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Anti-inflammatory Activity and Chemical Characterisation of *Opuntia ficus-indica* Seed Oil Cultivated in Saudi Arabia

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Abstract

The fruits of *Opuntia ficus-indica* (L.) Mill. (OFI), a member of the family Cactaceae, is cultivated in Saudi Arabia and considered as a functional food with anti-inflammatory activity. The aim of current study was to chemically characterise OFI growing in Saudi Arabia by GC-MS and evaluate its anti-inflammatory activity in rat models *via* carrageenan-induced paw oedema and croton oil-induced ear oedema at two doses 100 and 200 mg/kg. Palmitic acid (10.68%), linoleic acid (5.9%), oleic acid (8.16%) and β-sitosterol (24.98%) are the major constituents in OFI seed oil. In carrageenan-induced rats, the OFI seed oil (100 and 200 mg/kg) produced significant inhibition of oedema by approximately 46% and 62%, respectively, and reduced PGE2 concentrations in exudates by 54% and 67%, respectively. Also, it significantly decreased the weight of punch from challenged ears by 20% and 33%, and myeloperoxidase (MPO) activity by 54% and 62% of the induced ear respectively. This was accompanied by amelioration of croton oil induced histopathological changes. In conclusion, the anti-inflammatory activity of the OFI seed oil might be attributed to the presence of USFA such as oleic acid (omega-9) in addition to β-sitosterol through decreasing PGE2 and MPO activity in the inflamed tissues which was supported by histopathological examination.

Keywords: *Opuntia ficus-indica*. Anti-inflammatory. β-Sitosterol. Fixed oil. Cactaceae

1 Introduction

Inflammation is a cascade of defense reactions produced by the host against external stimuli. These reactions cause redness, heat, swelling, and pain in the inflamed part [1]. The process includes increasing blood flow and vascular permeability resulting in accumulation of fluid as well as inflammatory mediators (viz. cytokines, eicosanoids and ROS) in the inflamed tissue [2]. The immune response activates feedback mechanisms through the secretion of anti-inflammatory cytokines to inhibit pro-inflammatory signaling cascades to keep homeostasis and maintain healthy tissue [2]. Classical anti-inflammatory drugs (non-steroidal; selective and non-selective) showed severe side effects including renal failure and showed harmful effect on GIT and cardiovascular system [1,3]. In this context; drugs of natural origin are widely used due to their effectiveness, lesser side effects and their relative economic cost [4]. Several plants and plant-derived active compounds demonstrated anti-inflammatory activity [5-8]. Fatty acids are naturally occurring dietary constituents in seeds and could be classified to saturated and unsaturated. Saturated one could be synthesized by mammalian tissue but unsaturated one especially polyunsaturated (PUFA) are obtained only from plants [9]. PUFA could be classified to omega-3, 6, and 9 (ω-3, ω -6 and ω -9) based on a number of double bonds and the position of the first double bond from the terminal methyl [9]. Omega-6 PUFA as linoleic acid is converted in mammalians into arachidonic acid (AA); an ω -6 PUFA, which is the source of inflammatory mediators' prostaglandins (PGE) and leukotrienes. Meanwhile, consumption of oleic acid (ω -9) and α-linolenic acid (ω -3) fatty acids from fish oil or edible seeds acts as AA antagonist and decrease the production of inflammatory mediators [9].

Plant-based drugs are widely used due to their proven effectiveness, reduced side effects and their relative economic cost [4]. The kingdom of Saudi Arabia is rich in plants; including about 2250 in a different region of Saudi Arabia [10]. Genus *Opuntia* follows family Cactaceae and comprises about 1500 species. Many of them produce edible favored fruits with thick coloured

pericarp and sweet pulp that include tiny seeds [11]. *Opuntia ficus-indica* L. (OFI) is native to Mexico but currently cultivated in many regions worldwide. The plant is cultivated in different regions of Saudi Arabia for its Prickly pear edible fruit that consumed by locals as a food source. The leaves and fruits of the plant are used by Mexican to treat arteriosclerosis, diabetes, gastritis, and used also as anti-inflammatory, and anti-viral [12-15]. Total ethanol extract of its stem and fleshy leaves showed anti-inflammatory activity and protective effect against gastric lesions [12]. Bio-guided fractionation revealed that β-sitosterol is responsible for the associated anti-inflammatory effect [16]. The fruit juice was used as anti-diarrhea due to its tannin contents [17]. Moreover, cladodes of the plant contain neutral mucilage (glucomannan) that delay glucose absorption and interfere with lipid metabolism [17]. Phenolic compounds were in the fruits of OFI [11], in addition to pigments [18] and essential oil [19]. The fixed oil content of the seeds from different regions [20-22] was analyzed and effect of habitat was also examined [21]. The oil obtained from the seeds of some other *Opuntia* species is rich in PUFA [23] and showed anti-oxidant, cytotoxic, anti-microbial, anti-fungal and analgesic effects. Therefore, the aim of current study was to characterise OFI seed oil growing in Saudi Arabia using GC-MS analysis and evaluate its *in vivo* anti-inflammatory activity in two rat models; carrageenan-induced paw oedema and croton oil-induced ear oedema.

2 Experimental

2.1 Plant material

Seeds of *Opuntia ficus-indica* (L.) Mill. (OFI) were purchased from Al-Baha region. The identity of seeds was confirmed by staff members of the Biology Department, College of Science, King Abdulaziz University, Saudi Arabia. A specimen (OFI-1241) was kept in the herbarium at the Faculty of Pharmacy, King Abdulaziz University.

2.2 Seed oil extraction

One Kilogram of the OFI seeds was grounded and extracted with hexane for four consecutive times, two hours each, using a Soxhlet apparatus. The solvent was evaporated under reduced pressure and the percentage of the collected oil was calculated. The resulting oil was kept away from light at low temperature for analysis and biological study.

2.3 Identification of fatty acids

Approximately 0.1 mg of the oils sample (hexane extract) was dissolved in 1mL hexane, filtered and loaded into HPLC vial. Then 5µL was analyzed on an Agilent 7820A gas chromatography system coupled to Agilent 5975 series quadrupole mass spectrometer working in EI mode and resolved on a Termo BPX70 column (50 m × 250 µm × 0.25 µm) (J&W Scientific, USA). Compounds were desorbed at 260 °C injection port. The analysis was performed in programmed temperature: 150 °C for 1 min, then 5°C/min to 300 °C over 20 min, then hold at 300 °C till 51 min, using He as a carrier gas with a flow of 0.5 mL/min. Gas chromatography/mass spectrometry (GC/MS) interface temperature was set to 280 °C. Compounds were identified using NIST 11 library of mass spectra on mass hunter software. For quantitative analysis, the same sample, same column and same conditions were used on Agilent 6890N gas chromatography equipped with FI detector.

2.4 Anti-inflammatory activity

2.4.1 Animals

Adult male Wister rats with body weight range (175-200 g) were obtained from the vivarium, Faculty of Pharmacy, King Abdulaziz University. Rats were acclimatised for one week preceding the experiment under the standard conditions (24 ± 2ºC) with free access to water & standard food pellets. The study was approved by the ethical committee of the Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia (PH-101-40).

2.4.2 Experimental Protocol

Initially, OFI seed oil was investigated for its anti-inflammatory activity via measuring paw volume in carrageenan-induced paw oedema model, subsequently assessing PGE2 concentration in the inflammatory exudates. Thereafter, the croton oil-induced ear oedema model was utilized to further confirm the anti-inflammatory activity by measuring ear oedema, tissue myeloperoxidase (MPO) activity as well as histopathological examination. The experiments were conducted as a dose-response study in which OFI seed oil was tested at two doses (100 and 200 mg/kg). The control group animals were handled under the same experimental condition as those of the test groups except that they received appropriate volumes of corn oil. Indomethacin was used as reference drug at a dose of (10 mg/kg) solubilised in sodium carboxymethyl cellulose (Na-CMC at 0.5% w/v)

2.4.3 Evaluation of paw volume and PGE2

Adult 30 rats were grouped equally (6 rats/group) numbered (I – V). Fasting of rats was obligate 16 hours previous to the experiment, but with free access to water. The first and second groups were administered corn oil (po). Animals in group III had been administered indomethacin (10 mg/kg po). The fourth and fifth groups were treated with the seed oil at (100 & 200 mg/kg po, respectively). Dosing volume was 10 mL/kg for all groups. After 30 minutes of oral treatment, the first group (control) received a volume 50 µL saline, while all remaining groups (II–V) have been injected 50 µL carrageenan (1% solution in saline) subcutaneously on the plantar surface of the right rear paw. Immediately after carrageenan injection, the paw volume was measured then after 1, 2 & 3 hour-intervals, by saline displacement using a plethysmometer (Ugo Basile, Comerio, Italy) as previously discussed [24].

Then, the concentration of PGE2 was assessed in the inflammatory exudates using PGE2 ELISA kit from R & D systems Inc. (Minneapolis, MN, USA). Briefly, rat paws were dissected, followed by injection of (0.1 mL) saline containing (10 µM) indomethacin in order

to remove the eicosanoid-containing fluid and to stop further production of PGE2. Subsequently, paws were incised with a scalpel and suspended off the bottom of polypropylene tubes with Eppendorf pipette tips to facilitate drainage of the inflammatory exudates, then centrifuged at 1,800 *g* for 15 minutes [25].

2.4.4 Evaluation of ear oedema, tissue MPO activity, and histopathology in croton oil-induced ear oedema model.

The experiment was performed as described previously [26]. An irritant mixture was prepared by mixing 4 parts croton oil (the irritant) in a solvent combined of ethanol (10 parts): pyridine (20 parts): ethyl ether (66 parts). Again, 30 rats were equally grouped as previously mentioned in the former model. The irritant mixture was applied topically (20 µL) on both sides of the right ears. The left ear was untreated as a control. The first group was considered as negative control, and received only irritant-free solvent, while group II received corn oil orally. The third group was administered indomethacin (10 mg/kg orally) as a reference. Groups IV-V received seed oil at (100 & 200 mg/kg orally), respectively. After one hour, all groups except the first one received croton oil topically. Four hours afterwards, rats were euthanized and discs were punched from both the treated as well as the control ears using 8-mm cork borer. The two punches were weighed immediately and the weight difference was calculated to check the inflammatory response. A representative ear tissue from each group was fixed in 10% formalin saline for histopathological examination. The whole tissue of the right ear was homogenized and MPO activity was quantified kinetically [27]. Results were expressed as units of activity per milligram protein. Protein content was determined as previously reported [28].

2.4.5 Statistical analysis

Data are presented as mean ± SD of 6 animals per group. Statistical analysis was performed using one-way ANOVA followed by Tukey's *post-hoc* test at "0.05" as the criterion for

significance. All statistical analyses were performed using GraphPad InStat software version 3 and graphs were sketched using GraphPad Prism software version 5 (ISI_ Software, La Jolla, CA, USA).

3 Results and discussion

In the present study, fixed oil obtained from OFI seeds was chemically characterized for its contents of fatty acids and sterol by GC/MS analysis. Moreover, OFI was pharmacologically tested for its anti-inflammatory activity in carrageenan-induced rat paw oedema and croton oil-induced ear oedema animal models.

Carrageenan injection in the plantar surface of rats' hind paws caused noticeable inflammation after 3 hours and significantly increased the mean volume of the challenged paw by about 52%, compared to the control (Table 1). All treatments have shown time-dependent inhibition of paw oedema. No significant effect was detected after 1 hour of carrageenan challenge. Only indomethacin showed significant oedema inhibition after 2 hours, however, the optimum anti-inflammatory effect of seed oil occurred after 3 hours of carrageenan injection. Indeed, the standard anti-inflammatory drug "indomethacin" demonstrated the highest inhibition of the induced oedema by about 76% after 3 hours. Pre-treatment of rats with OFI seed oil showed significant inhibition of the carrageenan-induced increase in the oedema volume in a dose-related manner, causing oedema inhibition after 3 hours of carrageenan induction by about 46% and 62%, at doses 100 and 200 mg/kg, respectively.

Table 1

These findings were confirmed by measuring PGE2 in the inflammatory exudates of incised paws. Carrageenan challenge caused a significant 18-fold increase of PGE2 concentration, compared to animals in the control group, as shown in Figure (1). The standard indomethacin

was able to decrease PGE2 concentration by about 83% compared to carrageenan-challenged. Animals received OFI seed oil (100 & 200 mg/kg) showed a significant reduction of the PGE2 concentration in exudates by about 54% and 67% of the carrageenan-challenged rats, respectively.

Figure 1

Application of croton oil to rat ears led to an enormous (2-fold) increase in the weight of ear punches from control animals as shown in Figure (2). Indomethacin pre-treatment significantly reduced punch weight to the extent that no significant difference from the control group. In addition, pre-treatment of rats with OFI seed oil at doses (100 & 200 mg/kg) caused a significant decrease of punch weight from challenged ears by about 20% and 33%, respectively.

Figure 2

In a similar pattern, MPO activity in ear tissues homogenates pointed out the ability of croton oil to significantly elevate the enzyme activity by more than 19 folds, compared to the unexposed animals, as implicated in Figure (3). The standard indomethacin pre-treatment significantly protected against croton oil-induced enhancement of MPO activity by about 83%. However, pre-treatment with OFI seed oil at doses of 100 and 200 mg/kg reduced MPO activity by about 54% and 62% of the induced ear, respectively.

Figure 3

Histopathological examination of the ear tissue verified those findings of MPO activity. Figure (4A) showed the normal histological structure of the skin layers, subcutaneous tissue, musculature and cartilaginous structures with no apparent infiltration of neutrophils that

correlated with very little MPO activity previously assessed. The croton oil-exposed rats demonstrated tissue oedema and enormous neutrophil infiltration (Figure 4B) that was related to previously measured MPO activity that rose by about 19 folds. Figure (4C) represented indomethacin pre-treatment (10 mg/kg) with almost intact dermal and cartilaginous structures with only a few inflammatory cells infiltration in subcutaneous tissue. Furthermore, Figures (4D & 4E), which represented ear tissue from animals pre-treated with OFI seed oil at 100 & 200 mg/kg, respectively, showed a dose-related improvement of tissue with regard to cellular infiltration and blood vessel congestion, when compared to croton oil-exposed group. This effect also reflected the dose-response difference in MPO activity between the two doses of OFI seed oil.

Figure 4

The promising anti-inflammatory activity of the oil could be attributed to its major constituents of USFA and sterols. The obtained oil recovered from the seeds reached up to 88 gm/kg. The yield was within the range of different species of prickly pear seeds that varied from 50 in Turkish cultivar -117 g/kg in Tunisian one [22,23,20,21,29,30]. The composition of the oil (Table 2) revealed the presence of palmitic acid as a major saturated fatty acid (10.68%); whereas linoleic (w-6) (5.9%) and oleic (w-9) (8.16%) were the major unsaturated fatty acids (USFA), while β-sitosterol (24.98%) was the major sterol in the oil followed by campesterol (6.584%).

Table 2

The GC-MS analysis of the oil constituents and their percentage showed some differences with the previous published data on different seed varieties. The obtained results showed a great similarity to a previous study on the Saudi variety, except in the value of linoleic acid that was 73% while β-sitosterol was not detected in the analysed oil [30].

In Turkish variety [21,23], the concentration of palmitic acid reached up to 11% while it was 17% in South African variety [29]. Meanwhile, the concentration of linoleic acid in this study was lower than studied varieties that ranged from 49% to 67% in Turkish [20] and South African varieties [29], respectively. Similarly, oleic acid concentration was lower than all previous studies that ranged from 15% in South African variety [29] to 24% in Algerian variety [22]. Moreover, our study showed high concertation of β-sitosterol and campesterol in comparison with published data which reached up to 21% for β-sitosterol and 3.7% for campesterol in Morocco variety [31]. The differences in the concertations of the various oil constituents could be attributed to the effect of the climate, time of collection and habitat.

Sitosterol is known for its strong anti-inflammatory effect [32] through different mechanisms including inhibition of MPO, IL-1B, TNF-α levels [33]. Meanwhile, it was reported that USFA as oleic acid (omega-9) fatty acids from fish oil or edible seeds acts as arachidonic acid antagonist and decreases the production of inflammatory mediators like prostaglandins (PGE) and leukotrienes [9], which could be another strategy or act by synergistic effect with sitosterol to reduce inflammation.

4 Conclusion

In conclusion, the anti-inflammatory activity of the OFI seed oil might be attributed to the presence of USFA such as oleic acid (omega-9) in addition to β-sitosterol through decreasing PGE2 and MPO activity in the inflamed tissues which was supported by histopathological studies. This study highlighted the importance of using prickly pear seeds as a potential new source of edible lipids/sterols that could be used as a nutraceutical / food supplement in inflammatory diseases. The results of this study can serve as a basis for further detailed chemical investigations and biological evaluation of the OFI seed oil growing in the Kingdom

of Saudi Arabia. Further pharmacological and clinical studies required to utilise OFI seed oil in the treatment of inflammatory disease is recommended.

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Disclosure statement

The authors declare no conflict of interest.

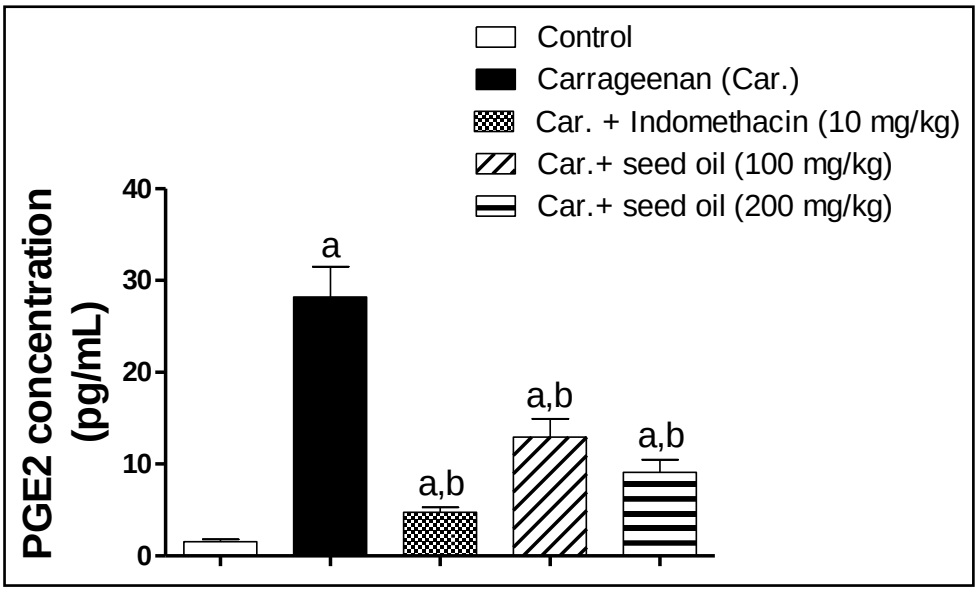

Fig. 1 Effect of OFI seed oil on PGE2 concentration in the inflammatory exudate of carrageenan-induced rat paw oedema model

Data are presented as mean ± S.D.

n = 6

Statistical analyses were carried out using one-way ANOVA, followed by Tukey's posthoc test

[a] Statistically significant difference from the control group at $p < 0.05$

[b] Statistically significant difference from the carrageenan-induced group at $p < 0.05$

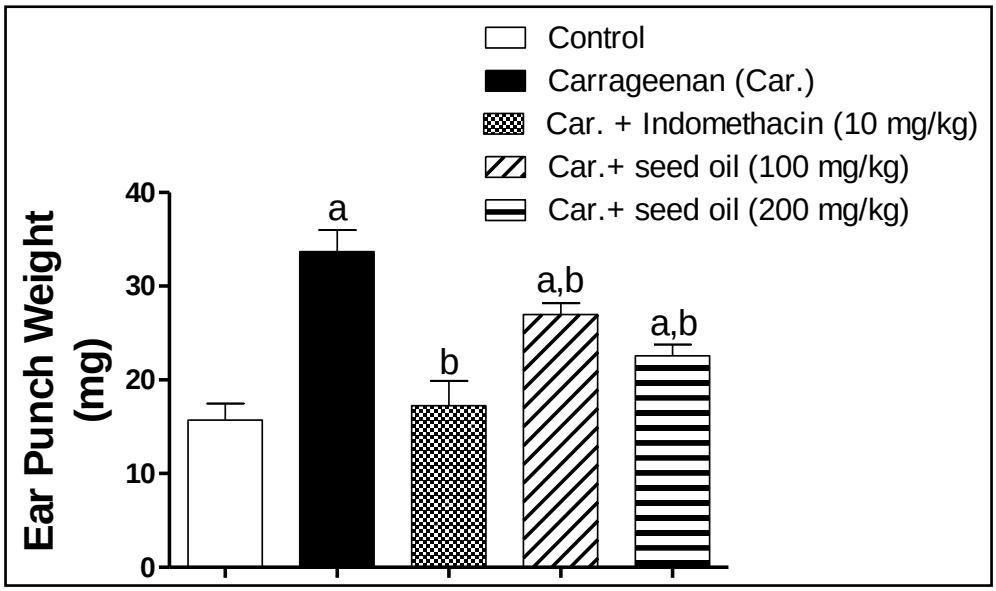

Fig. 2 Effect of OFI seed oil on-ear punch weight of croton oil-induced ear oedema model

Data are presented as mean ± S.D.

n = 6

Statistical analyses were carried out using one-way ANOVA, followed by Tukey's posthoc test

[a] Statistically significant difference from the control group at $p < 0.05$

[b] Statistically significant difference from the carrageenan-induced group at $p < 0.05$

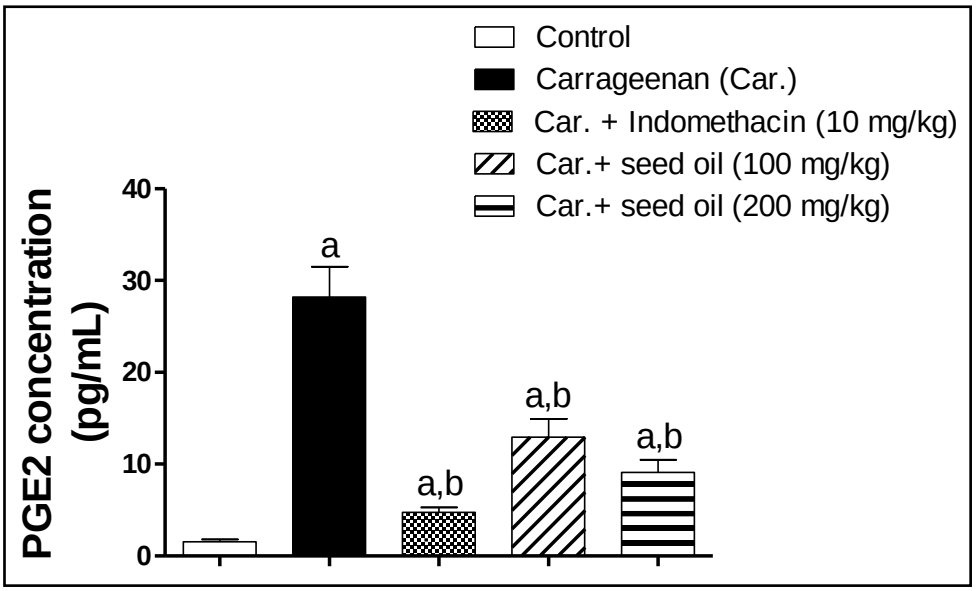

Fig. 3 Effect of OFI seed oil on MPO activity of croton oil-induced ear oedema model

Data are presented as mean ± S.D.

n = 6

Statistical analyses were carried out using one-way ANOVA, followed by Tukey's posthoc test

[a] Statistically significant difference from the control group at $p < 0.05$

[b] Statistically significant difference from the carrageenan-induced group at $p < 0.05$

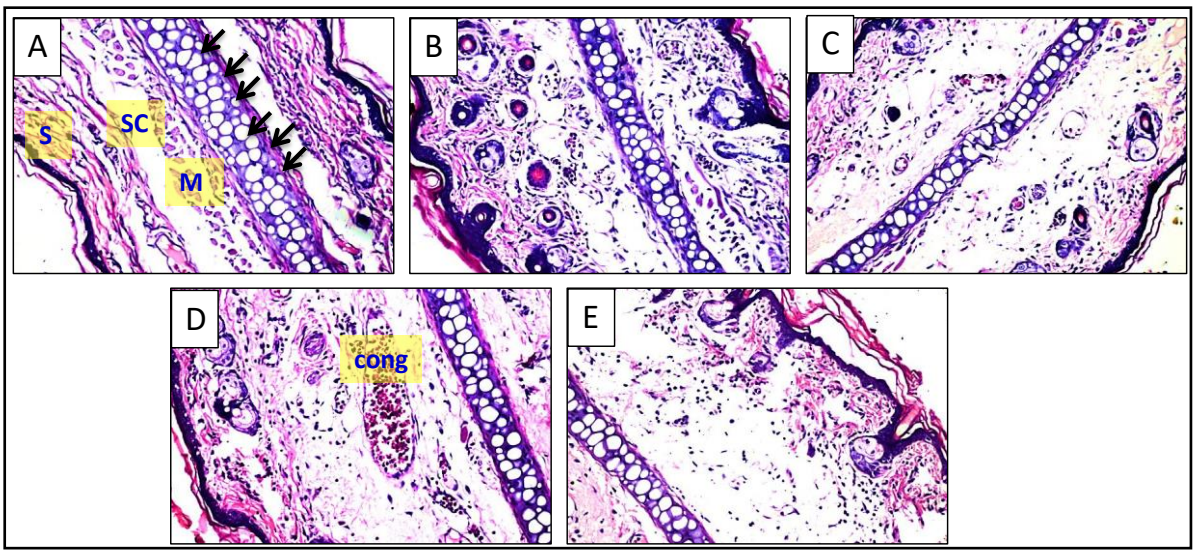

Fig. 4 Effect of OFI seed oil on histopathological changes in the croton oil-induced ear oedema model (X40):

A) Control ear tissue: no histopathological alteration and the normal histological structure of the skin layers (S), subcutaneous tissue (SC), musculature (M) and cartilaginous structure (arrows); B) Ear tissue from Croton oil–exposed rats with massive inflammatory cells infiltration (blue dots) with oedema; C) Ear tissue from rats treated with Indomethacin (10 mg/kg) shows almost intact dermal and cartilaginous structures with only a few inflammatory cells infiltration in subcutaneous tissue; D) Ear tissue from rats treated with F. opuntia seed oil (100 mg/kg) showing congestion of blood vessels with oedema and few inflammatory cells infiltration; E) Ear tissue from rats treated with F. opuntia seed oil (200 mg/kg) showing few cellular infiltrations that is comparable to indomethacin effect.

Table 1 Effect of OFI seed oil on paw volume in carrageenan-induced rat paw oedema

	Paw volume after 1 Hr. (mL)	Oedema Inhibition (%)	Paw volume after 2 Hr. (mL)	Oedema Inhibition (%)	Paw volume after 3 Hr. (mL)	Oedema Inhibition (%)
Control	0.828 ± 0.053	--	0.833 ± 0.028	--	0.796 ± 0,034	--
Carrageenan (Car.)	1.028[a] ± 0.071	--	1.105[a] ± 0.062	--	1.212[a] ± 0.057	--
Car. + Indomethacin (10 mg/kg)	1.001[a] ± 0.067	**14.30**	0.96 [a,b] ± 0.094	**53.13**	0.897 [a,b] ± 0.036	**75.80**
Car. + seed oil (100 mg/kg)	1.016 [a] ± 0.041	**6.40**	1.033[a] ± 0.084	**26.47**	1.022[a,b] ± 0.067	**45.68**
Car. + seed oil (200 mg/kg)	0.985[a] ±0.041	**21.45**	1.005[a] ± 0.043	**36.58**	0.956[a,b] ± 0.063	**61.62**

$$\%\ Edema\ inhibition = \frac{(mean\ paw\ volume\ of\ Carrageenan - mean\ paw\ volume\ of\ Treatment)}{(mean\ paw\ volume\ of\ Carrageenan - mean\ paw\ volume\ of\ Control)}\ X\ 100$$

Data are presented as mean ± S.D.

n = 6

Statistical analyses were carried out using one way ANOVA, followed by Tukey's posthoc test

a Statistically significant difference from the control group at $p < 0.05$

b Statistically significant difference from the carrageenan-induced group at $p < 0.05$

Table 2 GC/MS analysis of hexane extract of OFI seeds

RT (min)	Tentative identification *	Retention index	NIST probability %	Content [%]
7.31	2E,4E-Nonadienal	1188	91	2.235
8.06	2E-Decenal	1236	88	1.942
8.55	2E,4E-Decadienal	1288	93	2.314
12.27	2-Dodecanol	1417	87	0.743
14.45	5,8-Diethyldodecane	1572	88	0.279
14.59	Undec-10-enoic acid, t-butyl ester	1585	89	0.349
14.95	1-Tetradecanol	1675	90	0.372
16.76	2-Hexadecanol	1702	93	0.429
17.58	Myristic acid	1748	97	0.372
18.67	Octadecane	1799	87	0.401
20.08	n-Cetyl alcohol	1864	90	0.936
20.22	Methyl isohexadecanoate	1877	83	0.641
21.82	9-Hexadecenoic acid (Palmitoleic)	1916	90	0.713
22.76	Palmitic acid saturated	1942	95	10.683
24.39	5-Octadecenal	1987	91	0.627
24.74	Methyl octadecadienoate	2012	90	0.162
24.89	Heptadecanoic acid	2038	92	0.956
24.99	Ethyl linoleate	2059	89	0.763
25.70	Linoleic acid	2095	97	5.988
25.86	Oleic Acid	2113	98	8.165
26.24	Stearic acid	2157	93	1.859

29.10	Phytol	2194	90	0.941
29.52	1-Eicosanol	2275	90	0.413
31.69	Arachidic acid	2359	86	0.329
32.24	E-13-Eicosenoic acid	2492	91	0.596
32.62	Methyl retinoate	2528	90	0.398
33.49	Docosanoic acid	2564	86	1.132
35.53	Tetracosanoic acid	2685	87	1.149
39.04	Cholesta-4,6-dien-3-ol	2779	88	1.521
39.29	Stigmastan-3,5-diene	2940	91	0.686
39.59	Cholesterol	3001	96	0.784
40.98	Campesterol	3005	97	6.584
41.36	Stigmasterol	3038	95	2.194
41.49	γ -Sitosterol	3073	91	1.312
42.19	β -Sitosterol	3094	98	24.985
42.68	Stigmastan-3-en-6-ol	3103	91	1.852
42.83	3β-24-methylene-9,19-Cyclolanostan-3-ol	3108	88	1.467
42.93	β-Tocopherol	3043	90	1.558
43.13	13,14-Epoxyoleanan-3-ol acetate	3177	95	0.398
43.56	Ethyl iso-allocholate	3194	87	2.358
43.90	Lupeol	3205	90	0.337
44.34	3β-Lanost-8-en-3-ol	3233	89	0.653
45.14	Lup-20(29)-ene-3β,28-diol	3290	85	0.746
45.36	5α-Stigmastane-3,6-dione	3375	93	0.349
45.66	α-Amyrin	3396	86	0.412

53.03	Pseduosarsasapogenin-5,20-diene	3956	91	1.291
53.56	2-Hydroxy-3-(palmitoyloxy) propyl (9E)-9-octadecenoate	4204	87	4.626

* Identification method: MS comparison of the mass spectra with those of the internal reference mass spectral library (NIST 11).

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