Journal of Ethnopharmacology 147 (2013) 530–535

Contents lists available at SciVerse ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Ethnopharmacological communication

The effect of the extract of *Crocus sativus* on tracheal responsiveness and plasma levels of IL-4, IFN-γ, total NO and nitrite in ovalbumin sensitized Guinea-pigs

Goltaj Byrami [a], Mohammad Hossein Boskabady [a,*], Sedigheh Jalali [b], Tahereh Farkhondeh [c]

[a] Applied Physiology Research Centre and Department of Physiology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, IR Iran
[b] Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, IR Iran
[c] Department of Toxicology, Faculty of Veterinary Medicine, University of Tehran, Tehran, IR Iran

ARTICLE INFO

Article history:
Received 20 September 2012
Accepted 7 March 2013
Available online 15 March 2013

Keywords:
Crocus sativus
Asthma
Tracheal responsiveness
Cytokines
Sensitization
Inflammation

ABSTRACT

Ethnomedical relevance: Anti-inflammatory, anti oxidant and effect of *Crocus sativus* (*C. sativus*) on Th_1/Th_2 balance were described previously.
Aim of the study: The preventive effects of the extract of *Crocus sativus* on tracheal responsiveness and plasma levels of IL-4, IFN-γ, total NO and nitrite were examined on sensitized guinea pigs.
Materials and methods: Five groups of sensitized guinea pigs to ovalbumin (OVA), were given drinking water containing three concentrations of the extract of *Crocus sativus*, dexamethasone (S+D) or alone (group S). Tracheal responses (TR) of control animals (group C) and sensitized guinea pigs ($n=6$, for each group) to methacholine, OVA and the levels of IL-4, IFN-γ, total NO and nitrite in serum were examined.
Results: The TR to both methacholine and OVA, the levels of serum IL-4, total NO and nitrite in S guinea pigs were significantly increased but that of IFN-γ and IFN-γ/IL-4 ratio (Th_1/Th_2 balance) were decreased compared to the controls ($p<0.05$ to $p<0.001$). In the treated animals with dexamethasone and all concentrations of the extract, TR to both methacholine and OVA, IL-4, total NO and nitrite were significantly decreased but IFN-γ and IFN-γ/IL-4 ratio increased compared to S group ($p<0.05$ to $p<0.001$). The effects of the highest concentration of the extract was greater than those of other concentrations and the effect of dexamethasone ($p<0.05$ to $p<0.01$).
Conclusions: These results not only showed a preventive effect of C. sativus extract on tracheal responses and serum levels of inflammatory mediators in sensitized guinea pigs but also showed increased Th_1/Th_2 balance.

1. Introduction

Airway inflammation (Dodig et al., 2011) and airway responsiveness (AHR) (Meurs et al., 2008) are main characteristic future of asthma. T helper 2 (Th2) is activated in asthma disease, and its mediators cause airway inflammation (Cohn et al., 2004; Wegmann, 2009) but Th1 can inhibit Th2 and thus one goal of asthma therapy should be focused on increasing the activity of Th1 (Randolph et al., 1999). Increased nitrite, nitrate, and nitrotyrosine concentrations in asthma (Robroeks et al., 2007) was shown which could be ccontribute in physiopathology of the airways inflammation in asthma (Ricciardolo et al., 2004).

Crocus sativus L., commonly known as saffron, is a small perennial plant from the iridaceae family which is widely cultivated in Iran and some other countries. The medicinally used part of the plant is its stigma (central part of the flower, female sexual organ). Constituents of the plant include; picrocrocin and its derivatives including safranal, flavonoid derivatives and crocin (Srivastava et al., 2010).

Saffron is used in folk medicine as; antispasmodic, eupeptic, gingival sedative, anticatarrhal, nerve sedative, carminative, diaphoretic, expectorant, aphrodisiac and emmenagogue (Rios et al., 1996). Pharmacological effects including; anti-inflammatory (Hosseinzadeh and Younesi, 2002), anti-oxidant (Abe et al., 1999), antitumour, radical scavenger effects (Rios et al., 1996; Abdullaev and Espinosa-Aguirre, 2004; Tavakkol-Afshari et al., 2008), chemopreventive, genoprotective and protection from genotoxins-induced oxidative stress (Premkumar et al., 2003;) have shown for this plant.

Our previous study also showed the relaxant effect of saffron on tracheal smooth muscle (Boskabady and Aslani, 2006), inhibitory effect on histamine (H_1) receptor (Boskabady et al., 2010) and its stimulatory effect on β-adrenoceptors (Nemati et al., 2008).

Crocus sativus (saffron) is widely used as a traditional medicinal plant for treatment of various diseases. In addition, a wide range of

* Correspondence author. Fax: +98 511 88828564.
E-mail addresses: mhboskabady@hotmail.com, boskabadymh@mums.ac.ir (M.H. Boskabady).

0378-8741/$ - see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jep.2013.03.014

pharmacological effects including anti-inflammatory, antioxidant properties and relaxant effect on tracheal smooth muscle were demonstrated. In the present study the effects of *Crocus sativus* on tracheal responsiveness (main characteristic of asthma), serum IL-4/IFN-γ ratio (as an indicator of I Th1/Th2 balance), total NO and nitrite levels (important inflammatory mediators in asthma) in sensitized guinea pigs were examined.

2. Material and methods

2.1. Plant, extract and drugs

Crocus sativus was collected from Torbat Heydarieh (northeast Iran), and its stigmas were dried at room temperature in the absence of sunlight. The plant was identified by botanists in the herbarium of Ferdowsi University of Mashhad (specimen number 293-0303-1). For preparation of hydro-ethanolic extract, three grams of chopped *Crocus sativus* stigmas were mixed with 50 ml ethanol 70% for 72 h at room temperature and the solution was separated by maceration method which was repeated for three times. The solutions were dried in room temperature and stored at −4 °C away from light. The chemical characteristics of the same plant were identified previously (Hadizadeh et al., 2007; Boskabady et al., 2011).

2.2. Animal sensitization and animal Groups

Senitization of animals to OVA was performed according previous method (McCaig, 1987; Boskabady and Adel-Kardan, 1999). Briefly, guinea pigs were sensitized to 10 mg OVA (Sigma Chemical Ltd, UK) and 100 mg aluminum hydroxide ($Al(OH)_3$) dissolved in 1 ml saline (i.p.) in day one and further 2 mg OVA and 100 mg $Al(OH)_3$ (i.p.) in day 7. From day 14 sensitized animals were exposed to an aerosol of 4% OVA for 18 ± 1 days, 5 min daily. The aerosol was administered in a closed chamber, dimensions 30 × 20 × 20 cm. Control animals were treated similarly but saline was used instead of OVA solution. The study was approved by the ethical committee of the Mashhad University of Medical Sciences.

The study was performed in control animals (group C, treated the same as the sensitized group but normal saline was used instead of OVA and were given drinking water alone) and five different groups of sensitized animals which were given drinking water containing various substance during sensitization period as follows ($n=6$ for each group); 1) Drinking water alone (group S, an animal model of asthma), 2)10 mg/kg/day dexamethasone (group S+D), 3) 20 mg/kg/day extract (group S+CS1), 4) 40 mg/kg/day extract (group S+CS2), 5) 80 mg/kg/day extract (group S+CS3).

2.3. Tissue Preparations

Guinea pigs were sacrificed by a blow on the neck, and trachea was removed. Each trachea was cut into 10 rings (each containing 2–3 cartilaginous rings). All the rings were then cut open opposite the trachealis muscle, and sutured together to form a tracheal chain (Boskabady et al., 2004). Tissue was then suspended in a 20 ml organ bath (Schuler organ bath type 809, March-Hugstetten, Germany) containing Krebs-Henseliet solution of the following composition (mM): NaCl 120, $NaHCO_3$ 25, $MgSO_4$ 0.5, KH_2PO_4 1.2, KCl 4.72, $CaCl_2$ 2.5 and dextrose 11. The Krebs solution was maintained at 37 °C and gassed with 95% O_2 and 5% CO_2. Tissue was suspended under isotonic tension of 1 g and allowed to equilibrate for at least 1 h while it was being washed with Krebs solution every 15 min.

In all experiments the contractions responses were measured using an isometric transducer (MLT0202, AD Instruments, Australia) which was connected to a powerlab system (PowerLab 8/30, ML870, AD Instruments, Australia).

2.4. Assessment of tracheal response to methacholine

In each experiment a cumulative log concentration-response curves of methacholine hydrochloride (Sigma Chemical Ltd, U.K.) induced contraction of tracheal chain was obtained. Consecutive concentrations (including 10^{-7} to 10^{-3} M) were added every 2 min, and the contraction due to each concentration was recorded at the end of 2 min, and the effect reached a plateau in all experiments. The percentage of contraction of the tracheal smooth muscle due to each concentration of methacholine in proportion to the maximum contraction obtained by its final concentration was plotted against log concentration of methacholine. The effective concentration of methacholine, causing 50% of maximum response (EC_{50}) using methacholine response curve in each experiment, was measured.

2.5. Measurement of tracheal response to ovalbumin

The tracheal response to 0.1% solution of OVA was measured as follows: 0.5 ml of 4% OVA solution was added to the 20 ml organ bath and the degree of tracheal chain contraction was recorded after 15 min and was then expressed as proportion (in percentage) to contraction obtained by 100 μM methacholine.

2.6. Evaluation of plasma Levels of inflammatory Mediators

Five ml of blood samples was taken by cardiac puncture immediately after sacrificing and exposing the animals chest and were collected into test tube and placed at room temperature for 1 h. The samples were then centrifuged at 3500 × *g* at 4 °C for 10 min. The supernatant was collected and immediately stored at −70 °C until analyzed.

Serum IL-4 (platinum ELISA BMS628/BMS628TEN, Bender Med Systems GmbH, Austria) and IFN-γ (platinum ELISA BMS621 /BMS621TEN, Bender MedSystems GmbH, Austria) were measured using Elisa sandwich method according to the manufacturer's instructions. The ratio of IFN-γ/IL-4 as an index of Th1/Th2 was also calculated.

Serum NO level was determined by Nitric Oxide Calorimetric Assay kit (nitric oxide colorimetric assay kit k262–200, BioVision Research Products, USA) according to the manufacturer's instructions. Nitrate in the samples and standards were converted to nitrite by adding nitrate reductase and Griess reagent in the each well plate. Absorbance was determined at 540 nm. The concentration of total NO (nitrite+nitrate) was calculated according to a standard curve of known nitrite concentrations. For measuring nitrite, a nitrite standard curve was prepared in the absence of Nitrate Reductase in the standard and assay samples.

2.7. Statistical analysis

The data were quoted as mean ± SEM. According to the Kolmogorov Smirnov test the data had normal distribution. The data of sensitized group were compared with control guinea pigs using unpaired "t" test. The data of three groups of animals treated with the extract and dexamethazone were also compared with sensitized guinea pigs using unpaired "t" test. Statistical comparison between three treated groups with the extract was performed using one way ANOVA with Tukey Kramer hoc post test. Significance was accepted at $p < 0.05$.