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# Investigation for anti-inflammatory and anti-thrombotic activities of methanol extract of *Capparis ovata* buds and fruits

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ABSTRACT

*Ethnopharmacological relevance:* *Capparis ovata* Desf. has wide natural distribution in Turkey and it is consumed in pickled form. Flower buds, root bark, and fruits of the plant are used traditionally due to their analgesic, anti-inflammatory, wound healing, anti-rheumatismal, tonic, and diuretic effects.
*Aim of the study:* The aim of this study was to investigate the possible anti-inflammatory and anti-thrombotic effects of methanol extracts prepared from flower buds (CBE) and fruits (CFE) of *C. ovata*.
*Materials and methods:* Anti-inflammatory effects of CBE and CFE were assessed using carrageenan-induced and prostaglandin $E_2$-induced mouse paw edema models. For the anti-thrombotic effect evaluation, carrageenan-induced tail thrombosis model was performed in mice. The extracts were administered intraperitonally (*i.p.*) at the doses of 100, 200, and 300 mg/kg. The anti-inflammatory effect of *Capparis* extracts were tested in comparison to 10 mg/kg diclofenac and anti-thrombotic activity to 10 and 100 IU heparin.
*Results:* CBE at the doses of 200, and 300 mg/kg and CFE at the doses of 100, 200, and 300 mg/kg showed significant anti-inflammatory activity and CFE reached therapeutic concentration early than CBE in carrageenan inflammation model. In prostaglandin $E_2$ inflammation model, CBE and CFE exhibited significant inhibitory effects. The *C. ovata* extracts did not show remarkable anti-thrombotic effect.
*Conclusions:* Based on the results obtained, it can be concluded that fruits of *C. ovata* have more potent anti-inflammatory effect than flower buds. It has been suggested that inhibition of cyclooxygenase pathway is one of the mechanisms of the activity. *C. ovata* may be potentially used as therapeutic agents for inflammatory diseases.



## 1. Introduction

The caper (*Capparis* spp.), belonging to the Capparaceae family, is a native Mediterranean perennate shrub that is cultivated as it is an economically important plant (Baytop, 1999; Bagci et al., 1999). Various parts of caper have been used for pharmaceutical, cosmetic, and nutritional purposes as well as preventing soil erosion and landscaping (Castro Ramos and Nosti Vega, 1987; Akgul, 1993; Ozcan and Akgul, 1998; Baytop, 1999). In Turkey and other countries, the flower buds, fruits, roots, and seeds of the caper have been used in folk medicine as an anti-rheumatic, tonic, expectorant (Jiang et al., 2007), anti-spasmodic (Ozcan, 2005), diuretic (Jain et al., 1993) and analgesic (Bagci et al., 1999; Jiang et al., 2007) agents. There are some scientific reports about the genus of *Capparis* related to its traditional therapeutic effects. *C. zeylenica* L., known as Indian caper, possesses analgesic and antipyretic properties (Ghule et al., 2007). *C. spinosa* L. and *C. decidua* (Forssk.) Edgew. both exhibit anti-inflammatory activities but no analgesic effects (Ageel et al., 1986; Al-Said et al., 1988; Zhou et al., 2010). *C. spinosa* has anti-arthritic activity (Feng et al., 2011).

Although the genus of *Capparis* consists of nearly 80 species, only *C. ovata* and *C. spinosa* have wide natural distribution in Turkey and they are consumed as pickles (Davis, 1965; Baytop, 1999). Capparaceae family members contain glucosinolates, alkaloids, and phenolics as flavonoids. They have phytochemical composition differences in constituents from different plant parts and these plant parts show various pharmacological effects (Ghule et al., 2007; Yang et al., 2008; Tlili et al., 2011). Flower buds and fruits of *Capparis* spp. contain biologically active compounds such as flavonoids, glucosinolates, and glycosides (Satyanarayana et al., 2008; Tlili et al., 2011). Various studies have suggested that plant materials containing these compounds possess anti-inflammatory activities (Mills and Bone, 2000; Morteza-Semnani et al., 2006). There are limited studies about pharmacological effects of *C. ovata* although there are more studies about *C. spinosa* in scientific reports,

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and anti-inflammatory activity of *C. ovata* has not yet been demonstrated. To provide scientific evidence for its anti-inflammatory activities known in folk medicine, the main purpose of the present study was to evaluate the effect of methanol extract of *C. ovata* flower buds (CBE) and fruits (CFE) using carrageenan-induced and prostaglandin $E_2$-induced ($PGE_2$-induced) paw edema model.

Carrageenan-induced inflammation model is useful for detecting the active of anti-inflammatory agents (Yesilada and Kupeli, 2002). Induction of edema by subcutaneous injection of carrageenan to the hind paw is a biphasic event (Vite et al., 2011). The first phase begins immediately after injection of carrageenan and diminishes within 1 h and the second phase begins at the end of first phase and persisted for at least 5 h after treatment. Different inflammatory mediators are responsible for inflammation at two phases (Holsapple et al., 1980). $PGE_2$-induced inflammation model is used for the assessment of anti-inflammatory mechanisms of effective agents (Kupeli Akkol et al., 2012).

κ-carrageenan, used in the inflammation model in this study, is also used to induce tail thrombosis *in vivo* and this model is useful to evaluate the effects of various anti-thrombotic drugs (Hagimori et al., 2009). Since thrombus formation occurred in tail and has an obvious border with the normal region, it is possible to observe appearing time, developing process, spreading range and length of the formed thrombus *in vivo* (Yan et al., 2009). As this agent is also used for generating thrombosis model, anti-thrombotic effect was evaluated synchronously with anti-inflammatory effect of *C. ovata*.

## 2. Materials and methods

### 2.1. Drugs and chemicals

Methanol (Merck, Darmstadt, Germany), DMSO (Merck), diclofenac (Sigma, St.Louis, USA), carrageenan (Sigma), $PGE_2$ (MP Biomedicals, France) were used in this study.

### 2.2. Plant material

Flower buds and fruits of *C. ovata* were kindly collected by Asci Murat Company, Burdur, Turkey. The company collected the plant material around Burdur in 2008 August and it was dried under shade. The plant samples were confirmed by Sevim Alan, Ph.D., and voucher specimens (ESSE-14487, ESSE-14486, respectively) have been placed in the Herbarium of Faculty of Pharmacy, Anadolu University, Eskisehir, Turkey.

### 2.3. Preparation of the methanol extracts of C. ovata flower buds and fruits

The methanol was used to extraction method due to methanol extracts biologically active polar phenolic compounds from the plant parts effectively. Powdered dried flower buds and fruits of *C. ovata* (10 g) were extracted separately with 250 mL methanol using the Soxhlet apparatus for 8 h. The solvent was removed from resulting solution under vacuum in a rotary evaporator at 40 °C. The dried extract yield was calculated as 8.5 g/20 g (42.5%) for flower buds methanol extract and 1.94 g/10 g (19.4%) for fruit methanol extract.

### 2.4. Phytochemical screening

The methanol extracts of flower buds and fruits of *C. ovata* were tested separately for the presence of alkaloids, tannins, reducing sugar, and flavonoids using standard phytochemical procedures (Evans, 2002). In each test, 10% (w/v) methanol solution of extract was used (Ahmed et al., 2007). The Folin–Ciocalteu method was used to determine the total phenolic content of extracts. Total phenols were estimated as gallic acid equivalents (GAE) and expressed as $mg_{GAE}/g_{extract}$. 6.0 mL $H_2O$ and 100 μL of appropriate concentration of sample were transferred in a 10.0 mL volumetric flask, to which 500 μL undiluted Folin–Ciocalteu reagent was subsequently added. After 1 min, 1.5 mL 20% (w/v) $Na_2CO_3$ was added and the volume was made up to 10.0 mL with $H_2O$. This volumetric flask was incubated at 25 °C for 2 h. The absorbance was measured at 760 nm and compared to a gallic acid calibration curve. The data are presented as the average of triplicate analyses (Kupeli Akkol et al., 2008).

### 2.5. Animals

Swiss albino mice (35–40 g) were obtained from Anadolu University Experimental Animals Research Centre. Animals were maintained in a room with controlled temperature (22 ± 2 °C) for 12 h light/12 h dark cycle with standard pellet diet and water *ad libitum*. Animal care and research protocols were based on the principles and guidelines adopted by the Guide for the Care and Use of Laboratory Animals (NIH publication No: 85-23, revised in 1985) and approved by the Local Ethics Committee of Anadolu University, Eskisehir.

### 2.6. Acute toxicity

Mice were divided into control and test groups ($n=6$). The first group served as a normal control. *C. ovata* extracts were administered intraperitoneally (*i.p.*) to different groups at the increasing doses of 200, 400, 500, 1000, and 2000 mg/kg (final volume is 0.5 mL). After injections of extracts, mice were allowed food and water *ad libitum* and all animals were observed for possible mortality cases and behavioral changes for 72 h (Lorke, 1983).

### 2.7. Drugs and treatment

The dried methanol extracts and reference drugs were dissolved in the vehicle, 20% DMSO (ratio DMSO saline 1:4) and administered *i.p.* The carrageenan and $PGE_2$ solutions were freshly prepared in saline and Tyrode's solution, respectively, and administered by subplantar injection to the hind paw. The mice were divided into eight groups for each anti-inflammatory activity and nine for anti-thrombotic activity studies. The first group served as a control group and received 20% DMSO. CBE and CFE were injected to the six test group animals at the doses of 100, 200, and 300 mg/kg in fixed volume of 0.1 mL. Diclofenac (10 mg/kg), a non-steroidal anti-inflammatory drug, and 10 IU and 100 IU heparin sodium, anti-coagulant drug, were used as reference agents.

### 2.8. Anti-inflammatory activity

#### 2.8.1. Carrageenan-induced hind paw edema

The carrageenan-induced hind paw edema model was used to evaluate anti-inflammatory activity. 40 min after the injection of DMSO 20%, diclofenac (10 mg/kg) and CBE and CFE (at three dose levels, 100, 200 and 300 mg/kg), the paw edema was induced in the right hind paw by sub plantar injection of 40 μl of 1% Type I carrageenan solution (Yesilada and Kupeli, 2002). As the control, 40 μL saline solutions were injected into that of the left hind paw of each mouse. The thicknesses of injected paws were measured using a digital caliper in every 90 min over a 6 h period by two investigators; one of the investigators did not know which treatment was applied.