Anti-inflammatory Effect of *Thymus kotschyanus* Boiss. & Hohen. Extract on Rat's Hind Paw Edema Induced by Carrageenan

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Abstract

Background: Advantages of herbal drugs have been known to human being for many years and they have been used for treatment of many diseases. Nowadays, inflammation is known to be one of the basic pathologic causes of the diseases.

Objective: In this study the anti-inflammatory effects of *Thymus kotschyanus* Boiss & Hohen was investigated.

Methods: Forty two Wistar rats were used into three divided groups. In the first group, extract of *Thymus kotschyanus* Boiss & Hohen in doses of 50,100,150,200 mg/kg was injected intra peritoneal and after one hour they were treated with hind paw edema test with carrageenan. In the second group, Indomethacin with the dose of 5 mg/kg was injected one hour before carrageenan and in the third one, saline was injected before carrageenan, every hours after carrageenan injection to rat's right hind paw; the inflammation was separately measured by the change in the volume of mercury (plethysmometer).

Results: Indomethacin and *Thymus kotschyanus* Boiss & Hohen extract had significantly reduced edema in all five measurements ($p<0.001$). There was no significant difference between Indometacin and the extract in reducing edema in the first to fourth hour, but in the fifth hour, the herbal drug showed a significant difference of anti-inflammatory effect with Indometacin in all doses ($p<0.05$).

Conclusion: The methanolic extract of *Thymus kotschyanus* Boiss & Hohen has anti-inflammatory effect in all doses which is dose dependant and can be used instead of or as a synergist for Indometacin. Higher doses of herbal extract may have more anti-inflammatory effects.

Keywords: Anti-inflammatory agent, *Thymus kotschyanus* Boiss & Hohen, Hind paw edema test, Carrageenan

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Introduction

The genus *Thymus* is divided into about 200 species which are among the well-known medicinal plants and are distributed through Mediterranean regions [1, 2, 3]. Fourteen species of *Thymus* are introduced as Iranian flora and among these, four are native of Iran. Different species of *Thymus* are different in content and type of components. Generally they contain thymol and carvacrol as main essential oil constituents, phenolic compounds such as rosmarinic acid which may have antiedemic and macrophage-inhibiting effects, and flavonoids [2-8]. In the recent centuries, many studies have been carried out to discover therapeutic effects of these components. On the other hand, increasing usage of anti-inflammatory and anti-microbial drugs for different causes (including inflammation which in known to be one of the pathologic basis of many diseases) and considering many side effects caused by these drugs, have necessitated more consideration to herbal drugs with equal effects [3]. Some of them have been in use as traditional medicines for centuries [9] and are known to have different therapeutic effects: anti-dyspepsia, anti-bacterial [9 - 13], anti-hypertension [14], anti-spasmodic and sedative [15], diuretic, treatment of pediatric enuresis and anti-acne [16-18]. Among different *Thymus* species, *Thymus vulgaris* is cultivated in many countries and is used more in pharmaceutical dosage forms because it contains more essential oil than other species with high amount of thymol which exhibits considerable antitussive and expectorant effects [19].

Considering the fact that *T. vulgaris* is less available and more expensive, some herbal industries tend to use other species of *Thymus* with different components [4]. One of the native species of Iran is *Thymus kotschyanus* Boiss & Hohen which has been experimented for anti-bacterial and anti-fungal activity, its chemical composition [2 - 4, 9, 20, 21], anti-arthralgia, reducing cough and asthmatic attacks [22, 23], and anti-depression effect [24], but there is no survey on its anti-inflammatory effect in the literature. As a result, the anti-inflammatory effect of *T. kotschyanus*, which seems to be necessary, was investigated in this study.

Materials and Methods

Animals

In this study, experiments were performed on Wistar male rats weighting 200-250 gr who have been bred and housed in animal house at the temperature of 21±2 and day/night cycle of 12/12 hours.

Drugs

Carrageenan was obtained from Sigma (USA) and dissolved in deionized water, Indometacin from Darou pakhsh company (Iran), respectively.

Plant material

Aerial parts of *T. Kotschyanus* were collected from Yazd, province of Yazd, Iran, in June 2009, during the flowering stage by Mr. Ajani. Voucher specimen (number TEH-6677) has been deposited at the Herbarium of the Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

Preparation of plant extract

Ground dried aerial parts of the plant (200 g) were perculated in MeOH–H_2O (80: 20) at room temperature for 2 days. The extraction procedure was repeated for 3 times on the plant material. The extracts were dried under vacuum and the residue was dissolved in normal saline to be injectable.

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Methods

In the first group, 24 rats (6 rats in each subgroup) were injected by 0.1 ml of carrageenan solution 1% in hind paw. This procedure was performed after one hour from intraperitoneal injection of 4 doses of *T. kotschyanus*, 50 – 100 – 150 - 200 mg/kg, and anti-inflammatory effect was measured by displacement of mercury. In the second group, Indometacin 5 mg/kg was injected one hour before carrageenan and in the third group only carrageenan was performed. All the assessments were performed by the same investigator in order to reduce any potential inter-operator differences.

Statistical analysis

The data were analyzed by ANOVA test and General linear model tests (Wilks' Lambda and Pillai's Trace).

Results

In the first hour to fifth hour after carrageenan injection, Indometacin and all doses of the herbal drug showed significant anti-inflammatory effect ($p<0.001$) (Figures 1 to 5). In the fifth hour, the herbal extract in dose of 50mg/kg had significant anti-inflammatory effect compared to Indometacin ($p<0.05$). In dose of 100mg/kg there was no difference with Indometacin. The dose of 150mg/kg had more anti-inflammatory effect in comparison with Indometacin ($p<0.05$). For the dose of 200mg/kg, the difference with Indometacin was significant as well ($p<0.01$) (figure 5). Increasing the dose of drug resulted in more anti- inflammatory effect ($p<0.05$).

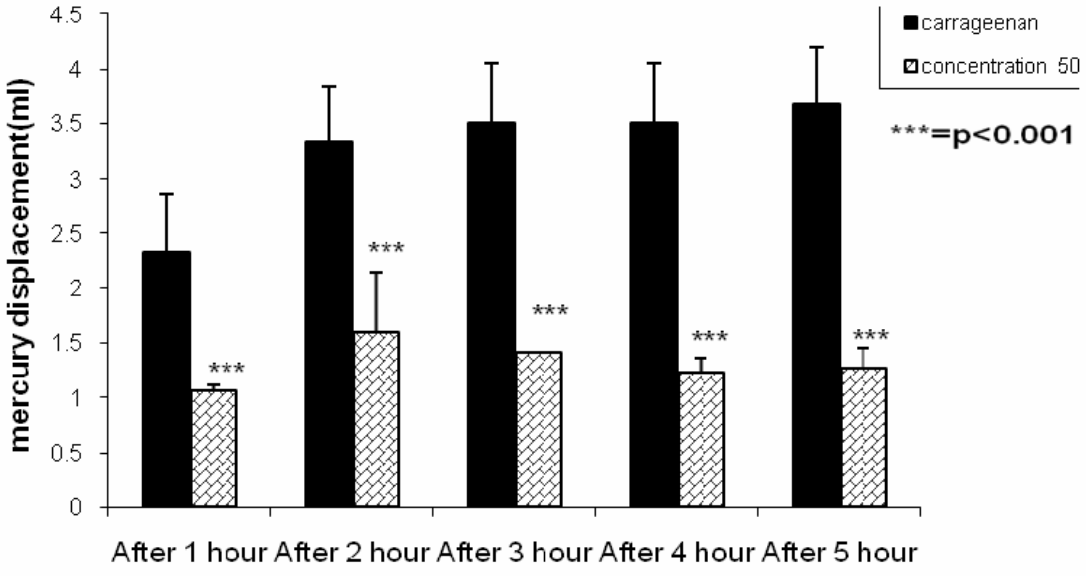

Fig.1- Anti- inflammatory effect of *Thymus kotschyanus* Boiss & Hohen in dose of 50 mg/kg after carrageenan injection

SD±Results are shown as Mean

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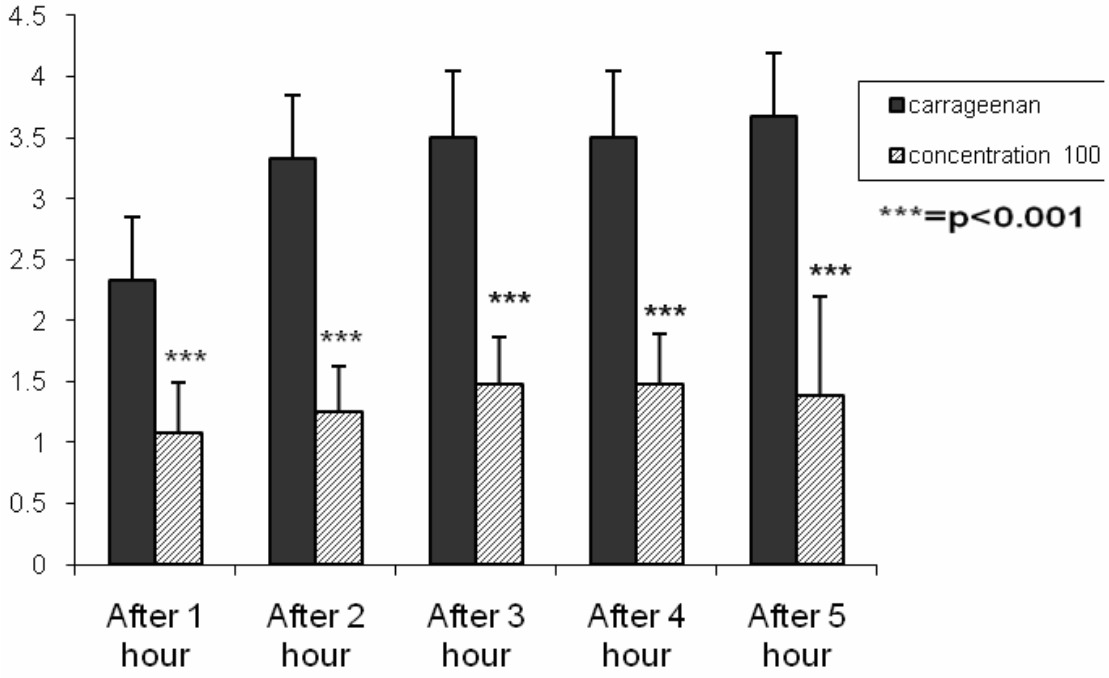

Fig. 2- Anti- inflammatory effect of *Thymus kotschyanus* Boiss & Hohen in dose of 100mg/kg after carrageenan injection

SD±Results are shown as Mean

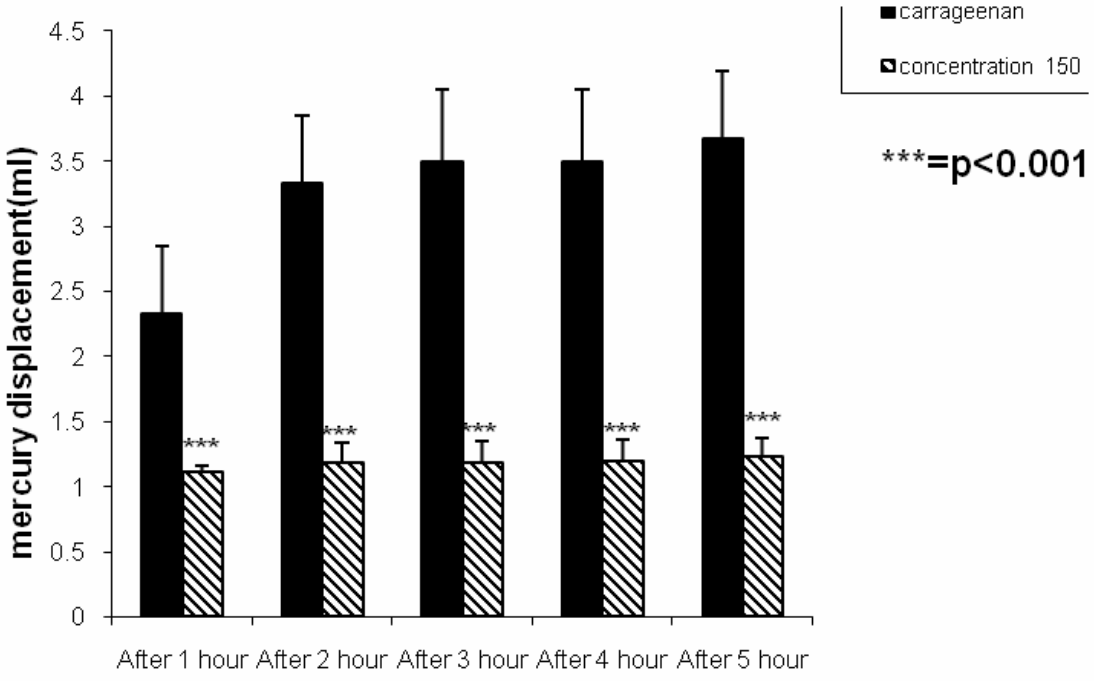

Fig. 3- Anti- inflammatory effect of *Thymus kotschyanus* Boiss & Hohen in dose of 150mg/kg after carrageenan injection

SD±Results are shown as Mean

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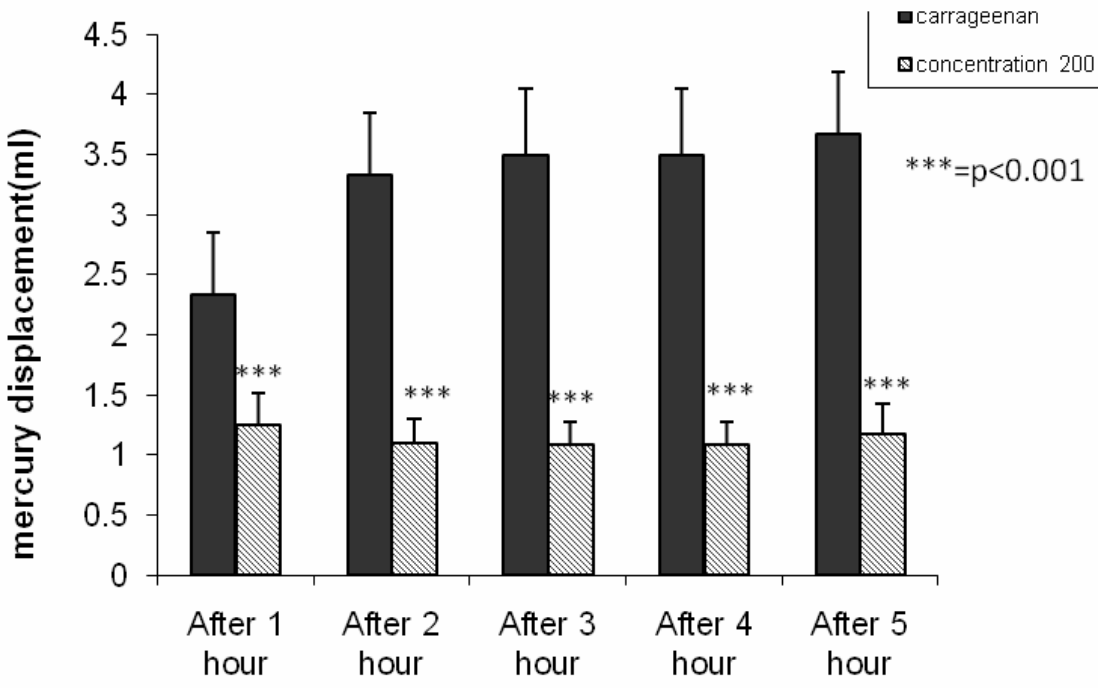

Fig. 4- Anti- inflammatory effect of *Thymus kotschyanus* Boiss & Hohen in dose of 200mg/kg after carrageenan injection

Results are shown as Mean ±SD

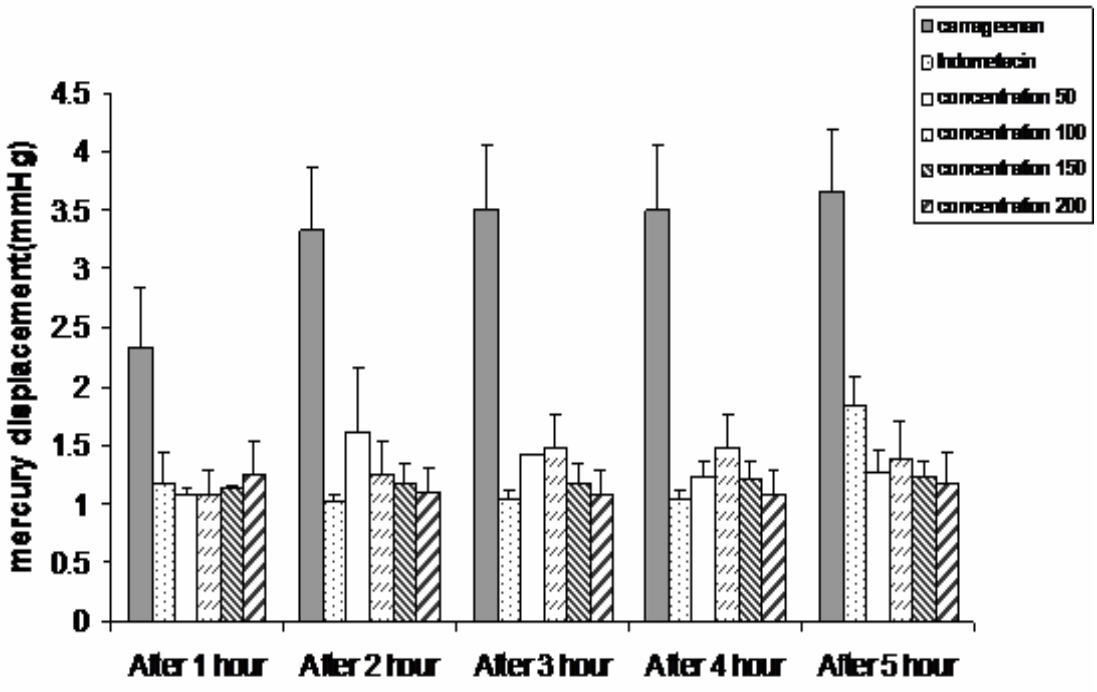

Fig. 5 - Anti-inflammatory effect of Indometacin and *Thymus kotschyanus* Boiss & Hohen in 4 doses after carrageenan injection

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Discussion

Inflammation has many clinical features like redness, hyperthermia, pain and edema. Edema is an important sign of inflammation. There are several mechanisms introduced in the literature for that, but still it's not well known. It is said that bradykinin, histamine, serotonin and specially prostaglandins are most important pharmacologic mediators in the process of inflammation [25, 26]. One of the models to assess edema induced by carrageenan in rat's paw is said to be maximum by 3 [27] to 6 [25, 26, 28] hours. Although there are other models like skin test for evaluating edema, rat's hind paw edema is more common because of its potency for re-evaluation, lower costs ,easy and available methods of measuring, and for these reasons it is used to introduce Indometacin as well [25]. The most common way to measure this method is plethysmometer (displacement of mercury). Inflammation is known to be a major cause of many diseases. For this reason and many side effects of non-herbal drugs, it seems to be necessary to do more researches on anti-inflammatory effect of plants [25]. One of these plants which is introduced as a famous herb in traditional medicine of Iran, is *T. kotschyanus.* A few researches have been done for evaluating its chemical composition [2, 3, 5 - 8] and anti-fungal activity [29, 30]. Most studies were reported on systemic and anti-bacterial properties [3, 5, 8, 22] but there is no survey on its anti-inflammatory effect in the published literature. Thus we investigated anti-inflammatory effect of 4 doses of *T. kotschyanus* extract by rat's paw edema test induced by carrageenan. In previous researches on anti-bacterial effect of plant extracts, lower doses are used [3, 5, 8, 22], but for intraperitoneal injection, it seems to be more effective to use higher doses [25]. To our findings, Indomethacin and the extract of *T. kotschyanus* had significantly reduced edema in every 5 hour of measurement ($p<001$). Increasing the dose of extract from 50 - 100 mg/kg didn't reduce inflammation more, but for higher doses 100 to 200 mg/kg, it showed that the increase in drug dose, resulted in more anti-inflammatory effect. In the fifth hour of experiment, the anti-inflammatory effect of herbal drug in all doses was more than Indomethacin.Therefore, it can be used as an alternative for Indomethacin for delayed treatment of inflammation. An important thing to note is about non-homogenous inflammation reduction by increasing herbal dose which can lead into a conclusion of slow-releasing particles of drug participating in this experiment.

Regarding the researches on chemical composition of *T. kotschyanus*, thymol and carvacrol were introduced as major components [2, 3, 5-8], so they might have an important role in anti-inflammatory effect of this plant [4]. These findings suggest that the methanolic extract of *T. kotschyanus* in doses of 50-100-150-200 has potential to reduce inflammation and maybe is an alternative agent whenever Indomethacin is contraindicated. Also it might be advantagous to use this herbal drug as a synergist for Indomethacin anti-inflammatory effects, to lower the dosage and consequently complications of NSAIDs.

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