ANTIEMETIC ACTIVITY OF DIFFERENT EXTRACTS FROM THE AERIAL PARTS OF *Thymus transcaspicus* KLOKOV

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Summary

The antiemetic effects of aqueous, methanolic and petroleum ether extracts of the aerial parts of *Thymus transcaspicus* Klokov in young chickens were studied. Emesis was induced by copper sulfate (60 mg/kg, orally) and ipecac (600 mg/kg, orally). The extracts were injected intraperitoneally (i.p.) at doses 600 and 1300 mg/kg. As a positive control, granisetron (0.1 and 0.4 mg/kg, i.p.) was used. Aqueous extract did not significantly reduce ipecac- or copper sulfate-induced emesis at any dose [percentages of retching inhibition: 23.6 % (increase in retching reflexes) and 1.9 % against ipecac for doses 600 and 1300 mg/kg, respectively and 141.5 % (increase in retching reflexes) and 13.3 % against copper sulfate, respectively]. Methanolic extract showed a significant antiemetic activity against ipecac- and copper sulfate-induced emesis at both doses ($p < 0.05$; percentages of retching inhibition: 44.5 % and 47.0 % against ipecac for doses 600 and 1300 mg/kg, respectively and 49.7 % and 59.7 % against copper sulfate, respectively). Petroleum ether extract showed an interesting and significant antiemetic activity against ipecac- and copper sulfate-induced emesis at both doses ($p < 0.001$; percentages of retching inhibition: 74.0 % and 88.0 % against ipecac for doses 600 and 1300 mg/kg, respectively and 69.0 % and 77.3 % against copper sulfate, respectively). These results indicate a moderate antiemetic activity for the methanolic extract and a strong activity for the petroleum ether extract of *T. transcaspicus* aerial parts in young chickens which is due to the peripheral and central mechanisms.

Keywords: Ipecac-induced emesis, copper sulfate-induced emesis, young chicken, petroleum ether extract, medicinal plant.

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Introduction

Thymus comprises 14 species, all of which are small perennial plants that are found wild in many regions of Iran (1,2). *Thymus* species as well as many other aromatic plants biosynthesize remarkable amount of volatile compounds such as thymol and carvacrol for which many biological activities have been reported such as antimicrobial (3,4), anti-inflammatory (5), anti-leishmanial (6), antioxidant (7), hepatoprotective (8) and anti-tumoral effects (9, 10). Terpenoids, flavonoid aglycones, flavonoid glycosides and phenolic acids were also found in *Thymus* spp. (11). In Iranian folk medicine, some *Thymus* spp. are used as an anthelmintic, antispasmodic, carminative, sedative, diaphoretic, usually in the form of an infusion or used externally in baths to cure rheumatic and skin disease (12). In some countries, these plants have also been used as a folk medicine against asthma, arteriosclerosis, colic, bronchitis, coughs, diarrhea, and rheumatism (13). On the other hand, according to the modern investigations, it has been revealed that *Thymus* spp. possess numerous biological activities including antispasmodic (14), antimicrobial (15), antioxidant (16) and antifungal (17) activities. *T. transcaspicus* is an aromatic and medicinal plant, which has been widely distributed in the north of Khorasan Razavi province, Iran, and southren areas of Turkemanistan as reported by Rechinger (2). According to a previous report, thymol, carvacrol, γ-terpinene and *p*-cymene constitute the major components of the oil of the aerial parts of this plant (18).

As a natural remedy, it has been observed that the infusion of *T. vulgaris* is effective against nausea (19). However, a bibliographical survey showed that there is no report on the antiemetic activity of other *Thymus* species. Therefore, in the present study, we aimed to investigate the antiemetic activities of aqueous, methanolic and petroleum ether extracts obtained from the aerial parts of *T. transcaspicus* klokov against ipecac- and copper sulfate-induced emesis in young chickens.

Materials and Methods

Animals

Male and female young chickens (45-50 g; 3-5 days old) were obtained from a poultry local store. The animals were housed in standard stainless steel cages in a temperature controlled room (24 ± 2°C) with a 12-12 hr light-dark cycle. After 24 h fasting, the antiemetic activity was evaluated. All animal experiments were carried out in accordance with the guidelines for the care and use of laboratory animals approved by the Japanese Pharmacological Society and acts of the Ethical Committee of Mashhad University of Medical Sciences.

Plant material

The aerial parts of *Thymus transcaspicus* were collected from Tandoureh national park (Dargaz, Khorasan province, Northeast of Iran) and dried in shadow and ground. A voucher specimen (No. 021-1622-35) was authenticated and deposited in the Herbarium of of Department of Pharmacognosy, School of Pharmacy, Mashhad.

Preparation of extracts

Dried powdered aerial parts (500 g) of the plant were extracted with petroleum ether (1200 cc) by maceration for 48 hours. The solvent was then filtered and evaporated to dryness under reduced pressure at 40 °C. The remained powder was then dried and extracted with methanol the same as above. The left powder was also dried and extracted with water. Finally, three different extracts were obtained. The yield values for aquous, methanolic and pethroleum dried extracts were 18.0, 17.5 and 19.0 g, respectively.

Acute toxicity test

Different doses of the extracts were injected to the separated groups of four young chickens. After 24 h, all of the mortalities were recorded.

Treatment

Young chickens were divided into 20 groups of 6 each. The antiemetic activity was evaluated using the method explained by Yang et al, with different emetic agents (20). The emesis was induced with copper sulfate, 60 mg/kg (0.5 cc, orally) or ipecac, 600 mg/kg (1 cc, orally) in young chickens. Two doses of each extract (600 and 1300 mg/kg), two doses of positive control (granisetron; 0.1 and 0.4 mg/kg) and negative control [normal saline for aqueous extract and dimethyl sulfoxide (DMSO) for methanolic and petroleum ether extracts] were administered to the chickens, i.p. at a volume of 0.2 ml, one hour prior to treatment with the emetic agents. The retching frequency was recorded for copper sulfate and ipecac 60 and 20 minutes after their treatments, respectively.

Statistical analysis

The data were expressed as mean values ± S.E.M. Analysis of variance followed by the multiple comparison test of Tukey-Kramer was used to compare data.

Results

Acute intoxication

To evaluate the acute toxicity and the maximum non-lethal doses of extracts, increasing doses of the three extracts were administered to young chickens beginning from 500 mg/kg. Up to 1900 mg/kg, no mortality was observed among chickens treated with any of the extracts. Doses higher than 1900 mg/kg were not administered to the chickens because at these doses the mortality can not be exclusively attributed to the toxicity of extracts and might be due to the changes in the electrolyte concentrations and osmotic pressure of biological fluids. Furthermore, at high concentrations, preparation of a homogenous and injectable solution of extracts would be very difficult because of the decreased solubility of extracts in solvents.

Antiemetic activity of aqueous extract

The administered doses of aqueous extract couldn't significantly reduce the emesis in chickens, either induced by ipecac or copper sulfate (Fig. 1 and Fig. 2). The percentages of retching inhibition, induced by ipecac were – 23.6 % and 1.9 % for doses 600 and 1300 mg/kg, respectively and induced by copper sulfate were – 141.5 % and 13.3 %, respectively.

Antiemetic activity of methanolic extract

The administered doses of methanolic extract could significantly reduce the emesis in chickens, either induced by ipecac or copper sulfate (Fig. 3 and Fig. 4). The percentages of retching inhibition, induced by ipecac were 44.5 % and 47.0 % for doses 600 and 1300 mg/kg, respectively and induced by copper sulfate were 49.7 % and 59.7 %, respectively.

Antiemetic activity of petroleum ether extract

The administered doses of petroleum ether extract could significantly reduce the emesis in chickens, either induced by ipecac or copper sulfate (Fig. 5 and Fig. 6). The percentages of retching inhibition, induced by ipecac were 74.0 % and 88.0 % for doses 600 and 1300 mg/kg, respectively and induced by copper sulfate were 69.0 % and 77.3 %, respectively.

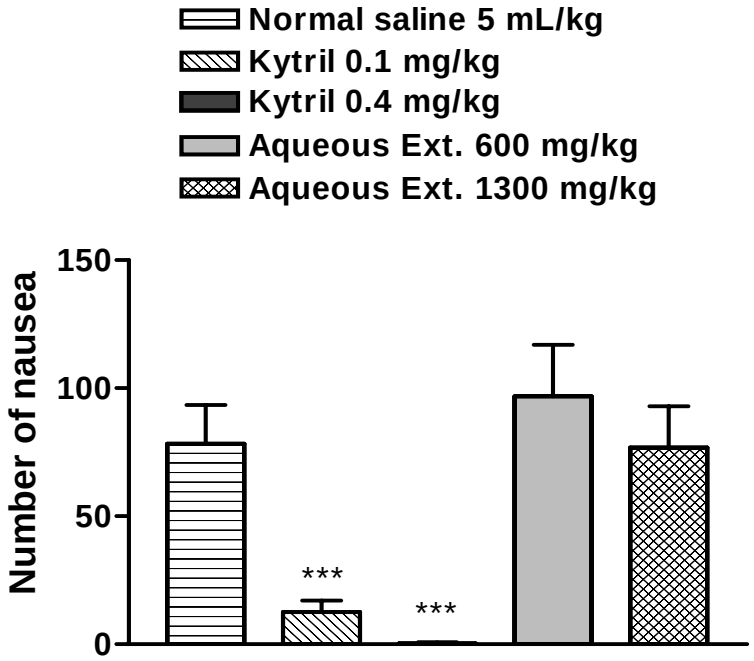

Fig. 1. The antiemetic effect of the aqueous extract of the of *T. transcaspicus* aerial parts and granisetron against ipecac (600 mg/kg, orally) induced emesis in young chickens.

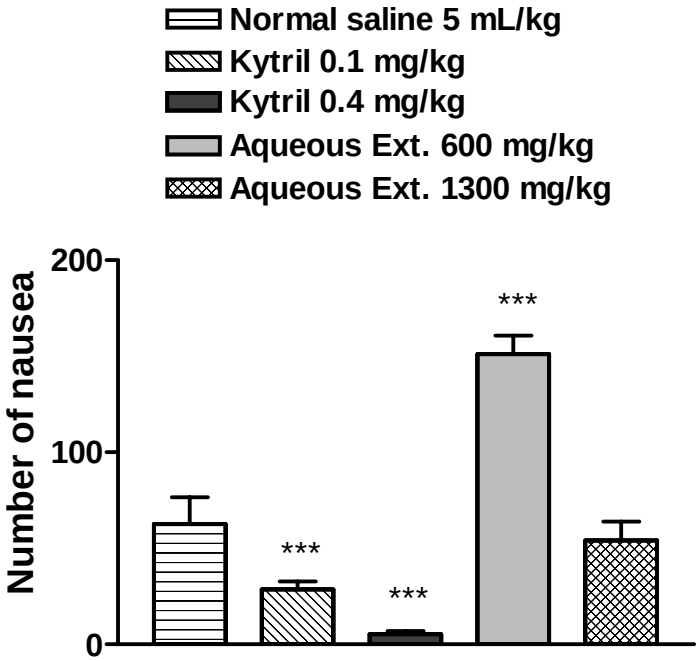

Fig. 2. The antiemetic effect of the aqueous extract of the of *T. transcaspicus* aerial parts and granisetron against copper sulfate (60 mg/kg, orally) induced emesis in young chickens.

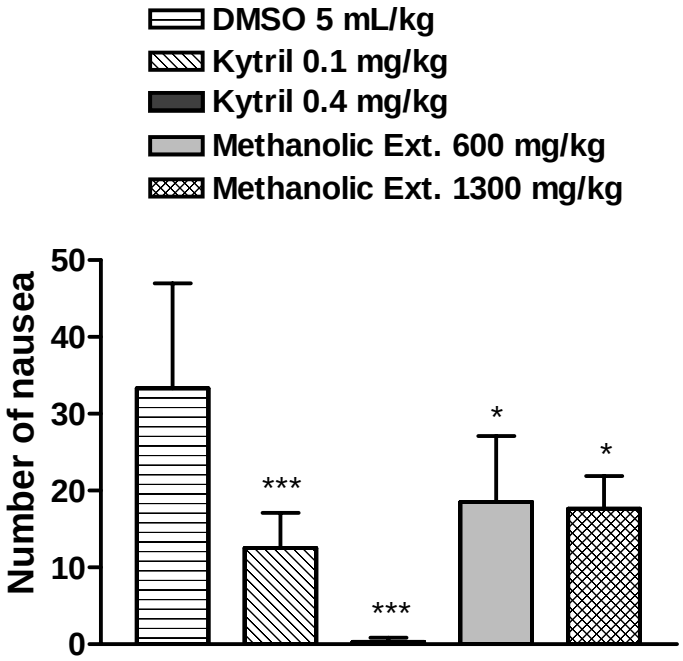

Fig. 3. The antiemetic effect of the methanolic extract of the of *T. transcaspicus* aerial parts and granisetron against ipecac (600 mg/kg, orally) induced emesis in young chickens.

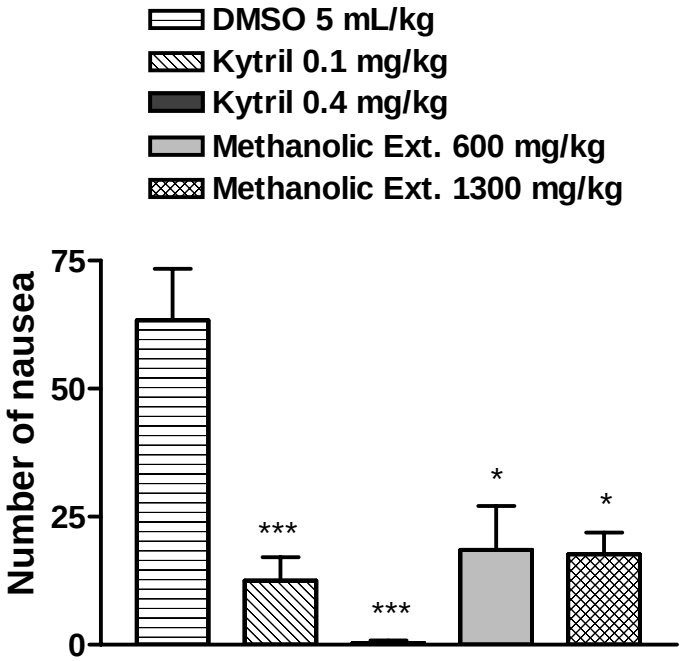

Fig. 4. The antiemetic effect of the methanolic extract of the of *T. transcaspicus* aerial parts and granisetron against copper sulfate (60 mg/kg, orally) induced emesis in young chickens.

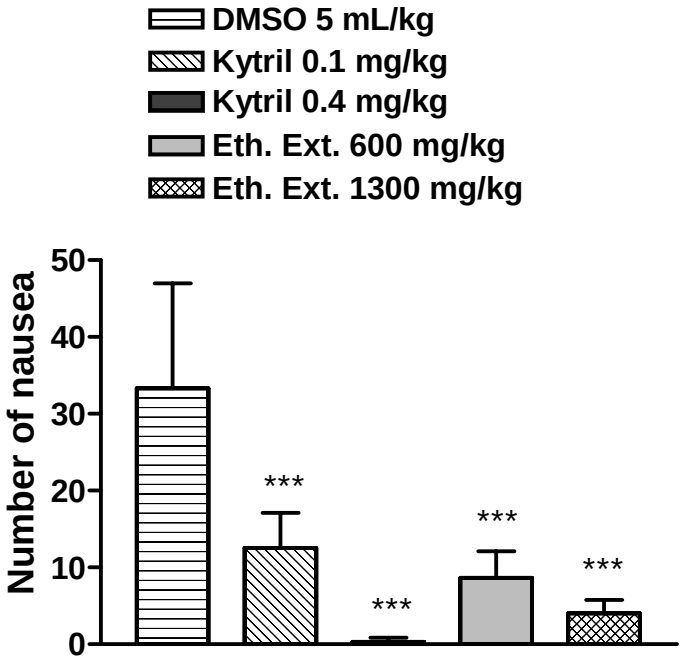

Fig. 5. The antiemetic effect of the petroleum ether extract of the of *T. transcaspicus* aerial parts and granisetron against ipecac (600 mg/kg, orally) induced emesis in young chickens.

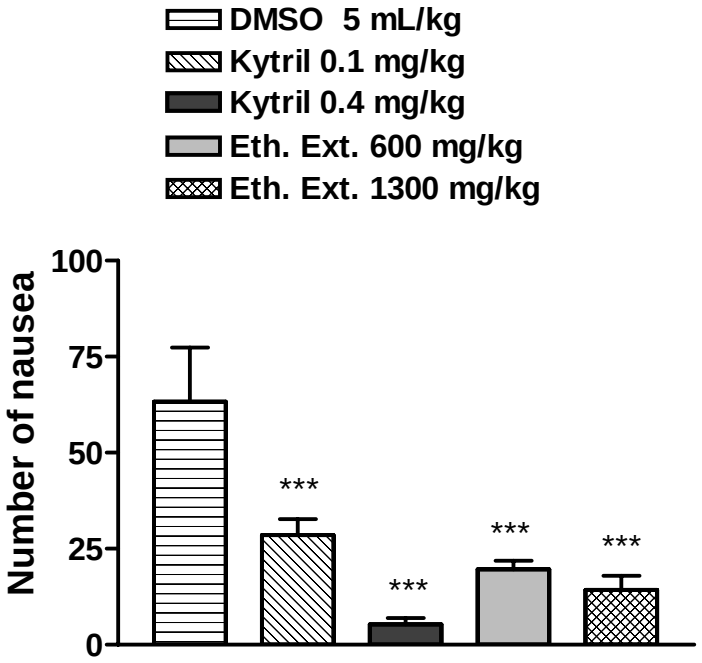

Fig. 6. The antiemetic effect of the petroleum ether extract of the of *T. transcaspicus* aerial parts and granisetron against copper sulfate (60 mg/kg, orally) induced emesis in young chickens.

Discussion

According to the findings of the current study, methanolic extract and particularly petroleum ether extract of *T. transcaspicus* aerial parts have protective effects against ipecac- and copper sulfate-induced emesis in young chickens.

Among the three extracts tested in the present study, siginificant antiemetic activity was observed with methanolic and petroleum ether extracts, being higher in the latter. Therefore, it could be implied that the antiemetic activity is dependent to the polarity of extracts. Besides, considering the increase in antiemetic activity with increasing dose (from 600 to 1300 mg/kg), the antiemetic activity of methanolic and petroleum ether extracts against ipecac and copper sulfate was dose dependent.

The actions of ipecac are mainly those of its major alkaloids, emetine (methylcephalin) and cephalin. They both act peripherally by irritating the gastric mucosa and centrally by stimulating the medullary chemoreceptor trigger zone (CTZ) to induce vomiting (21).

Due to the significant prevention of ipecac-induced emesis, it is plausible that methanolic and petroleum extracts act centrally (on CTZ) as well as peripherally (on gastric mucosa) to exert their antiemetic activities. Moreover, methanolic and petroleum extracts could also effectively prevent emesis induced by oral copper sulfate which indicates the peripheral antiemetic activity of these extracts, because the mechanism of emesis induction by copper sulfate is mainly peripheral (22). The peripheral antiemetic activity may be due to the chelation of copper sulfate by chelating compounds present in the extract (e.g. tannins, alkaloids and other negatively charged compounds) or the action of extract as a physical barrier which decreases the effect of copper sulfate on gastric mucosa.

The direct relationship between antiemetic activity and polarity of extracts is probably indicative of the non-polar nature of active constituents in the extracts which are responsible for antiemetic activity because the aqueous extract – in which non-polar compounds are supposed to be absent or occur only in very low concentrations – had almost no effect. On the other hand, petroleum ether extract which was the most non-polar of all three extracts – and probably contained the most concentration of non-polar compounds – showed a very promising antiemetic activity to the extent that it could even precede the positive control (granisetron) at dose 0.1 mg/kg, both in the ipecac- as well as copper sulfate-induced emesis.

According to the previous reports, the major components of the aerial parts of *Thymus* species including *T. transcaspicus* are phenolic compounds, especially thymol and carvacrol (13,18,23,24) for those many effects have been reported (3-10) including antiemetic activity (25).

In summary, the findings of the present study suggest that petroleum ether extract of aerial parts of *T. transcaspicus* has strong and dose dependent protective effect against both ipecac- and copper sulfate-induced emesis which is probably due to the central and peripheral mechanisms. Future investigations can be focused on the isolation and structure elucidation of bioactive compounds of the petroleum ether extract.

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