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Anti-Inflammatory Effect of *Kelussia odoratissima* Mozaff. Hydroalcoholicextract on Acetic Acid- Induced Acute Colitis in Rats

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A B S T R A C T

Kelussia odoratissima Mozaff. is an Iranian endangered endemic edible plant with numerous uses in the middle region of Iran as food and spice especially yogurt seasoning, and as medicinal herb for anti-inflammatory and cardiovascular purposes. Antioxidant, anti-inflammatory and antihyperlipidemic properties of *K. odoratissima* suggest that it may have beneficial effects on inflammatory bowel diseases like ulcerative colitis. In the present study, the effect of this plant on a model of acute colitis was evaluated. Different doses of hydroalcoholic extract of *K. odoratissima* (125, 250, 500 mg/kg) were administered orally (p.o.) to the separate groups of male Wistar rats (n = 6). It was started 4h before induction of colitis and continued on a daily basis for 3 consecutive days. Wet colon weight/ length ratio and tissue damage scores and area as well as indices of colitis and tissue myeloperoxidase (MPO) activity were evaluated for each specimen. Two lower doses of extract (125, 250 mg/kg) were effective to reduce all the indices of colitis and MPO activity in different assays. By increasing the dose, (500 mg/kg) the efficacy was declined suggesting a possible reverse dose related effect. It is concluded that *K. odoratissima* has anti-inflammatory action in rat model of colitis but further detailed studies are recommended to identify the mechanisms that are involved and the active constituents that are responsible for these findings.

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Introduction

Umbelliferae includes more than 450 genera and about 3700 species around the world [1]. *Kelussia*is one of the newest genera of this family and is represented by only one species, *Kelussia odoratissima* Mozaff. (*K. odoratissima*), which is found only in Iran [2]. This sweet- smelling, self-growing monotypic medicinal plant is endemic to a restricted area in western Iran and is locally called Karafse-Koohi. The aerial parts of the plant are commonly used as a popular garnish and a sedative medicinal plant. In Iran, this plant is consumed as a medicinal plant to treat hypertension, inflammation ulcers, and cardiovascular diseasesin folk medicine [3].

The antioxidant activity of the methanolic extract of the plant was evaluated by several methods including β-Carotene bleaching assay, reducing power, thiocyanate accelerated oxidation of sunflower oil, and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging which was effective in all assays [3,4].In spite of its in vitro and in vivo effectson lipid profile [5]the results could not confirm significant efficacy in clinical trials, except for an increase in high density lipoprotein (HDL) levels [6]. Furthermore, feeding rabbits with the *K. odoratissima*aerial parts extract exerted some beneficial effects on fatty streak prevention [7].

Besides, gastric acid secretion has been reduced meaningfully in rats fed with *K. odoratissima*, but pepsin secretion was unaffected [8]. Essential oil of the plant showed a pronounced anti-bacterial effect while ethnopharmacologic studies proposed anti-inflammatory action in carrageenan test in rat model [9, 10].

Inflammatory bowel disease (IBD) is characterized by chronic intestinal inflammation and could be found in two forms; Crohn's disease and ulcerative colitis. Although immunologic mechanisms have been postulated as important participants in ulcerative colitis, its etiology and pathophysiology are still unknown. Sulfasalazine, mesalamine and 5-ASA derivatives, glucocorticoides and immune-suppressive agents are among the current medications for which limited efficacy and various side effects are common [11]. Because of the lack of specific and curative treatments with limited toxicity, there is a growing need to develop safe and effective alternatives for IBD [12]. Anti- inflammatory, anti-ulcerative as well as antioxidant properties of *K. odoratissima* suggests that it may have beneficial effects on IBD [3, 4, 10].

The aim of this study is to determine protective effects of *K. odoratissima* hydroalcoholic extract using oral route administration in acetic acid model of colitis in rats.

Materials and Methods

Chemicals

Prednisolone powder was procured from Valeant Pharmaceutical Co. (Montreal, Canada). All of the organic solvents and acetic acid were purchase from Merck Company (Darmstadt, Germany).

Plant material and preparation of extract

Aerial parts of *K. odoratissima* were collected from Zard-Kooh Mountains, Charmahal-e-Bakhtiari province (south western Iran) at March 2012.

The plant identity was confirmed by Pharmacognosy Deportment of School of Pharmacy, Isfahan University of Medical Sciences. A voucher specimen of plant coded 2022 was deposited at Pharmacognosy Department of the Isfahan Pharmacy School.

For preparation of hydroalcoholic extract, air-dried and finely powdered aerial parts of plant (840g) were macerated with 2580ml of EtoH-H20 (7:3) for 24 hours. The extract was then shaken, filtered and evaporated in a rotary evaporator under reduced pressure until a semisolid extract was obtained. Moreover, the concentrated extract was freeze-dried to obtain a dry powdered extract with yield value of 52 % (W/W).

Animals

Thirty six male Wistar rats (225 ± 25g body weight) prepared from animal house of Isfahan School of Pharmacy (Isfahan, Iran) were allowed to adapt to our laboratory environment for one week. They had free access to tap water and rat chow pellets and were housed singly in wire- bottomed cages under uniform and controlled conditions of temperature (20-22°c), humidity and light/dark (12/12 h) cycles. The experiments were made according to the Ethics and Research Committee of Isfahan University of Medical Sciences, Isfahan, Iran.

Animal grouping

Six groups of ratswith6 animals in each group were studied. Sham group; Normal rats received vehicle

(1ml normal saline, *p.o.*) without ulcer induction. Control group; Rats with induced colitis received vehicle (1ml, *p.o*) 2h before ulcer induction. Reference group; Rats with induced colitis received prednisolone (4mg/kg, *p.o.*) 2h before ulcer induction. Test groups; Rats with induced colitis received increasing doses of *K. odoratissima* hydroalcoholic extract (KOHE) (125, 250, 500 mg/kg, *p.o.*), 2h before ulcer induction. The treatments were continued daily for 3 days and the animals were euthanized by ether overdose inhalation 24h after the last dose.

Experimental protocol

The test samples including solutions or suspensions of drug or plant extract were freshly prepared. The plant extract was prepared as a suspension in 0.2% V/V Tween 80. Acute colitis was induced by 2 ml acetic acid (4%) using a technique which was first introduced by Mascolo *et al.* [13]. Briefly, rats were fasted for 24h with free access to tap water and observed to ensure their health before induction of colitis. The rats were lightly anesthetized with ether. A flexible plastic rubber catheter with an outside diameter of 2mm was inserted 8cm into the colon and then the rats were maintained in a head-down position for 2min to prevent solution leakage. In sham operated and control group, normal saline (2ml/ rat) was instilled.

Colon biopsies were taken for macroscopic scoring of injured tissue, histopathological examination and measuring myeloperoxidase (MPO) activity.

Assessment of colon macroscopic damage

The abdomen was opened and the colon, 8cm in length and 2cm proximal to the anus, was excised and incised longitudinally and washed with normal saline. Wet colon was then weighed and weight/length ratio was determined for each specimen. Photos of colon segments were taken by Sony® camera, transferred to a personal computer and analyzed subsequently by Fiji Image Processor [14] for measuring the ulcerated areas. Then, macroscopic mucosal damage was evaluated using a validated grading scale according to Morris et al [15]. Scores were: O=no ulcer, 1=mucosal edema, slight bleeding or slight erosions, 3=moderate edema, bleeding ulcers or erosions, 4=severe ulceration, erosions, edema and tissue necrosis and perforation.

Ulcer index was determined by summing the ulcer score and the ulcer area for each colon [16]. For further assessments, the tissue samples were cut into two equal parts longitudinally, a part was stored immediately at-20°C till biochemical analysis (MPO determination) and the other part were stored in 10% formalin for pathological evaluation.

Assessment of colonpathology

Fixated colon tissue was dehydrated; paraffin embedded, processed, sectioned in 4μm thick sections, and stained with haemotoxylin and eosin (H&E). Inflammation and crypt damage were assessed on H&E- stained and coded sections using a validated scoring scheme set up in our laboratory[14].Total colitis score as the sum of the 3 following sub scores (inflammation severity, inflammation extent, and crypt damage) was measured for each specimen finally. Pathological evaluation and scoring was performed using a Zeiss® microscope equipped with a Sony® color video camera for digital imaging.

Assessment of colonic MPO activity

The colitis induced by acetic acid was associated with an increase in MPO activity. MPO activity, an indicator of polymorph-nuclear leukocyte accumulation, was determined by a previously described method [14]. To measure the enzymatic activity of MPO, samples were removed from the freezer and after melting, they were chopped into small pieces. Then one hundred milligrams of colon mucosal scraping were homogenized in a solution contained 0.5% hexadecyl trimethyl ammonium bromide dissolved in 50μm potassium phosphate buffer (pH=6), before sonication in an ice bath for 45s for four times. The homogenates were freeze-thawed three times, repeating the sonication after which they were centrifuged for 15 min at 15000 rpm.

The level of MPO activity was measured at 450 nm spectrophotometrically: for this 0.1ml of the solution was mixed with 2.9ml of 50μm phosphate buffer, pH 6.0, containing 0.167 mg/ml O-dianisidinedihydro-chloride.

MPO activity was defined as quantity of enzyme degrading 1μm of peroxide per minute at 25°C and was expressed in units per gram (U/g) of wet tissue.

Statistical analysis

Statistical analysis was performed using SPSS 14.0 statistical software. Differences among groups were examined using parametric one-way analysis of variance (ANOVA) with Tukey HSD as post hoc test. Non-parametric data was analyzed by Kruskal-Wallis followed by Mann-Whitney U test. Results are expressed as the mean±SEM. The minimal level of significance was identified at $P<0.05$.

Results

Macroscopic presentation

As it is shown in figure 1 and table1, macroscopic observation in control group showed maximum ulcer area, ulcer severity and weight/length ratio which are indicative of highest level of damage produced by acetic acid compared to sham (normal) group that showed no change. Data from the group treated with prednisolone orally as positive control showed significant healing ($P<0.001$) in all macroscopic parameters (table1). Pretreatment with KOHE 250 and 125mg/kg,*p.o.* was effective to attenuate all macroscopic parameters including ulcer severity, area and index and even weight to length ratio (at least $P<0.05$).Pretreatment with the largest dose of KOHE (500mg/kg, *p.o.)* was not effective on assessed parameters in comparison to control group while didn't aggravate the colitis indices too ($P>0.05$).

Table 1. Effects of *K .odiratissima* Mozaff. Hydroalcoholic extract (KOHE) on macroscopic parameters of colitis induced by acetic acid in rats.

Groups	Route of administration	Score (0-4)	Ulcer Area (Cm^2)	Ulcer Index (0-12)	W/L ratio
Sham	p.o	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	67.7 ± 5.6
Control	p.o.	4.0 ± 0.0	5.5 ± 0.6	9.5 ± 0.6	240.7 ± 3.6
Pred. 4	p.o.	1.3 ± 2.1	1.0 ± 0.2***	2.3 ± 0.4***	106.2 ± 2.3***
KOHE125	p.o.	3.2 ± 0.4*	2.3 ± 0.8***	5.5 ± 1.1***	136.6 ± 2.2***
KOHE250	p.o.	1.2 ± 0.2***	0.2 ± 0.1***	1.4 ± 0.1***	102.4 ± 2.2***
KOHE500	p.o.	3.5 ± 0.2	5.3 ± 0.1	8.8 ± 0.6	244.4 ± 3.2

Data are expressed as mean ± SEM. P.O.= Oral, W/L= Weight tolength ratio, Pred.= Prednisolone(4mg/ kg), KOHE (125, 250, 500mg/ kg) (n=6). *$P<0.05$, **$P<0.01$, ***$P<0.001$ denote significant difference versus control group.

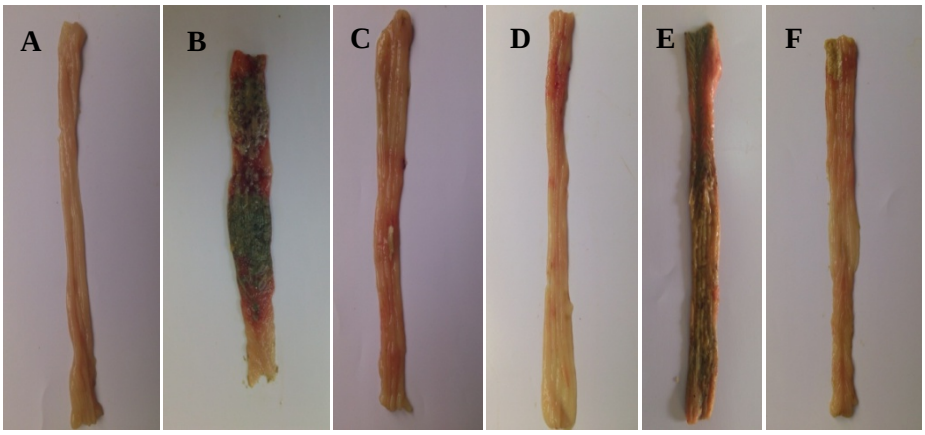

Fig. 1. Macroscopic presentation of acetic–acid-induced colitis in rats. A: Normal colon treated with vehicle, 2mg/kgB: Control colitis treated with vehicle 2mg/kgC: *K. odoratissima* hydro-alcoholic extract treated colitis 125 mg/kg p.o; D: *K. odoratissima*hydro-alcoholic extract treated colitis, 250mg/kg p.o. E: *K. odoratissima* extract treated colitis500mg/kg p.o ; F:Prednisolon treated colitis, 4 mg/kg.

Histological presentation

In accordance with table 2 and figure 2, no histological damage was observed in sham (normal) group. In control group in which colitis was induced by acetic acid instillation and treated with normal saline, microscopic assessments revealed severe inflammation and infiltration of white blood cells (leukocytes) in mucus and sub-mucosal layers(figure 2). Prednisolone by oral administration was effective to reduce inflammation severity and extent as well as crypt damage and total colitis index in injurious colons (table 2). As shown in table 2,hydroalcoholic extract of *K. odoratissima* at doses of 125mg/kg and 250mg/kg p.o., reduced all histological parameters of colon inflammation and edema (P<0.001), but pretreatment with the highest doseat 500 mg/kg couldn't significantly affect any of the histological parameters.

Table 2. Effects of *K. odiratissima* Mozaff. hydroalcoholic extract (KOHE) on microscopic parameters of colitis induced by acetic acid.

Groups	Route of administration	Inflam. severity	Inflam. extent	Crypt damage	Total colitis index
Sham	p.o	0.0 ± 0.0	0.0 ± 0.0	0.0 ±0.0	0.0 ± 0.0
Control	p.o	3 ± 0.0	3.0 ± 0.0	4.0 ± 0.0	10.0 ± 0.0
Pred.4	p.o	0.3 ± 0.2***	0 .8 ± 0.2***	0.3 ± 0.2***	1.5 ± 0.3***
KOHE125	p.o	1.3 ± 0.3***	1.7 ± 0.2***	2.2 ± 0.2***	5.2 ± 0.5***
KOHE250	p.o	0.8 ± 0.3 ***	0.3 ± 0.2***	0.2 ± 0.2***	1.33 ± 0.6***
KOHE500	p.o	2.7 ± 0.2	2.8 ± 0.2	3.7 ± 0.2	9.2 ± 0.4

Data are expressed as means ± SEM. P.O.= Oral, Inflam.=Inflammation, Pred.= Prednisolone (4mg/ kg), KOHE (125, 250, 500 mg/kg) (n=6). *P<0.05, **P<0.01, ***P<0.001 denote significant difference versus control group.

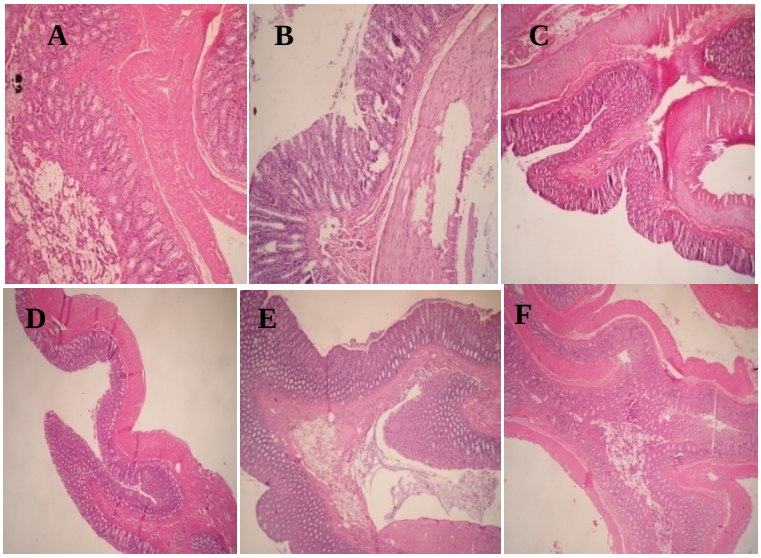

Fig. 2. Microscopic presentation of acetic acid-induced colitis in rats (H&E staining with **X**40 magnification).A: Sham, normal colon treated with normal saline, 2 ml/kg; mucus layer and crypts are normal and leukocyte infiltration is absent. B: Control colitis treated with normal saline, 2 ml/kg; mucosal and sub-mucosal inflammation as well as crypt damage and leukocyte infiltration are vastly evident; C: Colitis treated with*K. Odoratisma* hydro-alcoholic extract (KOHE), 125mg/kg .p.o.; D: 250 mg /kgE) 500 mg/kg administered p.o. F: Prednisolone treated colitis, 4 mg/kg.

Biochemistry Assessment

It has been reported that colitis caused by acetic acid was associated with an increase in MPO activity, a marker of neutrophil infiltration. Our data (figure 3) indicated that treatment with two doses of KOHE (125mg/kg and 250mg/kg, p.o.) could reduce MPO activity ($P < 0.001$) while at the highest test dose (500mg/kg) there was no change in its level ($P < 0.05$).

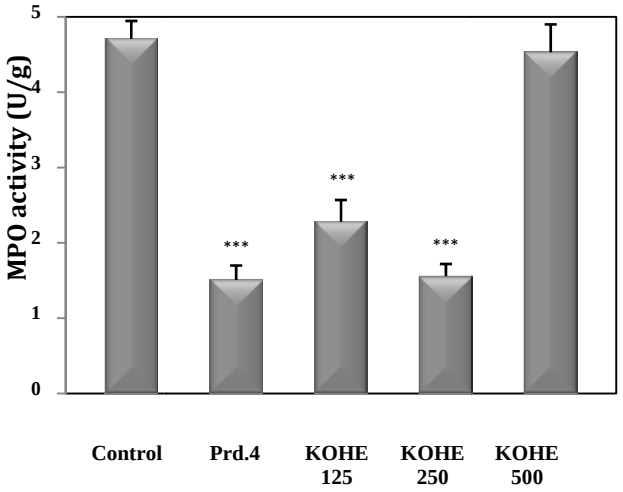

Fig. 3. Effect of oral administration of *K. odoratisma* hydro-alcoholic Extract (KOHE) (125,250,500mg/kg) and prednisolone (Prd. 4mg/kg) on myeloperoxidase activity (MPO, U/g wet tissue) of colon tissue, 4 days after acetic acid instillation in rats. The results are expressed as means ± SEM, (n=6). $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ denote significant difference versus control group.

Discussion

Acetic acid induced colitis is a model where inflammatory mediators such as reactive oxygen species, vasoactive amines and ecosanoides play important roles. The underlying pathophysiological mechanisms involved colon structure and mucosal barrier destruction by chemical stimulation, enhanced vessel permeability, increased inflammatory mediators, hydrolysis and disturbance of clotting process [17]. The wet weight of the inflamed colon tissue isconsidered a reliable and sensitive indicator of the severity and extent of inflammatory response [18]. In the present study, pretreatment with KOH at doses 125 and 250 mg/kg, p.o significantly reduced the wet weight of colon in acetic acid induced colitis.

With regard to the visual (macroscopic) and microscopic examinations, it was found that hydro-alcoholic extract of *K. odoratissima* (KOHE) orally could alleviate colitis at doses 125 and 250 mg/kg while the highest dose (500 mg/kg) was not effective. It seems that the healing effect of KOHE is related to the dose in which by increasing in dose the beneficial effects will be disappeared. This could be attributed to probably some active harmful constituents that exist in minute concentrations in KOHE which are absorbed in GI tract and oppose with therapeutic actions of other beneficial active ingredients.

According to the tables 1 and 2 it is concluded that KOHE at least in two doses 125 and 250 mg/kg were effective in colitis treatment after oral use. This is probably due toabsorption of active components that are beneficial in this model and/or may be due to a local effect produced by non-absorbable compounds which exist probably in plant extract reaching to distal colon by oral route of administration. It is supposed that treatment of animals with oral administration of *K. odoratissima* extract for a period of 4 days provide a suitable condition for systemic absorption and/or local availability of active plant constituents throughout the GI tract. To confirm this hypothesis, a non-oral route of test extract administration is strongly recommended in future studies. Moreover, by referring to tables 1 and 2 it is concluded that the highest test dose of extract (500mg/kg) could not aggravate colitis parameters in comparison to control group. This result could be pointed out with a combination of active ingredients with different mechanisms of action exist in applied extract have limited efficacy in this model. Similar findings obtained in a previous work conducted in our laboratory which indicated that *Helichrysum-oligocephalum* with lower dose (100mg/kg) was effective on acetic-acid induced colitis when administered both orally and parentrally while at higher doses of 200 and 400 mg/kg was not effective in comparison to control group [19]. As observed with the doses 125 to 250 mg/kg healing effects can be seen in all parameters. In agreement with previous phytochemical analyses carried out on total hydro-alcoholic extract of *K. odoratissima*, it is found that rutin, 3,4,7 trihydroxy flavonol, caffeic acid and phthalide are among its active constituents for which inhibition of prostaglandins (PGs) and leukotriene (LTs) synthesis has been shown [3].They have also anaglycon structure which could be rapidly absorbed

in GI tract [20]. In Iranian Traditional Medicine, the use of this plant has also proposed for inflammatory conditions like asthmatic cough. This property has also been examined in a rat paw edema model induced by carrageenan and the results were conclusive [10]. According to some more studies this plant has protective effects on liver [21], and free radical scavenging properties [3]. Additionally analysis of plant extract has shown that many different components exist in this plant like flavonoids, phenolic compounds, phthalide compounds and fatty acids [22, 23]. It is assumed that phthalide compounds like E-ligustilidephthalide, 3-E butyldiencephthalide and Z-ligustilideare responsible for cancer protective property of *K. odoratissima* [24, 25]. Regarding to the different active ingredients exist in KOHA for which many biological activities are probable, more studies are required to determine the exact mechanism of therapeutic effect of *K. odoratissima* extract on IBD .

Conclusion

In conclusion, our results suggest an advantageous therapeutic activity for hydro-alcoholic extract of *K. odoratissima*as an anti-inflammatory and anti-ulcerative medicinal plant for IBD condition and/or its prevention, especially when it is administered with critical dose monitoring. More fractionation of the extract can separate beneficial and harmful active constituents and may lead us to some pure active components which have more efficacious and suitable constituents.

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Conflict of Interests

Authors certify that no actual or potential conflict of interest in relation to this article exists.

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