drugs were calculated by the formula given below (Jacobs et al., 1994; Wood et al., 1995; Gicik, 1997).

The data were statistically analyzed in order to evaluate its significance, through analysis of variance test.

$$\text{Efficacy (\%)} = \frac{\left(\begin{array}{c}\text{Geometric mean number of}\\ \text{A. tetraptera in control group}\end{array}\right) - \left(\begin{array}{c}\text{Geometric mean number of}\\ \text{A. tetraptera in treated group}\end{array}\right)}{\text{Geometric mean number of A. tetraptera in control group}} \times 100$$

RESULTS

Table 1 shows results. At necropsy, There was severe parasite invasion (total 1402 *A. tetraptera*) in group I (control group). In group II, 234 parasites were detected and efficacy of ivermectin was calculated as 88.57%. The number of *A. tetraptera* was counted as 966 in group III and as 1120 in Group IV. Although the efficacy of aqueous extract of *P. major L.* leaves was 39.25%, the efficacy of *methanolic extract* was found lower (27.62%). Results showed that anthelmintic activity of *P.major L* leaves was much lower than that of ivermectin. The differences among efficacies of the drugs were statistically significant ($p<0.001$).

Table 1. The efficacy of *Plantago major* L and ivermectin against naturally infected mice with *A. tetraptera*

		Parasite counts recovered at necropsy (8th day)					Efficacy (%)
Groups	n	Total	Min-max	Geo-mean	SE	SEM	
Group I: Control	9	1402	58-299	134.43	87.421	27.140	
Group II: Ivermectin	10	234	3-74	15.36	22.579	7.140	88.57
Group III: Aqueous extract	10	966	32-236	81.66	61.430	19.426	39.25
Group IV: MeOH extract	10	1120	31-183	97.30	52.793	16.695	27.62

SE: Standard Deviation, SEM: Standard Error of Mean

DISCUSSION and CONCLUSION

The anthelmintics are widely used against different parasitic infections. Their low therapeutic indices and increasing resistance development to these drugs have led to the proposal of screening medicinal plants for their anthelmintic activity (Coles, 1997; Iqbal et al., 2004). There is a need for potent and less toxic anthelmintics. Anthelmintic plants offer a traditional alternative to manufactured anthelmintics that are both sustainable and environmentally acceptable. Such plants could have a more important role in the future control of helminthic infections (Hammond et al., 1997). A number of medicinal plants have been used to treat parasitic infections in man and animal in Turkey (Sezik et al., 2001; Kozan et al., 2006). We have previously shown that nettle (Turel et al., 2008) and garlic (Ayaz et al., 2008) had significant anthelmintic activities (88% and 91% respectively).

Samuelsen reported that *P. major* was used as anthelmintic in Argentina, Guatemala and Rodrigues (Samuelsen, 2000). But only one report on the anthelmintic activity of *Plantago lanceolata,* in which the efficacy of ethanolic and aqueous extracts of *P. lanceolata* was found as 44.5% and 35.9% respectively. These results were evaluated as significant anthelmintic activities (Kozan et al., 2006). Aqueous extract of *P. major L.* leaves had an anthelmintic activity 39.25%, which was similar to Kozan's result (Kozan et al., 2006). But efficacy of methanolic extract of *P. major L.* leaves was lower (27.62%) than efficacy of ethanolic extract of *P. lanceolata*. It may be interpreted that the difference between efficacies might be due to solvents and species.

It is concluded that *Plantago major* leaves possess anthelmintic activity. *Plantago major* is a widespread plant of pastures and may have a role to decrease the number of parasites in grazing animals. Therefore, further research is required to determine its anthelmintic effect in livestock grazing this plant.

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