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The essential oil from *Kelussia odoratissima* Mozaff. fruits exerting potent analgesic and anti-inflammatory effects

[El aceite esencial de frutos de Kelussia odoratissima Mozaff. ejerce potentes efectos analgésicos y antiinflamatorios]

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Abstract: *Kelussia odoratissima* fruits are utilized in Persian traditional medicine as a painkiller and to prevent inflammation-based disorders. Considering the higher content of essential oil in the fruits, the oil's anti-inflammatory and analgesic activities were investigated via the paw edema triggered in mice and the writhing test and hot plate technique, respectively. It was observed that the 100, and 200 mg/Kg doses of the oil revealed an analgesic impact (p<0.001) considering the increment in the reaction time needed for the hot plate approach. Furthermore, 100 and 200 mg/Kg doses of the oil caused a reduction in the frequency of writhes in the mice (p<0.01 and p<0.001, respectively). Using all examined doses of the oil (25, 50, and 100 mg/Kg) caused inflammatory reduction (p<0.001). The findings indicated that the oil possess significant activities against acute inflammation. It had both peripheral and central pain-killing impacts. The main components 3-n-butylphthalide (28.3%) and germacrene D (17.3%) can be considered as the responsible compounds to manage the inflammation and pain.

Keywords: Essential oil; *Kelussia odoratissima*; Fruits; Anti Inflammatory; Analgesic

Resumen: Las frutas de *Kelussia odoratissima* se utilizan en la medicina tradicional persa como analgésico y para prevenir los trastornos basados en la inflamación. Teniendo en cuenta el mayor contenido de aceite esencial en las frutas, se investigaron las actividades antiinflamatorias y analgésicas del aceite a través del edema de la pata desencadenado en ratones y la prueba de contorsiones y la técnica del plato caliente, respectivamente. Se observó que las dosis de 100 y 200 mg / kg del aceite revelaron un impacto analgésico (p<0,001) considerando el incremento en el tiempo de reacción necesario para el enfoque de placa caliente. Además, dosis de 100 y 200 mg / kg del aceite provocaron una reducción en la frecuencia de retorcimientos en los ratones (p<0,01 y p<0,001, respectivamente). El uso de todas las dosis examinadas del aceite (25, 50 y 100 mg/kg) provocó una reducción inflamatoria (p<0,001). Los hallazgos indicaron que el aceite posee actividades significativas contra la inflamación aguda. Tiene impactos analgésicos tanto periféricos como centrales. Los principales componentes 3-n-butilftalida (28,3%) y germacreno D (17,3%) pueden considerarse como los compuestos responsables del manejo de la inflamación y el dolor.

Palabras clave: Aceite esencial; *Kelussia odoratissima*; Frutas; Antiinflamatorio; Analgésico

INTRODUCTION

The Monotypic genus, *Kelussia* belongs to the Apiaceae family and represents by just one species, *K. odoratissima* which is found only in Iran (Mozaffarian, 2006). As an endemic aromatic plant, it is restricted just an area in west of Iran (Central Zagros). This sweet-smelling, self-growing medicinal plant is locally called "Karafs-e-Koohi" and its different parts including the aerial parts and the fruits are commonly used as a popular sedative and pain killer natural agent and to treat inflammation-based disorders (Rabbani *et al*., 2011).

Different parts of *K. odoratissima* contain noticeable amounts of essential oil which consist of mainly the phthalide derivatives like ligustilid isomers and 3-n-butylphthalide. The aerial parts and the fruits of the *K. odoratissima* are used as anti-inflammatory, analgesic natural agents and for rheumatic treatment and blood purification in Iranian traditional medicine (Ahmadi *et al*., 2020).

The anti-inflammatory and analgesic activities of *K. odoratissima* fruits were assessed considering the extensive utilization of different preparations of the plant fruits in Iranian traditional medicine for treating and pain relief as well as inflammatory based disorders. Since the plant's fruits comprise a higher quantity of essential oil (2% <, v/w), we tried to assess the antinociceptive and antiinflammatory impacts of *K. odoratissima* fruits essential oil (KOFEO) for the first time and assess the pharmacological basis for its folkloric utilization as a natural analgesic and anti-inflammatory agent. In this work, the analgesic and antiinflammatory features of KOFEO are explored utilizing different standard experimental test models. GC-MS was also used to analyze the fruit oil and recognize the compounds potentially in charge of the observed features. This is the first effort to state the ethnopharmacological features of KOFEO usage as a pian killer and manage the inflammation-based disorders in a complete mode.

METHODS AND MATERIALS

Plant material

In June 2019, fully ripen *K. odoratissima* fruits were purchased from local market in Kurdistan Province, west of Iran. Dr. N. Kazemivash (Director of the Herbarium; Tehran Medical Sciences, Islamic Azad University, Tehran, Iran) of the Herbarioidentified the sample and a voucher was placed in the Herbarium of Tehran Medical Sciences, Islamic Azad University (517-PMP/A).

Essential oil extraction

The powdered fruits were exposed to hydro-distillation in a Clevenger instrument for 2 hours. Then, extracting the essential oil and drying via anhydrous Na_2SO_4 were performed and it was maintained in glass vials at -18℃ for further analyses.

Animals

The experiments were conducted on male Swiss albino mice (20 to 25 g, 2 months old) and Wistar male rats (2.5 months old, 200 to 250 g). The animals were provided by the animal house of Tehran University of Medical Sciences. Maintaining the normal diet in the animal house was considered at the light/dark cycle of 12/12 hours and a temperature of 21 ± 1℃. All nutritional parameters of this diet exceed or meet the National Research Council (NRC) guidelines for rats and mice (National Research Council, 2011). Groups of five rats and eight mice were randomly selected for the tests. All processes were based on the Guidance for the Care and Use of Laboratory Animals (National Research Council, 2011). The study was performed after getting approval from the Research Ethics Committee of Tehran Islamic Azad University of Medical Sciences (approval number of IR.IAU.PS.REC.1398.34). In all tests, euthanizing the animals was performed through cervical dislocation.

Analgesic activity

Writhing triggered by acetic acid in mice

We performed a writhing acetic acid test following the description of Darabian *et al*. (2017). This approach was used for assessing the potential peripheral impacts of the fruit oil as a painkiller agent. Six groups each with eight mice fasted for one night before initiation of the experiment, however, they had access to water. Animals in all six groups were intraperitoneally (i.p.) injected with the studied oil (25, 50, 100 and 200 mg/kg), 10 mL/kg of sweet almond oil, and 50 mg/kg of diclofenac sodium. After 30 min, the animals in all groups were i.p. injected with the 10 mL/kg of 1% acetic acid. Diclofenac sodium as a peripheral painkiller agent was utilized as a positive control in the current work. The number of placing writhes in the mice was counted 30 minutes after injecting the acetic acid in an observation box. (Darabian *et al*., 2017).

Hot plate test

The hot plate assay approach was used to evaluate the

potential centrally mediated analgesic impact of KOFEO. In addition to the analgesic medicine, diclofenac sodium (50 mg/kg, i.p.) was employed as a positive control. Six groups, with eight mice in each, were fasted overnight prior to starting the test, however, they had access to water. The mice with a weight range of 20-25 g were put on a plate and kept at room temperature for 15 minutes daily for 3 consecutive days before the tests. The oil was intraperitoneally injected into the first, second, third, fourth and fifth groups at 25, 50, 100 and 200 mg/kg doses. The vehicle (10 mL/kg, sweet almond oil) and diclofenac sodium were provided to the fourth and fifth groups following the same path. Then, the animals were put onto a hot plate at 55℃. Delayed analgesic responses such as jumping the hot plate or licking the paws were measured 15, 30, 45, and 60 minutes followed by giving the test materials or tools (Khodadadian *et al*., 2016).

Anti-inflammatory action

Paw edema test triggered by carrageenan

Severe anti-inflammatory action was evaluated in terms of inhibition of the paw edema caused by injecting carrageenan (0.1 mL, 2%) into the sub-plantar area of the mice's right hind paw (Sharif *et al*., 2020). The male rats were assigned to 5 groups, along with five rats per group. The rats received 50 mg/kg diclofenac (i.p.), the sweet almond oil (10 mL/kg, i.p.), and the oil (25, 50, and 100 mg/kg, i.p.), 1 h prior to the injecting of carrageenan. A plethysmometer (model PM 4500, Borj Sanat Co., Iran) was used, 0.5, 1, 2, 3, and 4 h after injecting the carrageenan to measure the paw volume (Bakhtiarian *et al*., 2012).

The anti-inflammatory effects were measured as edema inhibition percentage in comparison to the control group received the standard drug, diclofenac sodium. Furthermore, the edema inhibition percentage was determined as follows:

% edema inhibition = 100 (1-Vtest/Vcontrol)

where Vtest and Vcontrol denote the edema volume in the test group and control group, respectively.

Essential oil assessment

Analysis of KAFEO was performed on aHp-6890 gas chromatograph (GC) equipped with a FID and a DB-5 capillary column, 30 m × 0.25mm, 0.25 μm film thickness, temperature programmed as follows: 60°-240°C at 4°C/min. The carrier gas Helium (He) was at a flow of 2.0 ml/min; the temperatures of injector port and detector were 250°C and 300°C, respectively. Samples were injected by splitting and the split ratio was 1:10. The analysis of GC-MS was performed on a Hewlett-packard 6890/5972 system with a DB-5capillary column (30 m × 0.25 mm; 0.25 μm film thickness. The mass spectra were prepared at 70 eV. The mass scan ranged within 40 to 400 m/z with a sampling rate of 1.0 scan/s. The quantitative data of this study were acquired through electronic combination of the FID peak regions. The oil components were determined based on their retention time and retention indexes related to computer matching with the WILEY 275.L library, C_9-C_{28} n-alkanes, comparing the mass spectra of the components with those of original samples, and comparing them with the results presented in previous works (Swigar, 1981; Adams, 2017). The detected compounds' composition percentages were estimated using the GC peak areas with no correction factor and computed relative to each other.

Statistical analysis

The writhing triggered by acetic acid was compared using post-hoc Tukey's test and one-way analysis of variance (ANOVA). Carrageenan data and hot plate test data were checked using the post-hoc Tukey's test and two-way ANOVA. The findings were stated as Mean ± Standard Error. Moreover, P values of less than 0.05, 0.01, and less than 0.001 were regarded as the statistically significant difference between the means. The statistical GraphPad Prism 6.0 software was utilized for data analysis.

RESULTS

The yellowish oil with the strong odor was obtained by hydrodistillation of *K. odoratissima* fruits along with the yield of 2.2% (v/w) in terms of the fresh weight. Table No. 1 represents the list of compounds with GC/MS concentrations higher than 0.1% of the overall peak concentration. According to the Table No. 1, 28 components were recognized in the fruits essential oil representing about 81.7% of the total composition. The main elements of KOFEO were characterized as 3-n-butylphthalide (28.3%) and germacrene D (17.3%).

Table No. 1
GC-MS analysis of *K. odoratissima* fruits essential oil

Compound[a]	KI[b]	KI[c]	Percentage
α-Phellandrene	1008	1005	0.3
β-Phellandrene	1027	1024	4.4
cis-β-Ocimene	1036	1040	0.3
β-Citronellol	1225	1222	0.4
Bornyl acetate	1281	1280	0.2
Lavandulyl acetate	1291	1296	1.3
α-Cubebene	1347	1337	1.4
Citronellyl acetate	1357	1362	0.4
α-Ylangene	1370	1369	1.1
α-Copaene	1379	1382	3.8
β-Cubebene	1388	1390	0.2
β-Elemene	1397	1401	2.0
β-Caryophyllene	1422	1423	1.8
γ-Elemene	1433	1432	0.8
α-Guaiene	1441	1448	0.5
α-Humulene	1453	1452	1.1
β-Farnesene	1460	1463	0.3
β-Acoradiene	1464	1466	0.5
Germacrene D	**1473**	**1470**	**17.3**
β-Selinene	1488	1491	1.5
α-Selinene	1493	1495	2.5
γ-Cadinene	1515	1512	1.7
Δ-Cadinene	1533	1530	1.5
α-Cadinene	1543	1544	3.4
Eudesma-3.7(11)-diene	1551	1555	0.4
Butylidiene phthalide	1624	1630	3.7
3-n-Butylphthalide	**1659**	**1662**	**28.3**
Butylidiene dihydrophthalide	1682	1683	0.6
Total			81.7

[a]Compounds presented in order of elution
[b]KI (Kovats index) measured relative to *n*-alkanes (C_9-C_{28}) on the non-polar DB-5 column under condition provided in the Materials and Methods section
[c]KI, (Kovats index) from literature

Analgesic activity of KOFEO

By the intraperitoneal presentation of the 100 and 200 mg/kg of the studied oil (p<0.01 and p<0.001, respectively), a considerable reduction was resultant in the number of acetic acid-triggered writhes in mice in comparison to those receiving only the vehicle (Figure No. 1).

The hot plate test revealed that the intraperitoneal injection of the examined doses of the oil (100, and 200 mg/kg) (p<0.001) increased the reaction time significantly at different time points 30 min after the treatment in comparison to the control groups. As can be seen from Figure No. 2, the

nociception delay at 30 to 60 was somehow near to the standard drug, diclofenac sodium.

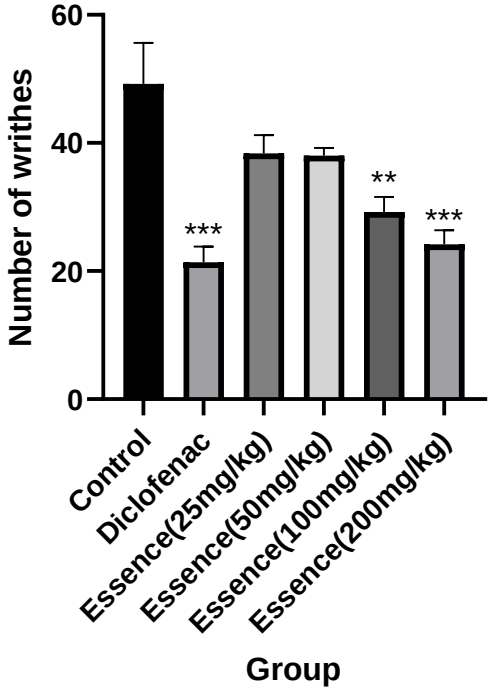

Figure No. 1
Impact of pretreatment with *K. odoratissima* fruits oil (25, 50, 100, and 200 mg/kg, i.p.), diclofenac, and the vehicle on acetic acid stimulated writhing in mice. The values are presented as the mean ± SEM (n=8 per group). **p<0.01 and ***p<0.001 compared to the control group using one-way ANOVA and Tukey's post hoc test

Antiinflammatory activity of FAFEO

The severe anti-inflammatory impacts of the fruit oil, carrageenan-triggered edema in rat paw were examined by injecting the essential oil intraperitoneally (Table No. 2). Based on the obtained results, by the essential oil (25, 50, and 100 mg/kg) and 50 mg/kg of diclofenac sodium, the carrageenan-triggered edema was significantly prevented in the rat paw.

The rat paw edema formation triggered by carrageenan was inhibited by all the examined doses of the studied oil (25, 50, and 100 mg/kg) (p<0.001) compared to diclofenac sodium (50 mg/kg) from the first to the fourth h of the experiment.

DISCUSSION

Various products of *K. odoratissima* aerial parts and the fruits are widely utilized in ethnobotanical measures for treating inflammation and pain in Iran. In the current research, the effectiveness of the essential oil extracted from *K. odoratissima* fruits was assessed because of its several volatile constituents. As we know, this is the first research explaining the analgesic and anti-inflammatory actions of the studied oil. The findings revealed that the essential oil of this plant reduced the abdominal constriction, suggesting prevention of the prostaglandin synthesis by the cyclooxygenase pathway. Moreover, the effect of this oil was identified on the central and peripheral levels, expressed as a biphasic licking response. The main action was approved in the hot plate experiment (100, and 200 mg/kg) representing the maximum impact 30 min after the response.

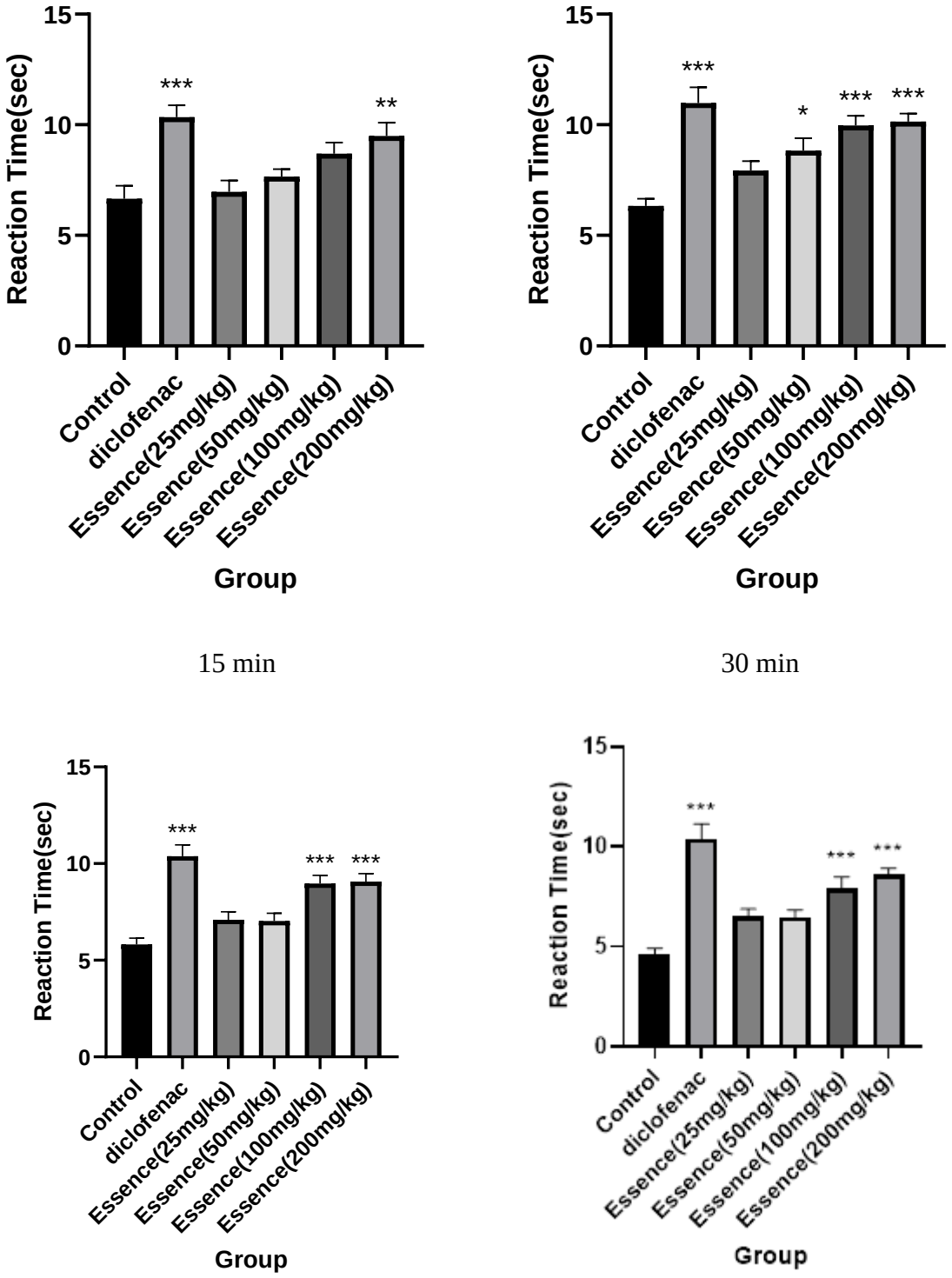

Figure No. 2
Effect of pretreatment with *K. odoratissima* fruits oil (25, 50, and 100 mg/kg, i.p.), diclofenac (50 mg/kg), and the vehicle (sweet almond) on hot plate test in mice; the values are stated as the mean ± SEM (n=5 per group). *p<0.05, **p<0.01 and ***p<0.001 compared to the control group using two-way ANOVA and Tukey's post hoc test

Table No. 2
Effects of KOFEO on rat paw edema stimulated by carrageenan; Each value represented the mean ± SEM (n=5)

Groups	Dose (mg/kg)	Edema inhibition percentages at different time intervals (%)				
		30 min	60 min	120 min	180 min	240 min
Diclofenac	50	2.34 ± 0.56	6.33 ± 1.82	74.83 ± 4.22	88.45 ±0.35	85.55 ± 0.96
FAFEO	25	1.56 ± 0.83	5.16 ± 1.53	59.35 ± 9.53	59.99 ± 5.54	64.19 ± 6.24
FAFEO	50	5.62 ± 1.82	4.20 ± 1.13	64.51 ± 5.31	66.91 ±4.91	76.83 ±7.38
FAFEO	100	3.12 ± 0.75	5.16 ± 1.33	69.67 ± 3.91	75.37 ± 10.61	65.78 ± 9.52

Since the essential oil is composed of many components with different portions and synergies between the compounds, it is not possible to determine the exact responsible compound for KOFEO observed activities impacts by not evaluation the compounds separately. Based on the obtained phytochemical results, 3-n-butylphthalide (28.3%) as the major component of KOFEO comprised about one third of the oil composition and might be guested to be one of the responsible compounds for painkiller impacts of the essential oil. 3-n-Butylphthalide is a compound initially isolated from the seeds of *Apium graveolens* (Apiaceae) and correspond to a family compounds named phthalides. Phthalides, and their derivatives are components of some genera from the Apiaceae family. They are bioactive secondary metabolites and possess important molecular and cellular activities including the inhibition of DNA methyltransferases, stimulation of glutathione transferase, antiproliferative effects on colon cancer cells and protective effects on focal cerebral ischemia in rats (Rabbani *et al*., 2011).

In the field of the botanical medicine, however there is no reports of any association of pure 3-n-butylphthalide in the essential oils with the analgesic activities, it is concluded from the results that presence of noticeable amounts of 3-n-butylphthalide in KOFEO could be guested the key responsible compound representing the significant analgesic effects of the studied oil. However, further evaluations on the analgesic and painkiller activities of the pure 3-n-butylphthalide are required to prove this conclusion.

Regarding the other germacrene D rich essential oils with significant analgesic activity (Del-Vechio-Vieira *et al*., 2009), germacrene D as the second major component (17.3%)of the studied oil might have a role in analgesic activity of KAFEO. However, it could not be concluded without evaluation on the pure germacrene D analgesic effects.

KOFEO also indicated activity against acute inflammation. Edema triggered by carrageenan was commonly utilized as an investigational and biphasic animal model for acute inflammation. A considerable reduction was found by the findings for paw edema at all studied doses particularly 100 mg/kg. The assessed oil showed significantly somehow equal inhibitory activity within 4 hours in the carrageenan-triggered paw inflammation test compared to the diclofenac sodium-treated group. The fruit oil's anti-inflammatory activity can be associated with the inhibition of NO formation or release. Based on several reports, NO, peripherally created by various NOS (nitric oxide synthase) isoforms has a role in edema formation (Rivot *et al*., 2002). According to the studies, the bradykinin liberation was triggered by injecting carrageenan into the rat paw later triggering the biosynthesis of prostaglandin and other autacoids. These autacoids were in charge of the inflammatory exudates formation (Ueno *et al*., 2000).

Previously studies on cultured astrocytes indicated that 3-n-butylphthalide possessed partial anti-inflammatory effects by acting as a potential inhibitor of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). It inhibits Aβ-induced activation of astrocytes and suppresses the upregulation of cyclooxygenase-2 (COX-2), prostaglandin E2 (PGE2), tumor necrosis factor alpha (TNF-α), and interleukin 6 (IL-6). In regards, the 3-n-butylphthalide could be guested to play a role against AD-associated neuroinflammation and astrocytes

activation (Wang *et al*., 2013).

Based on the reports of the previous investigations on the germacrene D rich essential oils (Del-Vechio-Vieira *et al*., 2009), it could be the other guested responsible compound to exert anti-inflammatory activities.

Further investigations are suggested to evaluate the analgesic and anti-inflammatory activities of the pure 3-n-butylphthalide and germacrene D and the possible probable synergies between them and comparing the results with those observed in the current study.

Such findings explain using *K. odoratissima* fruits in Iranian traditional medicine. Therefore, the plant essential oil is a potential option as an anti-inflammatory and analgesic agent particularly for use as a key active constituent in ointments, creams, and balms that are provided as topical anti-inflammation and analgesics.

CONCLUSIONS

The findings of this study show the existence of biologically active ingredients in KOFEO with considerable activity against acute inflammations. These constituents have peripheral and central anti-nociceptive effects justifying their use in relieving inflammation and pain.

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ETHICS APPROVAL AND CONSENT FOR PARTICIPATION

The work was performed after approval by the Research Ethics Committee of Tehran Islamic Azad University of Medical Sciences, with the approval number of IR.IAU.PS.REC.1398.34.

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