**The Effects of Mistletoe (Viscum album L. subsp. album) Extracts on Isolated Intestinal Contractions**

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**Abstract:** Throughout history folk medicine has served as an alternative or complimentary method of treating disorders like intestinal colic. Mistletoe is a common name for many species of semi-parasitic plants that grow on trees throughout the world, and has been used widely to treat intestinal colic. We conducted a study to evaluate the effect of freeze-dried (group 1) and heat-treated (group 2) extracts of Viscum album subsp. album leaves on contractions induced by acetylcholine in isolated hamster intestinal segments (duodenum, jejunum, and ileum). Freeze-dried extracts decreased intestinal contractions in all the intestinal segments ($P < 0.05$). The strongest relaxant effect was observed in the jejunum and the weakest was seen in the duodenum. Higher doses of heat-treated extracts were required to affect segmental contractions than that of freeze-dried extracts ($P < 0.05$); heat-treated extracts had the greatest effect on reducing contractions in the jejunum and the least effect in the duodenum. Mistletoe extracts affected the level of contractions in a dose- and extraction preparation-dependent manner. The results of this study show that mistletoe extracts could be effective against intestinal motility problems and that they have potential use as a therapeutic remedy for intestinal colic.

**Key Words:** Medicinal plant, colic, mistletoe, Viscum album subsp. album, organ bath

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**Introduction**

Herbs have been used for the treatment of various disorders throughout recorded human history. Among the disorders frequently treated with herbal drugs is intestinal colic, a common complaint that causes 1 in 6 families to consult a health professional (1). The incidence of infantile colic varies between 5% and 19% (2). Several drugs, such as simethicone, cimetropium, and anticholinergics, have been promoted as agents for decreasing intraluminal gas or colic episodes. Some of these drugs were either demonstrated to have some adverse effects, such as drowsiness and apnea (3), or to be no more effective than placebos (4). On the other hand, herbal teas containing mixtures of chamomile, vervain, licorice, fennel, and lemon balm used up to 3 times per day (150 ml per dose) have been shown to decrease crying in infants with colic (5,6).

Mistletoe is a common name for many species of semi-parasitic plants that grow on trees throughout the world (7). In Turkey, the genus *Viscum* (Loranthaceae) is represented by 1 species (*V. album* L.) and its 3 subspecies,
namely subsp. *album*, subsp. *abietis* (Wiesb.) Abromeit, and subsp. *austriacum* (Wiesb.) Vollmann (8,9). *V. album* has been reported to possess a number of therapeutic uses for curing or managing a wide range of diseases, such as diabetes mellitus, chronic cramps, stroke, stomach problems, heart palpitations, high blood pressure, breathing difficulties, and menopausal hot flashes, according to many folk medicine traditions (10). Hypotensive, vasodilator, cardiac depressive, sedative, antispasmodic, anticancer, and antidiabetic effects of *V. album* have been demonstrated in previous studies (7,11-14). In Turkey *V. album* has been used to treat hypertension in the eastern and southern parts of Anatolia (15), and to reduce blood sugar level in diabetics by the inhabitants of Beypazarı (Ankara, Turkey) (16).

Given the multitude of herbal products, the lack of standardization of strength and dosage, and potential interference with normal feeding, parents should be cautioned about their use. There continues to be a high demand for new antispasmodic drugs because the management of intestinal colic without side effects is still not possible with contemporary medicines; therefore, a selective search based on traditional knowledge would be more productive and certainly more economical. The present study investigated whether different doses of mistletoe extracts influence isolated intestinal contractility and compared the effects of heat-treated and freeze-dried extracts. Specifically, the aim of the study was to determine the pharmacological properties of *V. album* extracts on smooth muscle in order to understand their dilatational mechanism and to scientifically validate its popular use for the treatment of intestinal colic.

Materials and Methods

Plant Material

European mistletoe growing on oak trees (*Quercus pubescens* Wild.) was collected in April 2005 and identified according to *Flora of Turkey and the East Aegean Islands* (9). A voucher specimen (collection number AUT-2001) was deposited at the Herbarium of the Faculty of Arts and Sciences of Abant İzzet Baysal University, Bolu, for future reference.

Preparation of the Mistletoe Extracts

Collected European mistletoe leaves were dried in an oven at 40 °C and then ground into a powder. Two different preparations were made (freeze-dried aqueous extract for group 1) and heat-treated aqueous extract (for group 2)).

Heat-Treated Aqueous Extract Preparation

Thirty grams of mistletoe leaves were extracted 2 times with 200 ml of water at 80 °C in a water bath for 4 h and then filtered. Water was evaporated under vacuum (90-100 °C). The residue was then dissolved in saline (0.9% NaCl) to produce a final concentration of 100 mg/ml. The yield of extract (w/w) was 33.3%.

Freeze-Dried Aqueous Extract Preparation

Thirty grams of mistletoe leaves with 200 ml of cold water were incubated for 24 h at room temperature and then filtered. Frozen filtrate was lyophilized using a freeze-dryer at –65 °C. The residue was then dissolved in saline (0.9% NaCl) to produce a final concentration of 100 mg/ml. The yield of extract was 20.1%.

In order to investigate the dilatation effects of mistletoe, different concentrations (20, 60, 180, 360, 540, and 600 μg/ml) of both heat-treated and freeze-dried extracts were prepared from the final concentrations. These preparations, therefore, allowed us to compare how concentration affected the percentage of dilation by comparing the results with a control preparation (0 μg/ml).

Animals and the Organ Bath Procedure

Twenty adult male Syrian hamsters (~ 110 g, 120 days of age) were obtained from our laboratory colony maintained at Abant İzzet Baysal University. The procedures used in this study were carried out in accordance with the Animal Scientific Procedure Act of 1986 and were approved by the Institutional Animal Care and Use Committee. The hamsters were killed by cervical dislocation and 2-3-cm cross-sectional intestinal segments were isolated and placed immediately into cold (4 °C) Krebs solution of the following composition (mM): NaCl, 119; KCl, 4.75; KH₂PO₄, 1.2; NaHCO₃, 25; CaCl₂, 2.5; MgSO₄, 1.5; glucose, 11. The cross-sectional intestinal segments were trimmed free of adhering fat and connective tissues while in a petri dish filled with cold Krebs solution. Each isolated ring was suspended between 2 stainless steel L-shaped hooks (circular placement) and mounted in a 25-ml organ bath (Leticia Scientific Instruments, Panlab SL, Cornellà, Barcelona, Spain) filled with Krebs solution, maintained at 37 °C, and continuously aerated with a 95% O₂ and 5% CO₂ gas mixture. One hook was fixed to a
micrometric manipulator, allowing for adjustments in resting tension of the rings, and the other was connected to a force displacement transducer for the measurement of isometric force. Responses were recorded continuously with a computer-controlled polygraph system (ADInstruments, PowerLab Data Acquisition Systems, UK). All of the rings were allowed to equilibrate for 45-60 min at their optimum resting tension of 1 g and the bath solution was changed every 15 min. After stable control contractions evoked by acetylcholine (50 nmol/l) were recorded, responses were observed in the presence of increasing concentrations (20, 60, 180, 360, 540, and 600 μg/ml) of both heat-treated and freeze-dried mistletoe extracts. All fresh tissues were used on the preparation day. The extracts were added directly into each of the organ chambers in volumes not exceeding 1% of the total bath volume.

Statistical Analysis

Data were analyzed using SPSS v.11.0. Statistical differences between groups were examined by one-way ANOVA, followed by Student’s t-test. Significance was set at P < 0.05. Data are presented as mean ± SEM.

Results

In group 1, freeze-dried extracts were applied to duodenal (1A), jejunal (1B), and ileal (1C) segments of 10 Syrian hamsters. Freeze-dried mistletoe extracts decreased intestinal contractions in all of the intestinal segments. The strongest effect was observed in group 1B and the weakest effect was seen in group 1A. Figure 1 shows the percentage of contraction decrease in the 3 segments according to concentration. In group 1B final concentrations of freeze-dried extracts > 180 μg/ml reduced jejunal contractions significantly (P < 0.05), whereas lower concentrations did not significantly affect the contractions (P > 0.05). In group 1A and 1C, the results were similar to those of group 1B, but the level of decrease in contractions was not as pronounced as in group 1B.

In group 2, heat-treated extracts were applied to duodenal (2A), jejunal (2B), and ileal (2C) segments of 10 Syrian hamsters. The applied concentrations of the extracts were the same as in group 1. Figure 2 shows the decrease in the percentage of contractions according to concentration of extracts. Heat-treated extracts required higher concentrations to affect segmental contractions than freeze-dried extracts; the greatest decrease in contractions was again in the jejunum and was least in the duodenum. In group 2B final concentrations of heat-treated extracts > 500 μg/ml reduced jejunal contractions significantly (P <
Discussion

This study was performed in order to provide a scientific basis for the folk medicinal use of V. album for the treatment of intestinal disorders. Extracts of V. album leaves were tested in vitro using isolated segments of hamster duodenum, jejunum, and ileum. These extracts affected the contraction level in a concentration- and extraction preparation-dependent manner. As the concentration of both extracts (freeze-dried and heat-treat) increased, the contractions in the intestinal segments decreased.

The results show that European mistletoe was able to reduce acetylcholine-induced contractions, which are due to direct activation of muscarinic receptors located on smooth muscles. The ability of mistletoe to reduce the contractions induced by exogenous acetylcholine indicates a direct antispasmodic effect.

Mistletoe contains lectins, viscosotoxins, alkaloids, polysaccharides, flavonoids, phenyl propane, phenol carboxylic acid, and glucosides (17-22); however, lectins play the most active role in its cytotoxicity (23) and immunomodulation (24). We cannot, based on the present results, offer any explanation as to why the freeze-dried extract was more effective than the heat-treated extract. On the other hand, it might have been because the high cytotoxicity of isolated lectin completely disappeared after heating for 30 min. In addition to lectins, flavonoids, phenol carboxylic acid, and phenyl propane may have lost their effect after heat extraction. Viscotoxins are less cytotoxic, but more resistant to heat degradation when compared to lectins (25). Data from the present study show that higher concentrations of heat-treated V. album were necessary to reduce contraction in isolated intestinal segments. The dilatation effect of freeze-dried extracts started at the 180 μg/ml concentration versus 540 μg/ml for the heat-treated extracts. Therefore, lectins in the freeze-dried preparations were more effective on muscarinic receptors in inhibiting contractions, but were ineffective in the heat-treated preparations. The cytotoxic effects of viscosotoxins might be the effective component of the relaxing capability of the mistletoe extracts. The difference in responses to heat-treated and freeze-dried extracts is consistent with the results of our previous study (26). In that study, to test the hypothesis that the non-antineoplastic effects of mistletoe might be mediated by the circadian timing system, we applied mistletoe extracts and vehicle, and measured locomotor activity, feeding, and
drinking rhythms under constant darkness. Four groups (vehicle, 20 mg/kg injection, 40 mg/kg injection, and 6 g/kg oral administration) were tested with both heat-treated and freeze-dried extracts. None of the treatments changed locomotor activity, or feeding and drinking rhythms in the groups, except for the 40 mg/kg freeze-dried injection treatment (26).

In a recent study Radenkovic et al. (27) reported that mistletoe extracts increased intestinal contractility in rats. Their hypothesis was that the intestinal contractility increasing effect of *V. album* was due to effects on the muscarinic receptors found in the digestive tract (27). It is well known that muscarinic receptors are widely distributed in the digestive tract. On the other hand, Ergun et al. (28) demonstrated that an alcoholic extract of *V. album* produced marked relaxation in the rat aorta. Another study conducted by Tenorio et al. (29) confirmed that the aqueous extract of *V. album* leaves, at a dose of 0.8 mg/kg, had significant vasodilator activity on the Langendorff’s isolated and perfused guinea pig heart model. This vasodilator activity was also explained by the stimulation of muscarinic receptors (30). Therefore, it is an unexpected result to observe contraction in the intestinal segments with mistletoe extracts rather than relaxation (27). Our results are consistent with the results reported by Cook and Samman (31), Middleton (32), Deliorman et al. (30), and Tenorio et al. (29), but contradict the results published by Radenkovic et al. (27).

Radenkovic et al. (27) reported that mistletoe extracts had biphasic effects on the rat duodenum, short-lasting relaxation of tonus followed by long-lasting increase in tonus, and that mistletoe leaf extracts exhibited non-specific effects in the ileum. Their results are not sufficient to suggest that mistletoe leaf extracts increased the tonus of the intestines. In contrast, our results are sufficient to suggest relaxation of the intestine. There may be several explanations for the different responses of the intestines: 1) In their experiment, they used segments of the duodenum, ileum, and colon isolated from rats, whereas we used segments from the duodenum, jejunum, and ileum isolated from hamsters; 2) In their experiment they prepared longitudinal segments, whereas we used cross-sectional intestinal segments. Although the effect of acetylcholine was the same in both longitudinal and cross-sectional intestinal segments, there may be some differences when they were exposed to mistletoe extracts; 3) In their experiment, they used extracts prepared by methanol, whereas we examined the effect of 2 aqueous extracts of mistletoe–1 extracted with hot water, followed by evaporation under vacuum, the second extracted with cold water and lyophilization. The lyophilized product more effectively produced relaxation of isolated segments of various intestinal tissues than the hot extracts. Both products had an effect profile in the following order: jejunum > ileum > duodenum; 4) In their experiment, they used Tyrode solution, whereas we used Krebs solution.

Variation of the responses to the extracts in the different intestinal segments might have been related to differences in muscarinic receptor content. It has been shown that different intestinal segments contain varying quantities of muscarinic receptors (33). The differences might also have been due to denaturation of lectins in response to the heat applied during preparation. The present study did not identify the constituent in *V. album* responsible for the contractility reducing effect on intestinal segments. Determination of the exact mechanism involved was not the purpose of the present study. As such, we used impure extracts in our experiments. Supplementary studies are necessary in order to elucidate the mechanism of action of the different chemical components of *V. album* after being treated with heat and cold. This will aid in the understanding of the curative properties of this plant. Finally, we would like to add that in the light of the present study’s findings, *V. album* L. subsp. *album* could be considered effective against intestinal motility problems and that it has potential for use as a therapeutic remedy for intestinal colic.

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**References**

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8. Baytop T. Therapy with medicinal plants in Turkey (Past and Present). Publications of İstanbul University, 1984; No. 3255, Faculty of Pharmacy No. 40 İstanbul p. 203.


