



The Effects of *Ocimum Basilicum* Ethanol Extract on Carrageenan Induced Paw Inflammation in Rats

Maryam Rameshrad^{1,2}, Ronak Salehian², Fatemeh Fathiazad³, Sanaz Hamedeyazdan³, Mehroo Garjani², Nasrin Maleki-Dizaji^{1*}, Reza Vosooghi²

¹Department of Pharmacology and Toxicology, Tabriz University of Medical Sciences, Tabriz, Iran.

²Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran.

³Department of Pharmacognosy, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.

ARTICLE INFO

Article Type:

Original Research

Article History:

Received: 25 November 2014

Accepted: 29 December 2014

Keywords:

O. basilicum

Antioxidant

Carrageenan

MPO

Paw Inflammation

ABSTRACT

Background: Traditionally, *Ocimum basilicum* has been used for its anti-inflammatory activity. Here, the effect of *O. basilicum* ethanol extract was evaluated on carrageenan induced paw edema in male Wistar rats. **Methods:** *O. basilicum* ethanol extract was achieved through maceration procedure. Next, the paw inflammation was induced with 1% carrageenan, subcutaneous, to all groups after 1h of the intrapritoneal injection of *O. basilicum* extracts (0, 2.5, 5 and 10mg/kg; respectively). The paw thickness was measured at hourly intervals for 4h. Finally, histological examination and Myeloperoxidase (MPO) activity was assessed in the inflamed paw at the 4thh. Moreover, total phenolic and flavonoid content of the extract was determined. Besides, different *in vitro* antioxidant activities were detected by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, nitric oxide radical inhibition and reducing power methods. **Results:** The extract diminished paw inflammation as indexed by reduction paw thickness ($p < 0.001$) as well as MPO activity ($p < 0.001$), which was associated with a marked decrease in tissue edema and leukocyte infiltration. In addition, values for the total phenolic and flavonoids were calculated as 266 mg gallic acid equivalent and 65 mg quercetin equivalent per 100g of *O. basilicum* fresh plant material. Furthermore, the RC_{50} values for DPPH and nitric oxide antioxidant activity of the extract was determined as 118 μ g/mL and 929.7 μ g/ml. **Conclusions:** Ethanol extract of *O. basilicum* significantly decreased the inflammatory reaction induced by carrageenan which could be related to its antioxidant effects.

Introduction

Innate and adaptive immune systems are two equally important aspects of host defense which play crucial role in tissue repair and pathogen control. Inflammation is the immune system response to harmful stimuli.¹⁻³ Inflammatory responses are essential for immune surveillance, tissue repair, and regeneration after injury.^{4,5} Actually, dysregulation of the inflammatory system has detrimental effects on body function and may result in complex and chronic disease (e.g., infectious disease, autoimmune disorders, cancer, neurodegenerative disease, and metabolic disorders).^{4,6} Pain, redness, heat, swelling, and loss of function are the classic signs of inflammation.⁷ Plants have been used for medical treatments through much of human history.⁷ This tradition seems to be the basis of modern medicine, as many of the pharmaceutical drugs are derived from plants.⁷ *Ocimum* L. (Lamiaceae) is a genus of annual and perennial herbs which contains 30-160 species, including *Ocimum basilicum* (basil).⁸ Traditionally, the essential oil of

Ocimum basilicum was used as anti-inflammatory, anti-septic, and gastrointestinal protective medicine.^{9,10} Today, numerous studies have verified the fact and demonstrated that compounds produced by *O. basilicum* exert its prominent biological effects, namely hypolipidemic,¹¹ anti-tuberculous,¹² anti-proliferative,¹³ anti-inflammatory,¹⁴⁻¹⁶ and gastrointestinal-protective effects.¹⁴ *O. basilicum* derived compounds include mainly triterpenoids, polyphenols, steroids, and phenylpropanoids.^{17,18} Fathiazad *et al.* in 2012, argued that cardioprotective effects of *O. basilicum* are correlated with its antioxidant compounds.¹⁹ Antioxidants are compounds which restrain oxidative damage through variable mechanisms such as reacting with free radicals, chelating catalytic metals, and acting as oxygen scavengers. There is an increasing interest recently to elucidate the association of different antioxidants with stress-related conditions such as inflammatory disease.²⁰⁻²² Anti-inflammatory and antioxidant properties of *Ocimum* genus has been documented in some studies. In this study, we

investigated the effects of *O. basilicum* ethanol extract on carrageenan induced paw edema in rats and assessed the antioxidant activity of *O. basilicum* *in vitro*.

Materials and methods

Plant material

The aerial parts of *O. basilicum* were purchased before flowering in June from local market. The botanical identification was made by Dr. F. Fathiazad (Department of Pharmacognosy). A voucher specimen was deposited at the herbarium of the Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.

Extract preparation

The chopped fresh leaves (500 g) were extracted with ethanol (96%; 2 L×4) by maceration at room temperature and the solvent was removed at 40°C using a rotary evaporator. A greenish residue weighing 9.5 g was obtained and kept in air tight bottle in a refrigerator for further pharmacological study.

Animals

Male Wistar rats (180 to 200 g) were used in this study. The animals were given food and water ad libitum. They were housed in the Animal House of the Tabriz University of Medical Sciences at a controlled ambient temperature of 25±2°C and a 12 h light/12 h dark cycle. The present study was performed in accordance with the Guide for the Care and Use of Laboratory Animals of Tabriz University of Medical Sciences, Tabriz-Iran.

Carrageenan-induced paw edema

The extract was kept at 4°C until used. The dried extract was dissolved in solvent and passed through a weighed paper filter. The filtered solution was used for intraperitoneal injection. Following the filtration, the filter was dried and weighed again, and to obtain the real concentration of the extract, the unfiltered particles were calculated.

The animals were randomized into four groups each consisting of ten rats. Rat in group 1 (control) received 500 µl i.p injection of extract solvent and in group 2 to 4 (treatment groups) treated with 500 µl extract (2.5, 5 & 10 mg/kg, ip) one hour before S.C injection of 100 µl of carrageenan 1% (w/v) in the left hind paw.²³ The paw thickness was measured from the ventral to the dorsal surfaces using a dial calliper²⁴ immediately prior to carrageenan injection and then at hourly intervals from 1 to 4 h afterwards. Data were expressed as a percentage of increase in the paw thickness and were compared with those of pre-injection values. After measurement of the paw edema the rats were sacrificed by an overdose of diethyl ether and then the inflamed paws were excised with a guillotine.

MPO activity

Carrageenan edema was induced as described earlier. The rats were sacrificed 4 h later by an overdose of

diethyl ether and the inflamed hind paws were excised with a guillotine. Myeloperoxidase (MPO) activity was measured for quantifying the activity of neutrophils in tissue, as previously described²⁵ by some modification. Briefly, the chopped tissue (n=6) was homogenized in a solution containing 0.5% hexa-decyltrimethyl ammonium bromide (HTAB) dissolved in 50 mM potassium phosphate buffer (pH 6). The samples were then centrifuged at 3000 rpm for 30 min at 4°C. An aliquot of the supernatant (0.1 ml) or standard (Sigma, Germany) was then allowed to react with a 2.9 ml solution of 50 mM potassium phosphate buffer at pH 6 containing 0.167 mg/ml of O-dianisidine hydrochloride and 0.0005% H₂O₂. After 5 min, the reaction was stopped with 0.1 ml of 1.2 M hydrochloric acid. The rate of change in absorbance was measured by a spectrophotometer at 400 nm. Myeloperoxidase activity was expressed in mil-units (mU) per 100 mg weight of wet tissue.

Histological examination

For histopathological examination, biopsies of inflamed paws (n=3) from control and extracts treated rats (2.5, 5, 10 mg/kg) were taken 4 h after carrageenan injection, and the tissue slices were fixed in 10% neutral-buffered formaldehyde, embedded in paraffin, and sectioned. The sections were stained with haematoxylin and eosin for evaluating the severity of oedematosis.

Determination of total phenolic content

Folin-Ciocalteu reagent was used to determination total content of phenolic compounds.²⁶ The extract samples (0.5 ml of different dilutions) were mixed with Folin-Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) for 5 min and 4 ml aqueous Na₂CO₃ (1 M) were then added. The mixture was allowed to stand for 15 min and the phenols were determined by colorimetry at 765 nm. The standard curve was prepared by 0, 50, 100, 150, 200, and 250 mg/ml solutions of gallic acid in methanol:water (50:50, v/v). Total phenolic contents were calculated as gallic acid equivalent per gram of the extract from a calibration curve prepared with 0, 50, 100, 150, 200, and 250 µg/ml solutions of gallic acid in methanol.

Determination of total flavonoid content

The content of flavonoids was determined using colorimetric aluminum chloride method²⁶. Briefly, 0.5 ml solution of the extract were mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water, and were left at room temperature for 30 min. The absorbance of the reaction mixture was measured spectrophotometrically at 415 nm. Total flavonoids contents were calculated as quercetin from a calibration curve.

Assay for in vitro antioxidant activity

DPPH radical scavenging assay

One of the rapid and simple inexpensive methods to measure antioxidant capacity involves the use of the free radical, DPPH.²⁷ The free radical scavenging capacity of *O. basilicum* ethanol extract was measured from the bleaching of the purple-colored DPPH solution. The stock concentration 1 mg/mL of the ethanol extract was prepared followed by dilution, in order to obtain different concentrations of the extract. The obtained concentrations in equal volumes of 2 mL were added to 2 mL of a 0.04% of DPPH solution. After a 30 min incubation period at 25 °C, the absorbance was read against a blank at 517 nm. Tests were carried out in duplicate where the average absorption was noted for each concentration. In addition, the same procedure was repeated with quercetin as the positive control. Inhibition of free radical by DPPH in percent was calculated as follows:

$$I (\%) = 100 \times [(A \text{ blank} - A \text{ sample}) / A \text{ blank}]$$

Where A blank is the absorbance of the control reaction (containing all reagents except the plant extract) and A sample is the absorbance of the sample. Furthermore, the IC₅₀ value, the concentration of the plant extract inhibiting 50% of the free radical concentration, was calculated from the graph of inhibition percentages against *O. basilicum* extract concentrations in mg/mL.

Nitric oxide radical inhibition assay

In this procedure sodium nitroprusside was used at physiological pH to spontaneously generate nitric oxide, which interacts with oxygen to produce nitrite ions that could be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced nitric oxide production.²⁸ For the experiment, sodium nitroprusside (10 mM) in phosphate buffered saline (pH 7.4) was mixed with different concentrations of *O. basilicum* ethanol extract. After incubation for 150 min at 25°C, 1 ml of

Griess reagent (1% sulfanilamide in 20% glacial acetic acid and 0.1% naphthylethylenediamine dihydrochloride) was added. The absorbance of the pink chromophore formed during diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine was measured spectrophotometrically at 548 nm against a blank sample. Quercetin was used as a standard in this method. Besides, IC₅₀ value, the concentration of the extract required to inhibit 50% of nitric oxide radicals was calculated.

Reducing power assay

The Fe³⁺ reducing power of *O. basilicum* ethanol extract was determined according to the method of Yen and Chen (1995).^{22,29} Different concentrations of the extract and quercetin as the standard were mixed with 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL of potassium hexacyanoferrate [K₃Fe(CN)₆] (1%, w/v), followed by incubating at 50°C for 20 min. Next, 2.5 ml of trichloroacetic acid (10%w/v) was added for termination of the reaction. After 10 min of incubation at the room temperature, 2.5 mL of the solution was mixed with distilled water (2.5 ml) and FeCl₃ (0.5 ml, 0.1% w/v). Finally, the absorbance of the reaction mixture was measured at 700 nm against an appropriate blank solution spectrophotometrically. In this method, increased absorbance of the reaction mixture indicates increased reducing power of the extract.

Statistic

All results are expressed as mean±standard error of the mean (SEM). We assessed carrageenan-induced inflammation data by one-way analysis of variance (ANOVA), and the significant differences were examined by the LSD post-hoc test.

Differences between groups were considered significant at a level of p <0.05.

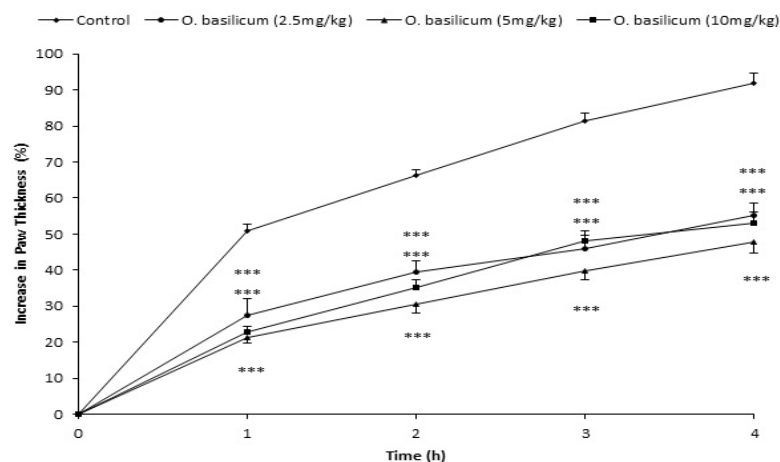


Figure 1. The effect of intraperitoneal injection of *O. basilicum* (2.5, 5, 10 mg/kg) on carrageenan-induced paw edema in rats compared to control group. *O. basilicum* was injected 1 hour before induction of inflammation by carrageenan; Results are expressed as percentage of increase in paw thickness from values. Each point represents mean±SEM of nine rats per group. Asterisks indicate significant changes from the control value (***)p<0.001).

Results

Effect of O. basilicum on carrageenan-induced paw edema

Induction of acute inflammation in control rats resulted in a prominent increase in paw thickness, began 1 h after S.C injection of carrageenan and reached a peak of inflammation after 4 h (Fig. 1).

In comparison with pre-carrageenan control value, the paw thickness in the control group increased by $92.01 \pm 2.76\%$, at the 4th hour. As shown in Figure 1, intraperitoneal injection of animals with the ethanol

extract of aerial parts of *O. basilicum*, caused a potent inhibition of the carrageenan induced inflammation. The extracts with doses of 2.5, 5, 10 mg/kg induced a significant ($p < 0.001$) anti-inflammatory effect after carrageenan injection at all hours. As demonstrated in Figure 2, pretreatment with extract by 2.5, 5, and 10 mg/kg significantly decreased ($p < 0.001$) the total inflammatory response measured as area under the curve (AUC) from 244.74 ± 6.2 in control to 141.45 ± 11.03 , 115.6 ± 8.13 , and 133.58 ± 7.3 ; respectively.

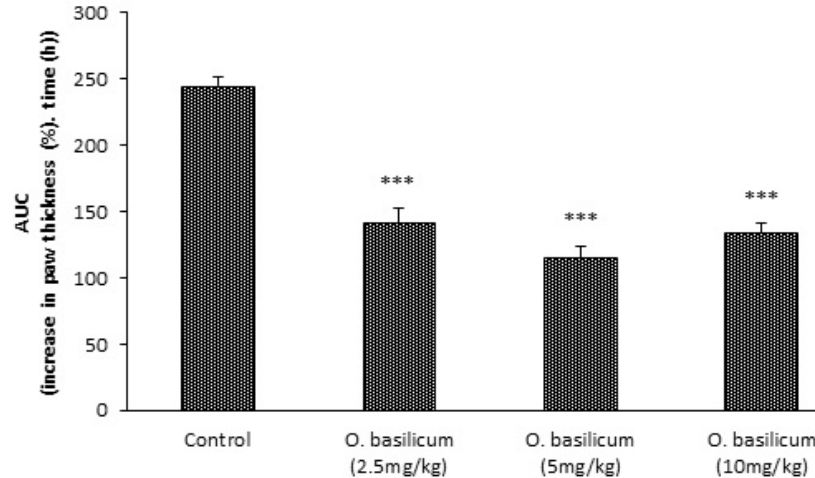


Figure 2. Total edema responses measured as area under the curve (AUC) of the treated rats compared to control. Each column represents mean \pm SEM of nine rats per group. Asterisks indicate significant changes from the control value ($***p < 0.001$).

Effect of O. basilicum on myeloperoxidase activity

Subcutaneous injection of the carrageenan into the rat hind paw induced an inflammation (swelling and erythema) that was maximal by the 4th hour following the carrageenan administration and produced a time dependent polymorphonuclear leukocytes (PMN) accumulation into the paw tissue. The treatment of rats

with intraperitoneal injection of single dose of *O. basilicum* (2.5, 5, 10 mg/kg) led to a marked reduction of MPO activity from 1352.276 ± 103.185 mU/100 mg wet tissue in control to 696.65 ± 109.52 , $p < 0.01$; 309.79 ± 67.82 , $p < 0.001$; and 460.39 ± 5.77 , $p < 0.001$; respectively in treated groups (Fig. 3).

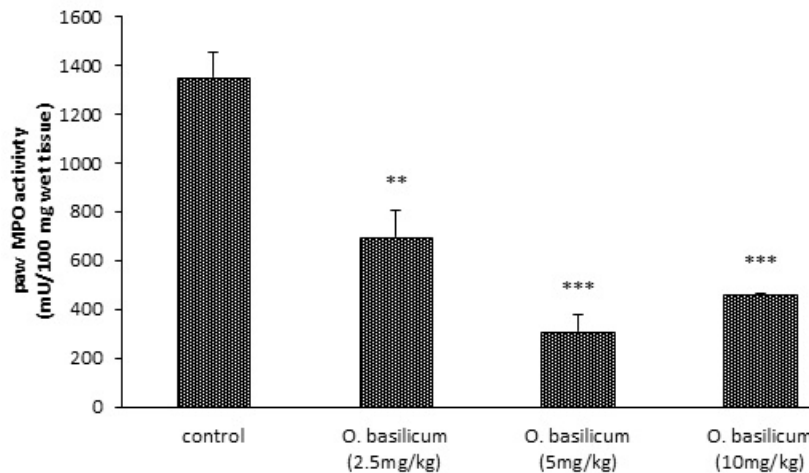


Figure 3. The effect of intraperitoneal injection of *O. basilicum* (2.5, 5, 10 mg/kg) on myeloperoxidase (MPO) activity in carrageenan-induced paw edema in rats compared to control group. *O. basilicum* was injected 1 hour before induction of inflammation by carrageenan; Results are expressed as mil-unit MPO activity in 100 mg wet tissue. Each point represents mean \pm SEM of 6 rats per group. Asterisks indicate significant changes from the control value ($**p < 0.01$, $***p < 0.001$).

Effect of *O. basilicum* on tissue damage

Histopathological study was carried out in paw tissue 4 h after induction of inflammation. A microscopic study of the tissue slices revealed acute oedematosis in epidermis and dermis after carrageenan administration

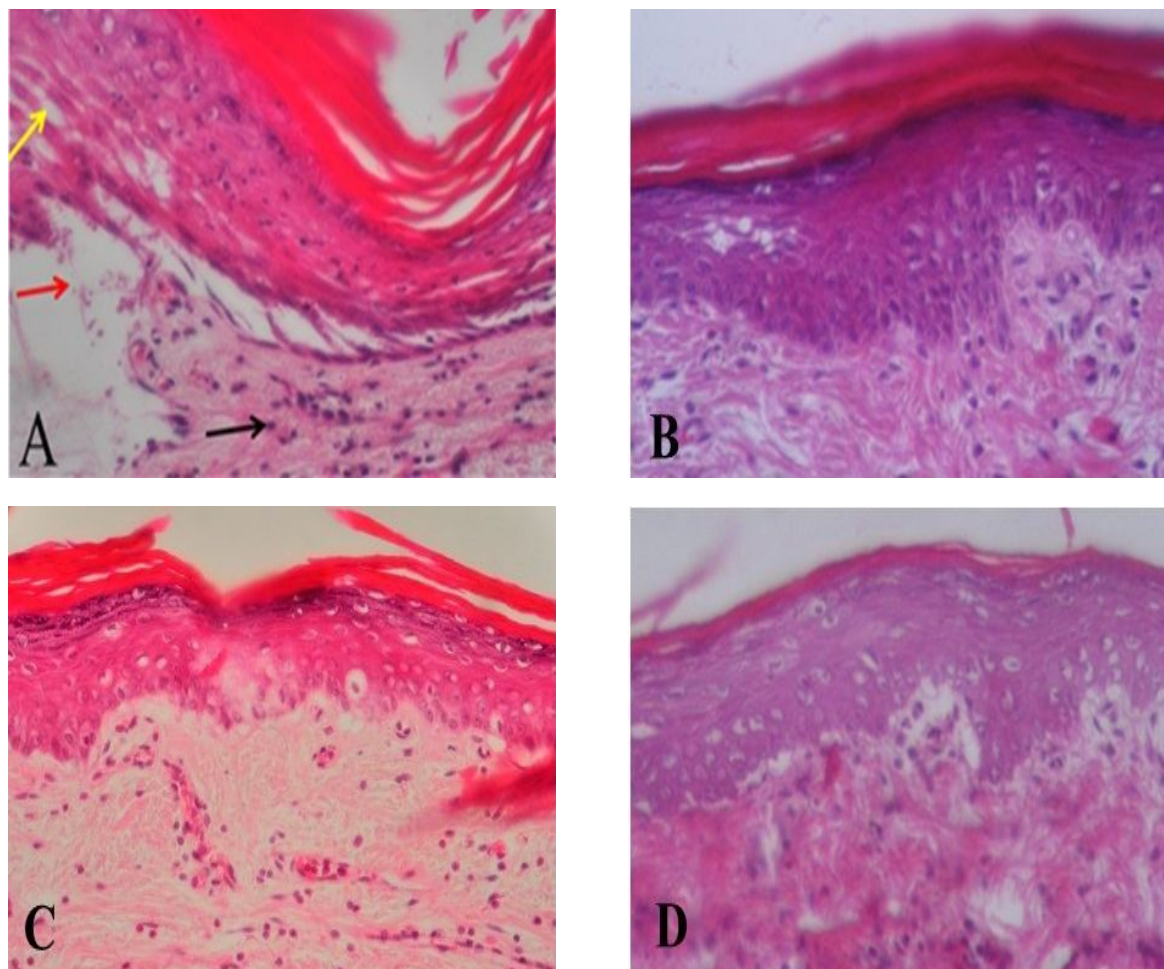


Figure 4. A paw tissue slices from a carrageenan-treated rat (A) shows a mild spongy-like appearance in epidermis and oedematosis in the dermis. The tissue section from the carrageenan-treated rats that received the extract (2.5, 5 and 10 mg/kg; B, C, D) demonstrate reduced oedematosis and spongy-like feature. There is a marked decrease in Polymorphonuclear infiltration in dermis vs. carrageenan-treated rat. $\times 40$.

Total phenols content of *O. basilicum* extract

The content for *O. basilicum* total phenolics showed the value 266 mg of gallic acid equivalent in 100 g of the fresh plant. The following equation obtained from

the gallic acid standard curve was applied in calculation of the phenolics content (Table 1).

Sample absorbance = $0.0063 \times \text{concentration } (\mu\text{g}) + 0.0432$; $R^2 = 0.999$.

Table 1. Total phenolic and flavonoids content of *O. basilicum* as well as its antioxidant activity *in vitro*.

	Total Phenolics *	Total Flavonoids **	IC ₅₀ ($\mu\text{g/ml}$) DPPH	IC ₅₀ ($\mu\text{g/ml}$) Nitric oxide
<i>O. basilicum</i>	266	65	118.1	929.7
Quercetin	-	-	3.8	55.3

* Values are reported as mg gallic acid equivalent in 100 g of fresh plant material

** Values are reported as mg quercetin equivalent in 100 g of fresh plant material.

Total flavonoids content of *O. basilicum* extract

Comparing the absorbance values for ethanol extract solution with the standard solutions of quercetin, value for the flavonoid content was calculated as 65 mg quercetin equivalent in 100 g of *O. basilicum* fresh plant with reference to the relative standard curve (Table 1).

Sample absorbance = $0.008 \times \text{quercetin } (\mu\text{g}) - 0.0625$;
 $R^2 = 0.9965$.

In vitro antioxidant activity of *O. basilicum* extract

Assay for the antioxidant activity of the extract was accomplished through three different methods. Regarding the results for the DPPH radical scavenging antioxidant assay, *O. basilicum* extract exhibited pleasant antioxidant activity with IC_{50} values of 118 $\mu\text{g/mL}$ for the extract and 3.8 $\mu\text{g/mL}$ for the control

quercetin (Table 1). Besides, for the nitric oxide radical inhibition assay it was established that incubation of aqueous sodium nitroprusside solutions at physiological pH resulted in nitrite production, which was reduced by the *O. basilicum* ethanol extract. It had been observed that the extract had moderate nitric oxide scavenging activity in a dose dependent manner with IC_{50} value of 929.7 $\mu\text{g/mL}$ compared to the quercetin standard with 55.35 $\mu\text{g/mL}$ value (Table 1). Considering the results of the reducing power assay, Fe^{3+} was transformed to Fe^{2+} in the presence of the reference compound quercetin whilst *O. basilicum* extract was almost inactive in reducing the Fe^{3+} to Fe^{2+} , as it has been demonstrated in Figure 5. Nonetheless, reducing power activity of the *O. basilicum* ethanol extract might be increased with increasing concentration of the extract.

Reducing Power Test

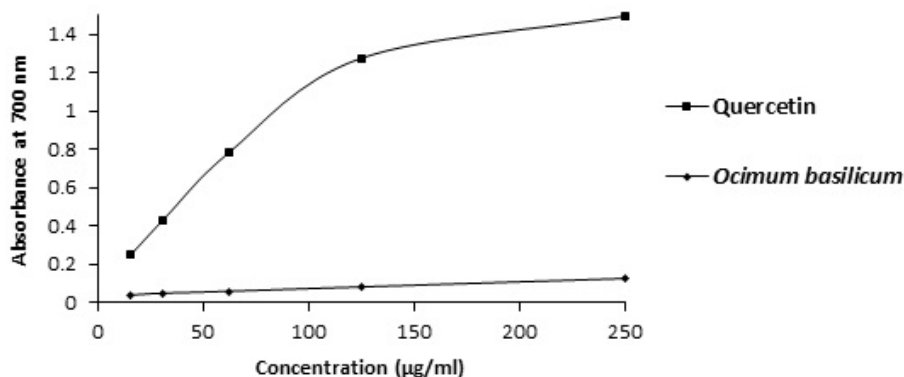


Figure 5. Reductive potential of different concentrations of Quercetin and *O. basilicum* ethanol extract using spectrophotometric detection of the Fe^{3+} - Fe^{2+} transformations.

Discussion

Although inflammatory responses are essential for survival, they can cause severe collateral damage if not tightly regulated, as in chronic states¹⁻³. *O. basilicum* has been used for the treatment of inflammatory disorders in traditional medicine¹⁴⁻¹⁶. Ethanol extract of *O. basilicum* contains high amounts of polyphenols and flavonoids which could attenuate oxidative stress and inflammatory reactions caused by reactive oxygen species (ROS)¹⁹. A recently published article demonstrated that ethanol extract of *O. basilicum* has cardioprotective and anti-inflammatory effects in isoproterenol-induced heart ischemia, partly due to high amounts of polyphenols and flavonoids¹⁹. Here, we investigated the therapeutic efficacy of the ethanol extract of the aerial parts of *O. basilicum* on carrageenan induced paw edema in rats. This is a reasonable model for evaluating the effects of different agents on acute inflammation. Oxygen-derived free radicals and arachidonic acid metabolites play an important role in acute inflammation³⁰. In this study, it was revealed that the non-flowering aerial parts of *O. basilicum* extract had potent anti-inflammatory effects. Besides, the extract noticeably reduced MPO activity

which was confirmed by histological examination.

Injection of carrageenan into the rat paw causes a biphasic inflammatory response through various mediators. In the initial phase (0-1 h) of inflammatory response, histamine, 5-hydroxytryptamine (5-HT), bradykinin, and prostanoids are released, and they increase the vascular permeability. Arachidonic acid metabolites and free radicals play a major role in the second phase of inflammation (1-6 h)^{31,32}. Recruitment of neutrophils from vessels to damaged area further augments inflammatory response due to oxygen-derived free radicals production³³. Nitric oxide (NO), produced by constitutive NO synthase (cNOS) and inducible NO synthase (iNOS), has a critical role in the development and maintenance of the inflammatory response³².

Considering the involvement of arachidonic acid metabolites in both phases of carrageenan-induced paw edema^{31,32}, we can assume that the anti-inflammatory effects of ethanol extract of *O. basilicum* could be due to the inhibition of release and/or synthesis of cyclooxygenase and/or lipooxygenase products. It is known that the anti-inflammatory effects of fixed oil of *O. basilicum* and *O. sanctum* are due to their

lipoygenase inhibitory and anti-histaminic effects^{34,35}. Moreover, antipyretic effect of the fixed oil of *O. sanctum* is due to anti-prostaglandin activity³⁵. The extract of *O. lamiifolium* aqueous leaf¹⁶, and butanol and aqueous fractions of the aqueous leaf extract of *O. suave* have been showed significant anti-inflammatory effects in histamine and serotonin induced mouse paw edema¹⁵.

In reviewing the literature, *Ocimum* genuse has anti-histaminic and anti-serotonin effects which may be mentioned as the other possible anti-inflammatory mechanism of ethanol extract of leave of *O. basilicum*. Involvement of flavonoids in the reduction of inflammation has been reported³⁶.

According to the established data of different methods of measuring antioxidant activity in this study, the order of activity for *O. basilicum* extract was: DPPH scavenging > nitric oxide scavenging > reducing power. Various antioxidant assays have been used to monitor and compare the antioxidant activity of the plant extract. These methods might give varying results depending on the specific free radical being used as the reactant and the nature of the phytochemicals within a specific plant extract³⁷. Plant extracts are complex mixtures of diverse compounds responsible for their antioxidant activity. In this regard, flavonoids and other phenolics present in the *O. basilicum* ethanol extract have promising role in donating a hydrogen atom to scavenge free radicals. Accordingly, the therapeutic properties of *O. basilicum* in a certain type of an oxidative stress condition like inflammation could be explained partly by its ability to scavenge free radicals and not the nitric oxide scavenging or its reducing power ability. Hence, radical scavenging activity of the *O. basilicum* extract has strong role for confronting with produced ROS free radicals during the second phase of inflammation. Overall, antioxidant properties of the extract could diminish carrageenan induced paw neutrophil infiltration and injury.

Conclusion

To conclude, the results of the present study revealed that the ethanol extract of *O. basilicum* (basil) exerts a potent action against inflammation and confirm that the extract contains an effective anti-inflammatory substance(s). Our results support the claim of traditional use of *O. basilicum* for the treatment of inflammation. Future studies may focus on the exact mechanism(s) responsible for its anti-inflammatory effect and identify the active compound(s).

Acknowledgments

The authors would like to thank the Research Vice-Chancellor of Tabriz University of Medical Sciences for financial support of this study. This article was written based on a data set of Pharm.D. thesis, registered in Tabriz University of Medical Sciences (NO. 3740).

References

1. Hummasti S, Hotamisligil GS. Endoplasmic reticulum stress and inflammation in obesity and diabetes. *Circ Res* 2010;107:579-591.
2. Hotamisligil GS. Endoplasmic Reticulum Stress and the Inflammatory Basis of Metabolic Disease. *Cell*;140:900-917.
3. Hotamisligil GS, Erbay E. Nutrient sensing and inflammation in metabolic diseases. *Nat Rev Immunol* 2008;8:923-934.
4. Vodovotz Y, Csete M, Bartels J, Chang S, An G. Translational systems biology of inflammation. *PLoS Comput Biol* 2008;4:e1000014.
5. Pawelec G, Goldeck D, Derhovanessian E. Inflammation, ageing and chronic disease. *Curr Opin Immunol* 2014;29:23-28.
6. Webb DR. Animal models of human disease: Inflammation. *Biochem Pharmacol* 2014;87:121-130.
7. Parveen A, Akash MS, Rehman K, Mahmood Q, Qadir MI. Analgesic, anti-inflammatory and anti-pyretic activities of *Caesalpinia decapetala*. *Bioimpacts* 2014;4:43-48.
8. Carović-Stanko K, Liber Z, Besendorfer V, Javornik B, Bohanec B, Kolak I, Satovic z. Genetic relations among basil taxa (*Ocimum* L.) based on molecular markers, nuclear DNA content, and chromosome number. *Plant Systematics and Evolution* 2010;285:13-22.
9. Velázquez C, Calzada F, Torres J, González F, Ceballos G. Antisecretory activity of plants used to treat gastrointestinal disorders in Mexico. *J Ethnopharmacol* 2006;103:66-70.
10. Rahimi R, Shams-Ardekani MR, Abdollahi M. A review of the efficacy of traditional Iranian medicine for inflammatory bowel disease: *World J Gastroenterol* 2010;16:4504-4514.
11. Bravo E, Amrani S, Aziz M, Harnafi H, Napolitano M. *Ocimum basilicum* ethanolic extract decreases cholesterol synthesis and lipid accumulation in human macrophages. *Fitoterapia* 2008;79:515-523.
12. Siddiqui BS, Bhatti HA, Begum S, Perwaiz S. Evaluation of the antimycobacterium activity of the constituents from *Ocimum basilicum* against *Mycobacterium tuberculosis*. *J Ethnopharmacol* 2012;144:220-222.
13. Bayala B, Bassole IH, Gnoula C, Nebie R, Yonli A, Morel L, et al. Chemical composition, antioxidant, anti-inflammatory and anti-proliferative activities of essential oils of plants from burkina faso. *PLoS One* 2014;9.
14. Kamyab AA, Eshraghian A. Anti-Inflammatory, gastrointestinal and hepatoprotective effects of *Ocimum sanctum* Linn: an ancient remedy with

-
- new application. *Inflamm Allergy Drug Targets* 2013;12:378-384.
15. Masresha B, Makonnen E, Debella A. In vivo anti-inflammatory activities of *Ocimum suave* in mice. *J Ethnopharmacol* 2012;142:201-205.
 16. Wolde Sellasie M, Eyasu M, Kelbessa U. In vivo anti-inflammatory activities of leaf extracts of *Ocimum lamiifolium* in mice model. *J Ethnopharmacol* 2011;134:32-36.
 17. Siddiqui BS, Aslam H, Ali ST, Begum S, Khatoun N. Two new triterpenoids and a steroidal glycoside from the aerial parts of *Ocimum basilicum*. *Chem Pharm Bull* 2007;55:516-519.
 18. Strazzer P, Guzzo F, Levi M. Correlated accumulation of anthocyanins and rosmarinic acid in mechanically stressed red cell suspensions of basil (*Ocimum basilicum*). *J Plant Physiol* 2011;168:288-293.
 19. Fathiazad F, Matlobi A, Khorrami A, Hamedeyazdan S, Soraya H, Hammami M, Maleki-Dizaji N, Garjani A. Phytochemical screening and evaluation of cardioprotective activity of ethanolic extract of *Ocimum basilicum* L. (basil) against isoproterenol induced myocardial infarction in rats. *Daru* 2012;20:2008-2231.
 20. Kandaswami C, Lee LT, Lee PP, Hwang JJ, Ke FC, Huang YT, Lee MT. The antitumor activities of flavonoids. *In Vivo* 2005;19:895-909.
 21. Rietjens IM, Boersma MG, Van Der Woude H, Jeurissen SM, Schutte ME, Alink GM. Flavonoids and alkenylbenzenes: mechanisms of mutagenic action and carcinogenic risk. *Mutat Res* 2005;574:124-138.
 22. Dinis TCP, Madeira VMC, Almeida LM. Action of Phenolic Derivatives (Acetaminophen, Salicylate, and 5-Aminosalicylate) as Inhibitors of Membrane Lipid Peroxidation and as Peroxyl Radical Scavengers. *Archives Biochem Biophys* 1994;315:161-169.
 23. Bilici D, Akpınar E, Kiziltunc A. Protective effect of melatonin in carrageenan-induced acute local inflammation. *Pharmacol Res* 2002;46:133-139.
 24. Al-Haboubi HA, Zeitlin IJ. Re-appraisal of the role of histamine in carrageenan-induced paw oedema. *Eur J Pharmacol* 1983;88:169-176.
 25. Bradley PP, Priebe DA, Christensen RD, Rothstein G. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. *J Invest Dermatol* 1982;78:206-209.
 26. Ebrahimzadeh MA, Nabavi SM, Nabavi SF, Dehpour AA. Antioxidant activity of hydroalcoholic extract of *Ferula gummosa* Boiss roots. *Eur Rev Med Pharmacol Sci* 2011;15:658-664.
 27. Nahar L, Russell WR, Middleton M, Shoeb M, Sarker SD. Antioxidant phenylacetic acid derivatives from the seeds of *Ilex aquifolium*. *Acta Pharm* 2005;55:187-193.
 28. Marcocci L, Maguire JJ, Droy-Lefaix MT, Packer L. The nitric oxide-scavenging properties of Ginkgo biloba extract Egb 761. *Biochem Biophys Res Commun* 1994;201:748-755.
 29. Yen G-C, Chen H-Y. Antioxidant Activity of Various Tea Extracts in Relation to Their Antimutagenicity. *J Agric Food Chem* 1995;43:27-32.
 30. Boughton-Smith NK, Deakin AM, Follenfant RL, Whittle BJ, Garland LG. Role of oxygen radicals and arachidonic acid metabolites in the reverse passive Arthus reaction and carrageenin paw oedema in the rat. *Br J Pharmacol* 1993;110:896-902.
 31. Maleki N, Garjani A, Nazemiyeh H, Nilfouroushan N, Eftekhari Sadat AT, Allameh Z, et al. Potent anti-inflammatory activities of hydroalcoholic extract from aerial parts of *Stachys inflata* on rats. *J Ethnopharmacol* 2001;75:213-218.
 32. Salvemini D, Wang ZQ, Wyatt PS, Bourdon DM, Marino MH, Manning PT, et al. Nitric oxide: a key mediator in the early and late phase of carrageenan-induced rat paw inflammation. *Br J Pharmacol* 1996;118:829-838.
 33. Posadas I, Bucci M, Roviezzo F, Rossi A, Parente L, Sautebin L, et al. Carrageenan-induced mouse paw oedema is biphasic, age-weight dependent and displays differential nitric oxide cyclooxygenase-2 expression. *Br J Pharmacol* 2004;142:331-338.
 34. Singh S, Majumdar DK. Evaluation of the gastric antiulcer activity of fixed oil of *Ocimum sanctum* (Holy Basil). *J Ethnopharmacol* 1999;65:13-19.
 35. Singh S, Taneja M, Majumdar DK. Biological activities of *Ocimum sanctum* L. fixed oil--an overview. *Indian J Exp Biol* 2007;45:403-412.
 36. Okokon JE, Udoh AE, Frank SG, Amazu LU. Anti-inflammatory and analgesic activities of *Melanthera scandens*. *Asian Pac J Trop Biomed* 2012;2:144-148.
 37. Nehete J, Bhatia M, Narkhede M. In-vitro Evaluation of Antioxidant Activity and Phenolic Content of *Costus speciosus* (Koen) J.E. Sm. *Iran J Pharm Res* 2010;9:271-277.
-