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Research Article

Potential anti-inflammatory activity of the *Tamarix aphylla* essential oil

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Abstract

Aim: This study aimed to assess the anti-inflammatory activity and potential mechanisms of essential oil of *Tamarix aphylla* (EOTA).

Methods: The essential oils were extracted from the plant's aerial parts using hydrodistillation and analyzed through gas chromatography and mass spectrometry (GC/MS). The essential oils were assessed for their anti-inflammatory effects using well-established inflammation models, namely carrageenan-induced paw edema and peritonitis. To evaluate the antioxidant activity of the essential oil, measurements were taken for nitric oxide radical-scavenging activity and lipid peroxidation.

Results: The predominant components of the EOTA were 6,10,14-Trimethyl-2-pentadecanone (20.2%), β-Ionone (20.1%), Dodecanoic acid (12.2%), and trans-β-Caryophyllene (10%.1). The study found that EOTA significantly reduced edema, peritonitis, myeloperoxidase activity, and NOx-peritoneal lavage concentration induced by carrageenan. Additionally, the essential oil exhibited significant inhibition of nitric oxide radical production triggered by sodium nitroprusside. Furthermore, EOTA demonstrated the ability to prevent lipid peroxidation induced by Fe+2- or Fe+2 plus H2O2.

Conclusion: The findings suggest that EOTA possesses anti-inflammatory activity, potentially linked to its antioxidant capacity.

Keywords

Tamarix aphylla, essential oil, anti-inflammatory, antioxidant

Introduction

The inflammatory process involves a range of pathological and physiological activities (Park et al. 2017). One prominent feature of this reaction is the movement of leukocytes from the bloodstream to the affected tissues, which takes place in a series of sequential steps. Inflammation is closely linked to oxidative processes as they share similar pathways (Kunsch and Medford 1999). Any disruption in these mechanisms can contribute to the development of various diseases. In addition to inflammation, elevated levels of free radicals have been observed in various pathological conditions such as cancer and ischemic disorders (Harput et al. 2012). Consequently, researchers worldwide have shown great interest in exploring indigenous remedies and their potential effects and benefits (Süntar

2020). The search for new therapeutic agents heavily relies on the investigation of natural sources in the context of ethno-pharmacological studies. Essential oils (EO), which are naturally occurring in plants and primarily composed of monoterpenes, have gained widespread use in the treatment of inflammation and pain (Miguel 2010).

Tamarix aphylla (*T. aphylla*) L., a medium-sized tree found across Africa, the Middle East, and parts of Southern and Western Asia, belongs to the Tamaricaceae family (Jasiem et al. 2019). In Jordan and other countries in the region, the aerial parts of the *T. aphylla* plant have been traditionally used for wound healing, abscess treatment, as an astringent, for relieving abdominal pain, fever, rheumatism, and joint pain (Alzweiri et al. 2011). In Saudi Arabia, the alcohol extract derived from *T. aphylla* leaves has exhibited antioxidant, anti-inflammatory, and wound-healing properties (Abo-Dola et al. 2015). The plant has also demonstrated analgesic and antipyretic activities (Alrumman 2016). Previous studies have highlighted the antimicrobial properties of *T. aphylla* (Alshehri et al. 2021). Various secondary metabolites have been identified in *T. aphylla*, including tannins, flavonoids, alkaloids, isoferulic acid, and ellagic acid, which contribute to its high antifungal potential (Bibi et al. 2015). While the chemical compositions and antiproliferative effects of the essential oil obtained from the aerial parts of *T. aphylla* have been investigated and reported in relation to selected cancer cell lines (Alhourani et al. 2018), the anti-inflammatory effects of the essential oil remain unknown. Hence, this study aims to assess the anti-inflammatory activity and potential mechanisms of the essential oil extracted from *T. aphylla* aerial parts using classical models of inflammation.

Materials and methods

Plant material

In April 2022, the aerial parts of *T. aphylla* were collected from North Amman, Jordan. The plant material was authenticated by Jamil Salam, serving as a botanist. To ensure future reference, a voucher specimen was submitted to the Hashemite University herbarium in Zarqa, Jordan, with the assigned Herbarium number HU.No. 3884. For the extraction of essential oil (EO), 500 g of dried aerial parts of *T. aphylla* were subjected to hydrodistillation. A Clevenger-type apparatus was used for the extraction process, with different durations of 1,2,4 hours. The maximum yield of EO (0.4%) was obtained after 4 hours (Siani et al. 2004).

Determination of essential oil composition

The GC analysis of *T. aphylla* L essential oil was conducted using a Trace GC ULTRA with flame ionization detector (FID) gas chromatograph. The instrument was equipped with a column (30 m × 0.25 mm × 0.25 µm) type VB-5 (methylpolysiloxane with 5% of phenyl) and a split injection. Mass spectrometry (MS) analysis was carried out using a Polaris Q MS mass spectrometer (with an ion-trap at 70 eV). The column temperature was programmed from 40 °C for 2 minutes and raised to 180 °C at a rate of 4 °C/min. The carrier gas used was helium, with a constant flow rate of 1.4 mL/min. The volatile constituents of the essential oil were identified by automated comparison of their mass spectra with that of the NIST (National Institute of Standards and Technology) library (Stein et al. 1987). Quantitative data were obtained from peak areas using FID, and the areas were normalized using an internal standard (ethyl octanoate). Essential oil samples were analyzed in triplicate.

Experimental animals

The study utilized male albino rats (n = 126), 7–8 weeks old and weighing between 120 and 190 grams. These rats were obtained from the animal house at the Hashemite University. They were housed in a controlled environment with constant access to food and water, following a 12-hour light/dark cycle. Before the experiments, the rats were allowed to acclimate to the laboratory conditions for a minimum of 2 hours. Each rat was used only once to ensure data integrity. All in vivo animal studies were conducted in compliance with the Ethics Committee for Animal Experiments at the Hashemite University, Jordan. The care and handling of the animals adhered to the guidelines set by the International Association for the Study of Pain for the ethical use of animals in pain research (Zimmermann 1983).

Acute toxicity test

In order to determine the lethal dose (LD50) of *T. aphylla* essential oil , rats were randomly divided into seven groups, each containing five rats. The experiment followed the protocol described by Lorke 1983 (Lorke 1983). The rats in six of the groups were orally administered different doses of the essential oil of *T. aphylla* (EOTA), specifically 10, 100, 500, 1000, 2000, and 3000 mg/kg. The seventh group, serving as the control group, received 1% v/v Tween 80 in sterile saline at a volume of 10 ml/kg. Throughout the experiment, the rats had unrestricted access to food and water. The rats were observed for signs of toxicity and mortality over a period of 72 hours, and these observations were recorded for analysis.

Assessment of *Tamarix aphylla* essential oil anti-inflammatory effect through Carrageenan-induced rat paw edema model

To evaluate the in vivo anti-inflammatory effect of the EOTA, the experiment was conducted on rats. The rats were fasted for 24 hours prior to the experiment but had free access to water during this period (Amanlou 2005). A total of six groups were formed, with each group consisting of six rats.

Group 1 served as the negative control and received the vehicle, which was administered orally as 0.2% Tween 80. Groups 2 to 5 received varying concentrations of the essential oil orally at doses of 10 mg/kg, 31.6 mg/kg, 100 mg/kg, and 316 mg/kg, respectively. doses were calculated to be located at approximately 0.5 log units from each other on a log scale. Group 6, the positive control, received dexamethasone subcutaneously at a concentration of 2 mg/kg. To assess the anti-inflammatory effect of EOTA, a carrageenan-induced rat paw edema model was employed (Morris 2003). One hour after administration of the desired concentration of EOTA or the control substances, acute paw edema was induced by injecting 0.1 ml of a 1% freshly prepared carrageenan suspension in normal saline into the right hind paw of each rat. The paw volume was measured before (0 h) and at intervals of 1, 2, 3, 4, 5, and 6 hours after the carrageenan injection using a water displacement method with a plethysmometer (Choi et al. 2005) (type 7140 Ugo Basile, Italy. The anti-inflammatory action of EOTA was calculated as a percentage using the following formula: (paw final volume – initial volume) / initial volume × 100.

Leukocyte migration into the peritoneal cavity

Rats (n = 6/group) were subjected to intraperitoneal injection of carrageenan (1% 250 µL, i.p.) one hour after oral administration of EOTA at concentrations of 10, 31.6, 100, and 316 mg/kg. To assess leukocyte peritoneal infiltration, dexamethasone (2 mg/kg, s.c.) and vehicle (0.2% Tween 80 orally) were used as positive and negative controls, respectively. After four hours of carrageenan injection, the rats were euthanized, and 4 mL of saline containing EDTA (1 mM) were injected into the peritoneal cavity to collect peritoneal lavage. The resulting cell suspension was chilled on ice, centrifuged at 1500 rpm for 10 minutes, and the supernatant was discarded. The cell pellets were then resuspended in 1 mL of saline. Total cell count (number of cells/mL) was determined using a Neubauer chamber, and the cells were stained with Diff-3 stain to differentiate between leukocytes (polymorphonuclear and mononuclear cells).

Measurement of myeloperoxidase activity

The ability of EOTA to reduce inflammation was further demonstrated by its ability to inhibit the activity of myeloperoxidase enzyme. At the end of the 4-hour edema measurement, the paw tissue of the rats was homogenized in 50 mM phosphate buffer (pH 6.0) and 0.5% hexadecyl-trimethylammonium bromide. The resulting supernatants were mixed with o-dianisidine dihydrochloride (0.167 mg/mL, in 50 mM phosphate buffer) and 0.005% hydrogen peroxide. The samples' absorbance was measured at 460 nm using a spectrophotometer, and the results were expressed as units of myeloperoxidase (MPO) (UMPO)/mg of paw tissue. One UMPO represents the amount of enzyme that degrades 1 µmol of H2O2 per minute (Loftus et al. 2011).

Total nitrate/nitrite (NOx) concentration

To investigate the potential anti-inflammatory mechanisms of EOTA, rats were injected intraperitoneally with carrageenan (1% 250 µL) one hour after oral administration of EOTA (316 mg/kg), subcutaneous administration of Dexamethasone (2 mg/kg) as a positive control, or oral administration of the vehicle (0.2% Tween 80) as a negative control. After six hours of carrageenan injection, the concentration of NOx in the peritoneal lavage was measured spectrophotometrically at 546 nm using the Griess reagent (consisting of 5% H3PO4, 2% sulphanilamide, and 0.1% napthylethylenediamine dihydrochloride) (Bradley et al. 1982). A standard curve was constructed using known concentrations of sodium nitrite.

Determination of lipid peroxidation *in vitro*

The antioxidant capacity and free radical scavenging effect of EOTA were evaluated by measuring the inhibition of lipid peroxidation induced by FeSO4 (0.145 mM) or FeSO4 (0.145 mM) in combination with H2O2 (0.4 M). The concentration of oxidized lipids was determined by quantifying the level of thiobarbituric-acid reactive substances (TBARS), as described by Kizil and colleagues. The extent of lipid peroxidation was assessed by measuring the formation of malondialdehyde (MDA), which is a final product generated during lipid peroxidation (Tsikas 2017). Rat's liver was isolated and homogenized in a phosphate buffer (0.1% w/v in 50 mM phosphate buffer, pH 7.4). The homogenate was then incubated with different concentrations of EOTA (0.001, 0.01, 0.1, 1, 10, 100, 316 µg/ml) along with either 100 µl of 0.145 mM FeSO4 or 100 µl of 0.145 mM FeSO4 combined with 0.4 M H2O2. The mixture was incubated at 37 °C for 1 hour. After incubation, 0.3 mL of the samples was mixed with 0.6 mL of trichloroacetic acid (20% v/v), followed by centrifugation at 10,000 g at 4 °C for 15 minutes. Then, 0.5 mL of thiobarbituric acid (0.67% w/v) was added to the supernatant, and the mixture was heated at 100 °C for 30 minutes. The samples were transferred to a 96-well plate in triplicate, and the absorbance was measured at 532 nm using a spectrophotometer. The experiment included the same range of concentrations of ascorbic acid as a positive control. A standard curve was generated by serial dilution of freshly prepared MDA, which was used to determine the concentrations of TBARS in the samples (Feldman 2019).

Assessment of nitric oxide (NO) radical scavenging activity

The spectrophotometric quantification of nitric oxide (NO) radical scavenging activity was performed. Various

concentrations of EOTA (0.001, 0.01, 0.1, 1, 10, 100, 316 µg/ml) were added to separate test tubes before the addition of 0.5 ml of sodium nitroprusside in phosphate buffer saline (10 mM). After incubating all the tubes at 37 °C for 60 minutes, an equal volume of freshly prepared Griess reagent (consisting of 5% H3 PO4, 2% sulphanilamide, and 0.1% napthylethylenediamine dihydrochloride) was added to each tube. A control sample without plant essential oil, prepared in the same manner as the test samples, was used for comparison. As a positive control, the same range of concentrations of ascorbic acid was employed. Subsequently, 150 µL of the reaction mixture from each tube was transferred in triplicate to the wells of a 96-well plate, and the absorbance was measured at 546 nm

Nitric oxide scavenging activity was calculated as: Scavenging activity (%) = (1- [A sample /A control]) ×100 (Rajesh and Natvar 2011):

A sample: The absorbance values obtained from the samples containing plant essential oil.
A control: The absorbance values obtained from the control samples without any plant essential oil.

Statistical analysis

The collected data was presented as mean ± SEM and subjected to statistical analysis using GraphPad Prism 6. One-way analysis of variance (ANOVA) or two-way ANOVA was performed, followed by a Tukey post hoc test. The significance level was set at $P < 0.05$.

Results

Chemical characterization of essential oils

Table 1 presents the analysis and chemical compositions of the EOTA. A total of twenty-two compounds were detected in the essential oil derived from the plant, constituting 93.2% of the overall oil content. The predominant compounds identified were 6,10,14-Trimethyl-2-pentadecanone (20.2%), β-Ionone (20.1%), Dodecanoic acid (12.2%), and trans-β-Caryophyllene (10%.1).

Acute toxicity of plant essential oil

Rats were given varying doses of EOTA to evaluate their safety and estimate potential toxicity. The doses administered were 10, 100, 500, 1000, 2000, and 3000 mg/kg body weight of the aerial parts essential oil. Results showed that up to 3000 mg/kg was deemed safe to be used for in vivo investigations of the anti-inflammatory effects of EOTA. However, for anti-inflammatory and antioxidant studies, different doses of the plant essential oil were employed (10, 31.6, 100, and 316 mg/kg).

Table 1. Essential oil composition of *Tamarix aphylla* identified by GC-MC.

No.	Substances	RI (exp.)	RI (Literature)	% Composition
1	γ-Terpinene	1053	1054	0.6
2	Linalool	1079	1081	5.5
3	cis-Decahydronaphthalene	1100	1099	1.5
4	α-Thujone	1102	1101	1.2
5	cis-4-Caranone	1201	1200	0.8
6	β-E-Damascenone	1369	1373	1.8
7	trans-β-Caryophyllene	1379	1376	10.1
8	Dodecanal	1406	1408	1.4
9	α-Caryophyllene	1453	1455	6.1
10	β-Ionone	1472	1477	20.1
11	Farenal	1505	1508	0.6
12	Tridecanal	1510	1509	0.8
13	α-Selinene	1517	1523	1.1
14	δ-Cadinene	1525	1523	0.4
15	Dodecanoic acid	1561	1565	12.2
16	n-Hexyl benzoate	1573	1579	2.8
17	β-Cedrene	1622	1619	0.9
18	α-Muurolen-15-al	1767	1767	1.2
19	Caryophyllene oxide	1820	1853	2.7
20	6,10,14-Trimethyl-2-pentadecanone	1840	1845	20.2
21	Methyl hexadecanoate	1920	1921	0.5
22	Docosane	2150	2200	0.7
93.2				

Tamarix aphylla essential oil reduces edema in carrageenan-induced rat paw edema model

After one hour of administering various doses of EOTA, dexamethasone, and the vehicle, rat paw edema was induced by injecting 0.1 ml of a 1% freshly prepared carrageenan suspension. It was observed that the paw edema increased over time. However, positive control dexamethasone (2 mg/kg) and EOTA significantly reduced rat paw edema at doses of 31.6, 100, and 316 mg/kg between 2 to 6 hours after inducing carrageenan edema, in comparison to the vehicle control. This reduction was statistically significant at all-time points (Fig. 1, $n = 6$, $p < 0.05$). The group treated with 316 mg/kg of plant essential oil demonstrated a 64% reduction in edema, while the group treated with 2 mg/kg dexamethasone showed a 75% reduction, both compared to the vehicle control group, 6 hours after inducing carrageenan edema (Fig. 1, $n = 6$, $p < 0.05$). However, the group treated with a dose of 10 mg/kg failed to reverse the edema induced by carrageenan in comparison to the vehicle control at all tested time points.

Tamarix aphylla essential oil reduces leukocytes migration in carrageenin-induced peritonitis model

The peritonitis induced by carrageenan was assessed by examining the migration of mononuclear leukocytes (lymphocytes, monocytes, and macrophages) as well as polymorphonuclear leukocytes (neutrophils, eosinophils, and basophils) into the peritoneal cavity (Bradley et al. 1982). Following intraperitoneal injection of carrageenan,

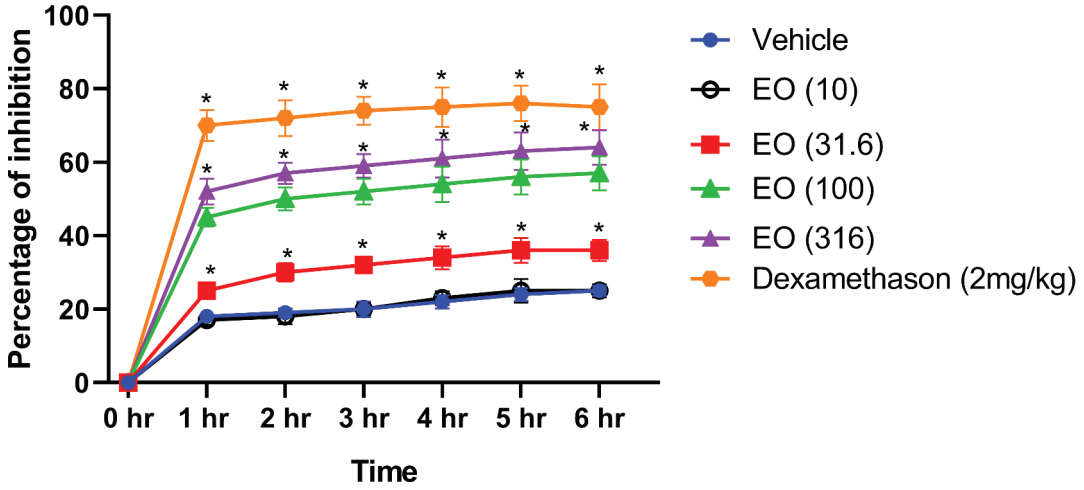

Figure 1. The effect of EOTA on rat paw edema was examined to assess its anti-inflammatory properties. Rats were given pre-treatment of either a vehicle, EO at various doses (10, 31.6, 100, 316 mg/kg), or Dexamethasone (2 mg/kg) prior to carrageenan-induced paw injection. The pre-treatment was administered at 6-hour intervals. EOTA demonstrated a significant inhibition of paw swelling induced by carrageenan, particularly at concentrations ranging from 31.6 to 316 mg/kg. The representative blot shown in the study represents a sample size of 6 rats for each experimental group. All values were expressed as the mean ± SEM (standard error of the mean) and analyzed using a two-way analysis of variance (ANOVA), followed by the Tukey Post Hoc test. A p-value less than 0.05 (*) was considered statistically significant when compared to the vehicle control group.

there was an influx of leukocytes into the peritoneal cavity over a 4-hour period. Positive control dexamethasone (2 mg/kg) significantly affected the migration of leukocytes and polymorphonuclear (PMN) cells, as indicated by statistical analysis (Fig. 2, $n = 6$, $p < 0.05$). Similarly, groups treated with EOTA at doses of 31.6, 100, and 316 mg/kg demonstrated a significant reduction in both total leukocyte and polymorphonuclear (PMN) cell migration (Fig. 2, $n = 6$, $p < 0.05$) compared to the vehicle control. However, neither the doses of plant essential oil nor dexamethasone influenced the migration of mononuclear (MN) cells. These findings suggest that EOTA exhibits an anti-inflammatory effect based on the presented results.

Tamarix aphylla essential oil reduces myeloperoxidase activity in carrageenan-induced rat paw edema model

MPO, also known as myeloperoxidase, is an enzyme released during the degranulation of monocytes and neutrophils. Its primary function is to catalyze the oxidation of various cellular and biological structures. Additionally, MPO plays a role in the development of inflammation, cardiovascular diseases, and immune-mediated conditions. The EOTA has been found to exhibit a significant anti-inflammatory effect by inhibiting MPO activity. This effect was observed after 4

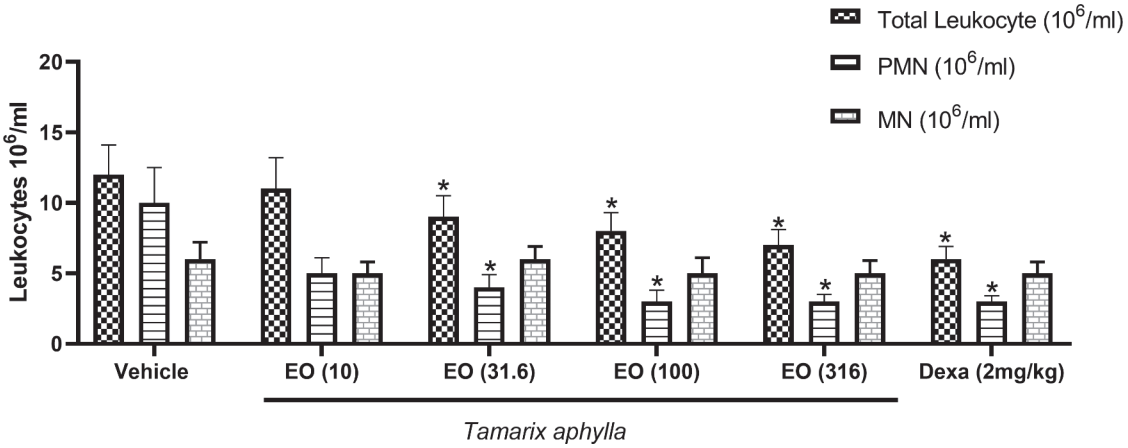

Figure 2. Anti-inflammatory effect of EOTA treatment on leukocyte migration. Rats were pretreated orally with different doses of EOTA (10, 31.6, 100, 316 mg/kg) or a vehicle containing 0.2% Tween 80, one hour before receiving intraperitoneal injection of carrageenan (1%, 250 µl). The experimental groups consisted of 6 rats each. After the injection, intraperitoneal lavage was performed to collect inflammatory cells. These cells were then assessed to evaluate the anti-inflammatory effect of EOTA using a Neubauer Chamber for cell counting and Diff-3 Stain for leukocyte differentiation. All values were presented as the mean ± SEM and analyzed using a one-way analysis of variance (ANOVA), followed by the Tukey Post Hoc test. Statistical significance was determined as $p < 0.05$ (*) when comparing the EOTA -treated groups to the vehicle control group.

hours of carrageenin induction. Among the different concentrations tested, namely 31.6, 100, and 316 mg/kg of EOTA, there was a significant decrease in MPO activity compared to the control group treated with a vehicle (Table 2, n = 6, $p < 0.05$). Moreover, these concentrations were comparable to the anti-inflammatory effects of the reference drug dexamethasone (Table 2, n = 6, $p < 0.05$). However, a dose of 10 mg/kg of EOTA did not show any significant alteration in MPO activity compared to the control group (Table 2, n = 6). Consequently, EOTA demonstrates potential antioxidant and anti-inflammatory effects by inhibiting the activation of inflammatory cells.

Table 2. The anti-inflammatory effect of EOTA on the generation of Myeloperoxidase (MPO) after carrageenan-induced paw injection in rats.

Treatment	Dose (mg/kg)	UMPO/mg tissue
Vehicle	————	6.88 ± 0.50
EO	10	4.1 ± 0.40
EO	31.6	1.5 ± 0.30 *
EO	100	0.74 ± 0.11 *
EO	316	0.51 ± 0.12 *
Dexamethasone	2	0.42 ± 0.11 *

The rats were pretreated with a vehicle, Dexamethasone (2 mg/kg), or different doses of EOTA (10, 31.6, 100, 316 mg/kg) one hour prior to the carrageenan paw injection. After four hours, the levels of MPO were measured in the paw that received the carrageenan injection. All values were expressed as the mean ± SEM and analyzed using a one-way analysis of variance (ANOVA), followed by the Tukey Post Hoc test. Statistical significance was determined as $p < 0.05$ (*) when comparing the EOTA -treated groups and the Dexamethasone group to the vehicle control group. This analysis allowed for the evaluation of the inhibitory effect of EOTA on MPO generation, indicating its potential anti-inflammatory properties.

Anti-Inflammatory effects of *Tamarix aphylla* essential oil through inhibition of peritoneal lavage NOx- generation

After pretreating rats with EOTA at a dose of 316 mg/kg before intraperitoneal injection of carrageenan, there was a significant reduction in the concentration of total NOx (nitrite and nitrate) compared to the control group treated with a vehicle (as indicated in Table 3, n = 6, $p < 0.05$). Similarly, pretreating rats with dexamethasone also led to a significant decrease in NOx concentration, indicating that EOTA exhibits an anti-inflammatory effect (Table 3, n = 6, $p < 0.05$).

Table 3. The anti-inflammatory effect of EOTA on the total concentration of nitric oxide (NOx) in peritoneal lavage after intraperitoneal injection of carrageenan.

Treatment	lavage NOx – concentration
Vehicle	7.9 ± 1.8 µM
EO 316 mg/kg	3.8 ± 0.5 µM *
Dexamethasone	3.4 ± 0.3 µM *

Both dexamethasone and EOTA (316 mg/kg) demonstrated a significant decrease in the lavage NOx concentration compared to the vehicle control group. All values were presented as the mean ± SEM and analyzed using a one-way analysis of variance (ANOVA), followed by the Tukey Post Hoc test. Statistical significance was determined as $p < 0.05$ (*) when comparing the EOTA-treated and dexamethasone groups to the vehicle control group. These results indicate that both dexamethasone and EOTA have a notable anti-inflammatory effect as evidenced by the reduction in NOx concentration in the peritoneal lavage after carrageenan injection.

Antioxidant activity of *Tamarix aphylla* essential oil

Antioxidants play a crucial role in preventing oxidative damage caused by reactive oxygen species to cellular DNA, lipids, and proteins. Reactive oxygen species such as hydrogen peroxide and nitric oxide can cause oxidative damage to cells (Tsikas 2017). Furthermore, lipid peroxidation resulting from the action of free radicals can lead to the destruction of cell membranes and eventual cell death (Grisham et al. 1996). Therefore, the use of antioxidants to scavenge free radicals can prevent lipid oxidation. The antioxidant activity of EOTA was assessed by comparing its ability to scavenge nitric oxide with that of ascorbic acid, which was used as a standard. The study revealed that both EOTA and ascorbic acid exhibited dose-dependent increases in scavenging capacity, using the same range of concentrations. EOTA significantly inhibited the generation of nitric oxide (NO) from sodium nitroprusside within the concentration range of 0.01–100 µg/ml, as compared to the vehicle control (Table 4, n = 6, $p < 0.05$). The highest inhibition was observed with the highest concentration of ascorbic acid, 100 µg/ml (Table 4, n = 6, $p < 0.05$). To assess lipid peroxidation, the level of thiobarbituric acid reactive substances (TBARS) is measured. This measurement is based on the reaction between thiobarbituric acid (TBA) and malondialdehyde (MDA), a breakdown product of lipid hydroperoxides. In the case of this study, the impact of EOTA on lipid peroxidation induced by FeSO4 (Fe2+) in liver homogenate was evaluated. The results demonstrated that EOAH exhibited an inhibition of lipid peroxidation that was dependent on its concentration. All tested concentrations of EOTA (ranging from 0.01 to 100 µg/ml) as well as ascorbic acid sig-

Table 4. Antioxidant effect of EOTA on Lipid peroxidation induced by Fe^{+2} and NO radical scavenging activity.

Sample (µg/ml)	NO scavenging activity	TBARS (FeSO4)	TBARS (FeSO4+ H2O2)
Vehicle	43.4 ± 3.1	47.2 ± 1.2	69.5 ± 1.8
EO 0.001	46.2 ± 4.2	46.3 ± 0.9	70.4 ± 1.6
EO 0.01	51.4 ± 3.2 *	52.5 ± 0.8 *	76.6 ± 1.3 *
EO 0.1	53.3 ± 2.3 *	61.3 ± 1.3 *	81.5 ± 2.4 *
EO 1	55.1 ± 3.2 *	66.6 ± 1.2 *	83.2 ± 2.3 *
EO 10	57.2 ± 4.3 *	68.4 ± 0.8 *	85.6 ± 2.6 *
EO 100	62.4 ± 3.6 *	74.6 ± 0.9 *	87.1 ± 1.2 *
EO 316	64.2 ± 2.4	75.2 ± 0.7 *	90.2 ± 1.3 *
Ascorbic acid 0.001	71.1 ± 1.5 *	46.3 ± 1.3 *	67.3 ± 0.5 *
Ascorbic acid 0.01	76.1 ± 2.3 *	54.4 ± 1.5 *	72.7 ± 0.4 *
Ascorbic acid 0.1	78.5 ± 2.5 *	59.6 ± 2.6 *	73.6 ± 0.9 *
Ascorbic acid 1	83.2 ± 1.5 *	64.8 ± 2.4 *	74.5 ± 1.1 *
Ascorbic acid 10	86.3 ± 1.9 *	73.3 ± 2.1 *	76.4 ± 1.2 *
Ascorbic acid 100	87.1 ± 1.2 *	77.8 ± 1.8 *	78.2 ± 0.8 *
Ascorbic acid 316	89.2 ± 1.4 *	76.1 ± 2.3 *	80.3 ± 0.9 *

Rat's liver homogenate was incubated with EOTA (0.001,0.01, 0.1,1,10,100, 316 µg/ml) together with same concentration of ascorbic acid as a positive control in the presence of 100 µl of 0.145 mM FeSO4 or 100 µl 0.145 mM FeSO4 plus 0.4 M H_2O. Essential oil antioxidant effect is assessed by the inhibition of thiobarbituric acid reactive substances (TBARS) generation as well as NO production. This representative data is from six independent replicates (n = 6). All values were represented as mean ± SEM and analyzed by one-way analysis of variance (ANOVA) followed by followed by Tukey post hoc test. $P < 0.05$* compared with the vehicle.

nificantly inhibited the generation of TBARS compared to the vehicle control (Table 4, $n = 6$, $p < 0.05$). This indicates that EOTA has a concentration-dependent inhibitory effect on lipid peroxidation induced by FeSO4, similar to the effect observed with ascorbic acid.

Discussion

We assessed the anti-inflammatory properties of the essential oil derived from the plant by conducting experiments on established acute inflammatory models such as paw edema and peritonitis. Our research findings demonstrate that EOTA effectively reduces the inflammatory response in rat models.

Carrageenan-induced rat/mouse model has been increasingly used, to evaluate the anti-inflammatory and antinociceptive effects of natural products as well as to study the mechanisms involved in inflammation process. Carrageenan is a strong chemical, when it is used, it enhances the release of inflammatory and proinflammatory mediators, such as histamine, bradykinin, TNF-α, leukotrienes prostaglandins (Ndrepepa 2019).

The development of edema in the hind paw of rodents following carrageenan injection is described as a biphasic process, characterized by the sequential activation of various mediators that contribute to the inflammatory response. During the early phase, which lasts for approximately 3 hours, the initial mediators detected are histamine, serotonin, and 5-HT. In the late phase of inflammation, sustained levels of TNF-α, leukotrienes, and bradykinin are observed, supported by increased levels of NO and prostaglandins (PGs). These elevated levels of NO and PGs are associated with enhanced vascular permeability in the injected area (Li et al. 2021). Conventional anti-inflammatory drugs available in the market are primarily designed to target the late phase of inflammation (Briganti and Picardo 2003).

In our study, we observed that the injection of carrageenan into the hind paw of rats resulted in a substantial and time-dependent edema, reaching its peak at 4 hours. Furthermore, the significantly increased levels of myeloperoxidase (MPO) at 4 hours, which is used as an indicator of neutrophil infiltration in the hind paw tissue, indicated not only swelling due to excessive plasma extravasation but also a significant influx of leukocytes in the injection area. However, when the rodents were treated with the essential oil derived from the plant, we observed a significant reduction in both edema and the elevated levels of MPO. These findings suggest that EOTA exerts its effects by suppressing vascular permeability (edema) and the influx of leukocytes.

To further investigate and validate the impact of the plant essential oil on the influx of inflammatory cells, particularly neutrophils, to the site of injury, we conducted experiments using an animal model of carrageenan-induced peritonitis. As anticipated, after 4 hours of carrageenan injection into the peritoneal cavity of rats, a robust infiltration of leukocytes was observed. However, EOTA exhibited significant inhibition of both total leukocyte and polymorphonuclear (PMN) cell migration, comparable to the effects of 2 mg/kg dexamethasone. Interestingly, neither the plant essential oil at any of the tested doses nor dexamethasone had any effect on mononuclear (MN) cell migration. This confirms the anti-inflammatory properties of EOTA. It has been reported that carrageenan can induce peritonitis by increasing the levels of prostaglandins (PGs), leukotrienes, and reactive oxygen species (ROS), which subsequently promote vasodilation, exudation, and leukocyte recruitment (Solanki et al. 2015; Barung et al. 2021). The inhibition of leukocyte and PMN migration by EOTA may be attributed to its ability to inhibit the production of PGs, leukotrienes, and ROS.

MPO is widely utilized as a marker for assessing neutrophil infiltration, inflammation, and oxidative stress in vivo (Gunter et al. 2016). Our results showed that EOTA at doses of 31.6, 100, and 316 mg/kg significantly reduced MPO activity in the inflamed tissues. This suggests that EOTA treatment may alleviate oxidative stress. Hence, another potential mechanism contributing to the anti-inflammatory effects of EOTA could be attributed to its antioxidative activity.

Consistent with the anti-inflammatory effects of EOTA, rats that were pre-treated with a dose of 316 mg/kg of EOTA before intraperitoneal injection of carrageenan exhibited a significant reduction in total NOx concentration (including NO2 and NO3).

Nitric oxide (NO) is a small molecule produced by various cells in mammals, and it plays crucial roles in signaling pathways in numerous physiological systems, including blood pressure regulation, neurotransmission, smooth muscle relaxation, and defense mechanisms (Barth et al. 2016). While NO has important functions, it can also act as a toxic agent with free radical properties. Elevated levels of NO have been implicated in a wide range of diseases, such as cancer, diabetes, and inflammation. During the inflammatory process, there is a release and sustained presence of high levels of NO in the injured tissues, leading to inflammation in joints, lungs, and the gastrointestinal tract. Therefore, plant extracts that can scavenge NO or inhibit its production may hold therapeutic potential in managing inflammatory diseases (Pace et al. 2017). In this particular study, it was observed that EOTA significantly inhibits the generation of NO radicals derived from sodium nitroprusside.

Free radicals can have detrimental effects on various biological molecules, including nucleic acids, proteins, and lipids. Lipid peroxidation is a chain reaction that occurs when unsaturated lipids in cell membranes are targeted by free radicals. This process can lead to significant changes in the biochemical properties of biomolecules, which may contribute to the development of various pathological conditions (Patil et al. 2019). In our study, we found that EOTA exhibited a concentration-dependent inhibition of lipid peroxidation induced by FeSO4 (Fe+2) in liver homogenate. This suggests that EOTA has

the ability to prevent or reduce the oxidative degradation of unsaturated lipids in cell membranes. Furthermore, EOTA demonstrated antioxidant properties, as evidenced by its ability to scavenge NO radicals and inhibit lipid peroxidation in the conducted assays. These findings indicate that EOTA has potential as an antioxidant agent, capable of mitigating the harmful effects of free radicals on biological molecules.

It is important to highlight that the primary constituents of essential oils may contribute to their biological effects. One such constituent is β-Ionone, which makes up 20.1% of the EOTA. In BV2 microglia cells, β-ionone has been found to reduce the expression of pro-inflammatory mediators induced by lipopolysaccharide (LPS), including nitric oxide (NO), prostaglandin E2 (PGE2), and tumor necrosis factor-α (TNF-α) (Kang et al. 2013). Another significant component of EOTA is trans-β-caryophyllene, which constitutes 10.1% of the essential oil. Studies have shown that trans-β-caryophyllene possesses the ability to diminish acute inflammation and paw edema in rats induced with carrageenan (Dahham et al. 2016). Additionally, experimental investigations have demonstrated that trans-β-caryophyllene can alleviate chronic inflammation and oxidative stress by reducing the levels of pro-inflammatory mediators, including tumor necrosis factor-α (TNF-α), interleukin 1β (IL-1β), interleukin-6 (IL-6), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) (Scandiffio et al. 2020). Linalool, accounting for 5.5% of EOTA, is a notable component that has demonstrated efficacy in reducing paw edema in a rat model of carrageenan-induced edema (Peana et al. 2002). Furthermore, linalool exhibits potent radical scavenging activity (Liu et al. 2012). Based on these findings, it can be concluded that the observed anti-inflammatory activity in our study may be attributed to certain major compounds present in EOTA. However, it is also plausible that the activity of these components is influenced by other major and minor constituents.

Conclusion

Our research provides novel evidence that the EOTA possesses anti-inflammatory properties. Additionally, the observed antioxidant activity of the EOTA may contribute to its anti-inflammatory effects.

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