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Antinociceptive and Anti-inflammatory Efficacy of Oleic and Linoleic Acid Application

Ahmet Cihat ÖNER[1, *] Esin AKARSU ORUNÇ[2]

[1] *Van Yüzüncü Yıl University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, 65080, Van, Türkiye*
[2] *Van Yüzüncü Yıl University, Van Health Services Vocational School, Department of Pharmacy Services, 65080, Van, Türkiye*

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ABSTRACT The present study aimed to comparatively evaluate the antinociceptive and anti-inflammatory effects of oleic acid (OA) and linoleic acid (LA), administered individually and in combination, and to investigate their impact on serum pro-inflammatory cytokines in an experimental animal model. A total of 96 rats were orally treated with OA (45–90 mg/kg), LA (45–90 mg/kg), OA + LA (45+45 mg/kg and 90+90 mg/kg), standard analgesics (indomethacin and acetylsalicylic acid, ASA), or vehicle control. The antinociceptive properties were examined through Tail Flick, Hot Plate, and writhing test induced by acetic acid injection, whereas the anti-inflammatory properties were examined through carrageenan-induced paw edema. The concentrations of TNF-α and IL-1β in serum were determined by ELISA. OA demonstrated statistically significant antinociceptive effects in both central and peripheral pain models, exhibiting efficacy comparable to ASA. In contrast, LA alone did not produce significant analgesic effects. Notably, co-administration of OA and LA attenuated the analgesic efficacy observed with OA alone. In the carrageenan-induced inflammation model, both OA and OA + LA significantly reduced paw edema, with effects comparable to indomethacin, whereas LA alone showed limited anti-inflammatory activity. Biochemically, both OA and LA significantly elevated serum TNF-α levels, while no significant differences were observed among groups in IL-1β levels. These findings suggest that OA possesses pronounced antinociceptive and anti-inflammatory properties. However, the reduced efficacy observed with combined administration and the elevation of cytokines indicate that OA and LA might work via different pathways, thus warranting further investigation into their dose-dependent interactions.

Keywords: *Anti-inflammatory, Antinociceptive, Interleukin-1 beta, Linoleic acid, Oleic acid, TNF alpha.*

ÖZ

Oleik ve Linoleik Asit Uygulamasının Antinosiseptif ve Antiinflamatuvar Etkinliği

Bu çalışmanın amacı, oleik asit (OA) ve linoleik asidin (LA) ayrı ayrı ve kombinasyon halinde uygulandığında ağrı kesici ve antiinflamatuar etkilerini karşılaştırmalı olarak değerlendirmek ve deneysel bir hayvan modelinde serum proinflamatuar sitokinler üzerindeki etkilerini araştırmaktır. Toplam 96 Sprague-Dawley sıçanına oral yolla OA (45–90 mg/kg), LA (45–90 mg/kg), OA + LA (45+45 mg/kg ve 90+90 mg/kg), standart analjezikler (indometasin ve asetilsalisilik asit, ASA) veya kontrol grubu olarak taşıyıcı madde uygulandı. Ağrı kesici aktivite, Kuyruk Sıçratma, Sıcak Plaka ve asetik asit kaynaklı kıvranma testleri kullanılarak değerlendirilirken, antiinflamatuar etkiler karagenan kaynaklı pençe ödemi modeli ile değerlendirildi. Tümör nekroz faktörü alfa (TNF-α) ve interlökin-1 beta (IL-1β) serum düzeyleri ELISA kullanılarak ölçüldü. OA, hem merkezi hem de periferik ağrı modellerinde istatistiksel olarak anlamlı antinosiseptif etkiler gösterdi ve ASA ile karşılaştırılabilir bir etkinlik sergiledi. Buna karşılık, LA tek başına anlamlı analjezik etkiler üretmedi. Özellikle, OA ve LA'nın birlikte uygulanması, yalnızca OA ile gözlemlenen analjezik etkinliği azalttı. Karagenan kaynaklı inflamasyon modelinde, hem OA hem de OA + LA, indometasin ile karşılaştırılabilir etkilerle pençe ödemini önemli ölçüde azalttı, oysa LA tek başına sınırlı anti-inflamatuar aktivite gösterdi. Biyokimyasal olarak, hem OA hem de LA serum TNF-α düzeylerini önemli ölçüde yükseltirken, IL-1β düzeylerinde gruplar arasında anlamlı bir fark gözlenmedi. Bu bulgular, OA'nın belirgin antinosiseptif ve anti-inflamatuar özelliklere sahip olduğunu göstermektedir. Ancak, kombine uygulamada gözlemlenen azalmış etkinlik ve pro-enflamatuar sitokinlerin yükselmesi, OA ve LA'nın etkilerini farklı moleküler mekanizmalar aracılığıyla gösterebileceğini ve doz bağımlı etkileşimleri ve altta yatan yollar hakkında daha fazla araştırma yapılmasını gerektirdiğini göstermektedir.

Anahtar Kelimeler: *Antiinflamatuvar, Antinosiseptif, İnterlökin-1 Beta, Linoleik asit, Oleik asit, TNF-alfa.*

✉ *Corresponding author: ahmetcihatoner@yyu.edu.tr

INTRODUCTION

Türkiye, with its rich biodiversity, hosts numerous herbal sources used in traditional medicine. The increasing interest in herbal products stems from their fewer side effects, multiple therapeutic actions, and economic advantages compared to synthetic drugs (Çoban 2003). Particularly, the long-term administration of non-steroidal anti-inflammatory drugs (NSAIDs), widely employed in the treatment of chronic pain and inflammation, is linked to serious adverse effects such as tolerance development, gastrointestinal toxicity, and renal toxicity, necessitating alternative therapeutic approaches (Bozkurt et al. 2017; Sohail et al. 2023).

Pain is a complex sensory experience resulting from the stimulation of receptors called nociceptors, transmitted from somatic or visceral tissues to the spinal cord, and then to the thalamus and cerebral cortex (Sunzini et al., 2023). Inflammation, on the other hand, is a complex biological response occurring in tissues in response to trauma, infection, or autoimmune damage, aiming to eliminate the damaging agent, limit harm, and facilitate the repair of damaged tissue (Guyton and Hall 2001). In these processes, soluble factors such as the pro-inflammatory cytokines Tumor Necrosis Factor-alpha (TNF-α) and Interleukin-1 beta (IL-1β) play a central role (van der Poll et al. 1999).

Oleic acid (OA) and linoleic acid (LA) are unsaturated fatty acids that constitute the main components of many vegetable oils, such as olive oil, sunflower oil, and walnut oil. OA is a monounsaturated fatty acid that is renowned for its antioxidant and anti-inflammatory properties. It reduces the susceptibility of cells to oxidation by decreasing the formation of pro-inflammatory molecules (Asif 2011; Santa-María et al. 2023). LA, a polyunsaturated fatty acid (PUFA), is an essential fatty acid. However, current literature suggests that high dietary LA intake may increase pain susceptibility by leading to the accumulation of arachidonic acid (AA)-derived pro-nociceptive lipid mediators (e.g., prostaglandins and leukotrienes) (Ramsden et al. 2016; Birkic et al. 2022).

This study aimed to evaluate the antinociceptive and anti-inflammatory efficacy of OA and LA in vivo and to elucidate their molecular interactions by examining changes in cytokine levels.

MATERIAL AND METHODS

In this study, a total of 96 Sprague Dawley rats were used, randomly divided into 16 different groups, with 6 healthy rats in each group. All animals were housed under standard laboratory conditions (22±2°C, 50-60% humidity, 12-hour light/dark cycle) and had ad libitum access to food and water. The study received ethical approval from the Van Yüzüncü Yıl University Animal Experiments Local Ethics Committee (YUHADYEK) with protocol number 2019/06 on 27/06/2019.

Chemicals and Drugs

The chemicals used in the experiments were:

• Pain Inducer: Acetic acid (Sigma 320099).

• Test Substances: Oleic acid (OA, AB 333867, abcr Germany), Linoleic acid (LA, BLA 100448, Boston USA Academy; USA).

• Reference Drugs: Indomethacin (Sigma I7378), Acetylsalicylic acid (ASA).

• Inflammation Inducer: 1% lambda carrageenan solution.

• Biochemical Kits: Rat TNF-α ELISA kit (BT lab, Cat no: E0764Ra), Rat IL-1β ELISA kit (BT Lab, Cat No: E0119Ra).

Experimental Groups and Administration

Study groups were arranged separately for antinociceptive and anti-inflammatory efficacy tests (n=6) (Table 1 and 2). All administrations were performed orally (p.o.). The doses of 45 mg/kg and 90 mg/kg were selected based on previously reported in vivo studies. Ramsden et al. (2016) and Hidalgo et al. (2021) demonstrated that fatty acid doses in the range of 40–100 mg/kg produced measurable pharmacological effects in rodent models without overt toxicity. The lower dose (45 mg/kg) was chosen to reflect a physiologically relevant intake level, while the higher dose (90 mg/kg) was included to assess potential dose-dependent effects and to align with established dose-escalation paradigms used in similar preclinical fatty acid studies.

Table 1: Groups for Antinociceptive Experiments.

Group No	Application (Oral)	Dose (mg/kg)
1	Control (Physiological Saline)	0.2 ml
2	Acetylsalicylic Acid (ASA)	300
3	Oleic Acid (OA)	45
4	Oleic Acid (OA)	90
5	Linoleic Acid (LA)	45
6	Linoleic Acid (LA)	90
7	OA + LA (Combined Low Dose)	45 + 45
8	OA + LA (Combined High Dose)	90 + 90

All test substances and reference drugs were administered 30 minutes before pain experiments. n = 6 animals per group. All substances were administered orally (p.o.) 30 minutes before pain tests. OA: oleic acid; LA: linoleic acid; ASA: acetylsalicylic acid; p.o.: per os (oral administration).

Table 2. Groups for Anti-inflammatory Experiments.

Group No	Application (Oral)	Dose (mg/kg)
1	Control (Physiological Saline)	0.2 ml
2	Indomethacin (Reference)	3
3	Oleic Acid (OA)	45
4	Oleic Acid (OA)	90
5	Linoleic Acid (LA)	45
6	Linoleic Acid (LA)	90
7	OA + LA (Combined Low Dose)	45 + 45
8	OA + LA (Combined High Dose)	90 + 90

n = 6 animals per group. Substances were administered to all groups except the control and indomethacin groups after carrageenan application. OA: oleic acid; LA: linoleic acid; p.o.: per os (oral administration).

Substances were administered to all groups except the control and indomethacin groups after carrageenan application.

Experimental Pain Models

Antinociceptive Efficacy

Three different models were used to evaluate central and peripheral antinociceptive efficacy:

The tail flick test assessed the pain response at the central spinal cord level using the D'Amour and Smith (1941) technique. Radiant heat was applied to a specific area on the rat's tail, and the amount of time taken for the rat to withdraw its tail (latency) was recorded. The maximum time of exposure was set at 30 seconds. Measurements of the withdrawal response were made each time before drug administration (baseline) and again 30, 60, and 90 minutes after drug administration.

The hot plate test assessed the pain response at the central supraspinal level using modifications of the Eddy and Leimbach (1953) technique. Rats were placed on a hot plate (55±0.1 °C) and the time taken for each rat to lick or withdraw its paw from the hot plate (latency), was recorded. If the rat did not lick or withdraw its paw from the plate within 30 seconds after being placed on the hot plate, the measurement was terminated (cut-off time). Measurements of the time to lick or withdraw a paw from the hot plate were made each time before drug administration (baseline) and were repeated 30, 60, and 90 minutes after drug administration.

The acetic acid-induced writhing test was conducted as per the methods of Koster et al. (1989). Five (5) minutes after receiving an intraperitoneal injection of 0.6% acetic acid (0.1 ml/10 g) rats were observed for 10 minutes for the number of writhing movements (bending of the back, abdominal muscle contraction, stretching, and extending).

Anti-inflammatory Activity Determination

Carrageenan-Induced Paw Edema Test: We used the method of Winter et al. (1962) to evaluate carrageenan-induced paw edema, with some modifications from the original protocol. The right rear paw of each rat was measured using a plethysmometer before the administration of the drug. After thirty minutes of drug administration, inflammation was induced by injecting 100 µL of a 1% solution of lambda-carrageenan into the subplantar region of the right rear paw. The volume of the paw was re-measured 1, 2 and 3 hours post-injection. The degree of inhibition of the inflammatory response was calculated as a percentage of the inflammatory response and was calculated using the following formula (Kouadio et al. 2000):

%I = [1 - dt/dc] x 100

%I = Percentage inhibition

dt = Volume difference in the paw of the drug-treated group

dc = Volume difference in the paw of the control group

Biochemical Analyses

At the end of the experiment, serum samples obtained from the blood of rats were used to measure TNF-α and IL-1β levels using commercial ELISA kits. Analyses were performed according to kit procedures, and results were detected using an ELISA reader.

Statistical Analysis

The collected data have been expressed as mean and standard error of mean (Mean±SE). Within the framework of ANOVA assumptions, the normality of the data distribution was examined using the Shapiro-Wilk test, and the homogeneity of variances was examined using Levene's test. Data from all groups showed a normal distribution, and the variances were found to be homogeneous. Duncan's multiple range test was used for multiple comparisons. Statistical analyses were carried out using IBM SPSS 22.0 software, and statistical significance was set at a p-value < 0.001.

RESULTS

Findings related to Antinociceptive Efficacy

Groups treated with Oleic Acid (OA) showed pain tolerance (increase in latency time) equivalent to the reference drug ASA in both Tail Flick and Hot Plate tests. The antinociceptive effect of Linoleic Acid (LA) alone was lower compared to OA. Combined applications yielded results similar to the effect shown by OA alone, while LA's combined use was observed to slightly reduce OA's efficacy.

OA application significantly reduced the number of writhes in the peripheral pain model (Acetic Acid Writhing Test) compared to the control group. This effect was found to be similar to that of ASA. While LA alone had a weak effect, combined groups yielded results similar to the effect of OA alone (Figure 1).

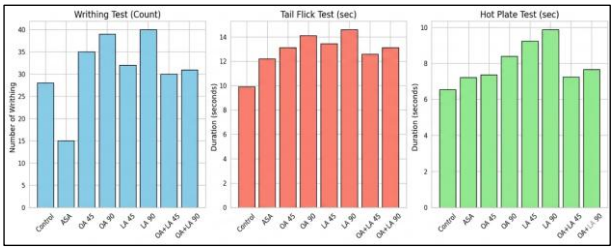

Figure 1: Group means for Writhing, Tail Flick, and Hot Plate tests.

Effects on Cytokine Levels (After Antinociceptive Experiment)

When TNF-α levels were examined, a statistically significant difference was found between the control group and all treatment groups ($p<0.001$). Compared to the control group, a statistically significant increase in serum TNF-α levels was observed with both OA and LA applications. Although the reference drug ASA showed a slight increase compared to the control group, it exhibited a significantly lower pro-inflammatory profile than the fatty acid groups.

There was no statistically significant difference in IL-1β levels between the control, ASA, LA (45) and combined (45) groups ($p>0.05$). However, it was determined that the groups in which high dose OA was administered alone and in combination were unable to reduce IL-1β levels yet were statistically different compared to the control group ($p<0.001$) (Figure 2, Table 3).

Table 3. Serum TNF-α and IL-1β Levels (Mean ± SE) after Antinociceptive Experiments

Experiment Group	TNF-α (pg/mL) (Mean ± SE)	IL-1β (pg/mL) (Mean ± SE)
Control	50.18 ± 3.66^{a}	333.32 ± 36.69^{a}
Acetylsalicylic Acid (ASA)	65.09 ± 6.19^{b}	395.76 ± 56.21^{ab}
Oleic Acid (OA) 45	78.42 ± 8.46^{cd}	488.11 ± 75.13^{bcd}
Oleic Acid (OA) 90	95.62 ± 7.36^{e}	678.45 ± 77.34^{e}
Linoleic Acid (LA) 45	80.99 ± 4.01^{cd}	436.51 ± 48.43^{abc}
Linoleic Acid (LA) 90	100.10 ± 4.52^{e}	538.84 ± 49.14^{cd}
OA + LA (45)	71.74 ± 5.81^{bc}	463.83 ± 66.62^{abcd}
OA + LA (90)	89.01 ± 6.11^{de}	591.52 ± 68.70^{de}
p value	p<0.001	p<0.001

Data are presented as Mean ± SEM (n = 6 per group). Intergroup differences were analyzed using One-way ANOVA and Duncan's multiple comparison test. There was no statistically significant difference between means sharing the same letter ($p>0.05$). Statistical significance was set at $p<0.001$. TNF-α: $p<0.001$ across groups; IL-1β: $p<0.001$ across groups. SEM: standard error of the mean; ANOVA: analysis of variance.

Anti-inflammatory Efficacy Findings

In the carrageenan-induced paw edema model, the edema formed in the first hour was significantly reduced by OA and combined applications compared to indomethacin. The effect of LA alone was not as strong as OA and combined applications. By the end of the 3rd hour, high-

dose combined application (OA 90 + LA 90 mg/kg) was determined to reduce paw edema to a degree similar to the reference drug indomethacin. These results indicate that OA and combined application have a strong inhibitory effect on acute inflammation (Figure 3).

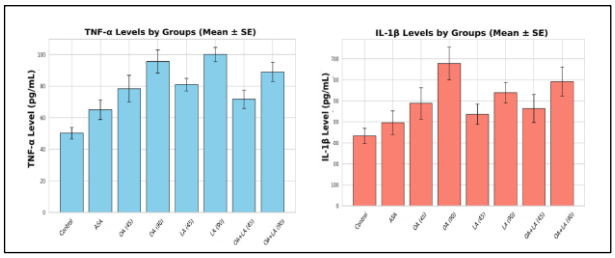

Figure 2: Changes in TNF-α and IL-1β levels in Antinociceptive efficacy with Oleic and Linoleic acid application.

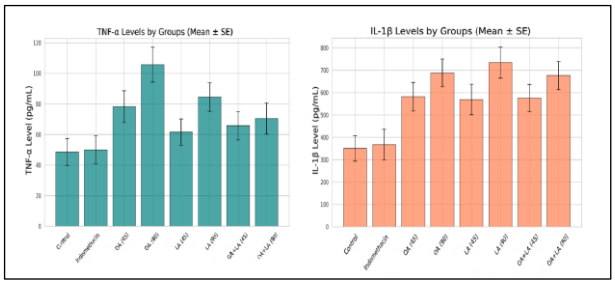

Figure 3: Paw increase graphs with Oleic and Linoleic acid application.

Effects on Cytokine Levels (After Anti-inflammatory Experiment)

In TNF-α measurements, the OA 90 group showed the most significant and distant deviation from the Control and Indomethacin groups ($p<0.001$). The LA 90 group showed a numerical increase compared to the control and indomethacin groups, although not as significant as the OA 90 group, and this increase was statistically significant. No significant difference was observed in the combined groups ($p>0.05$). It was observed that all other treatments did not reduce IL-1β levels and there was no statistically significant difference between the control group and the indomethacin group ($p>0.05$) (Figure 4, Table 4).

Table 4: Serum TNF-α and IL-1β Levels (Mean ± SE) after Anti-inflammatory Experiment.

Experiment Group	TNF-α (pg/mL) (Mean ± SE)	IL-1β (pg/mL) (Mean ± SE)
Control	48.57 ± 8.72^{a}	350.97 ± 57.25^{a}
Indomethacin	50.06 ± 9.24^{a}	367.72 ± 68.78^{a}
Oleic Acid (OA) 45	78.27 ± 10.34^{b}	581.46 ± 63.19^{bc}
Oleic Acid (OA) 90	105.68 ± 11.34^{c}	688.34 ± 62.19^{bc}
Linoleic Acid (LA) 45	61.59 ± 8.67^{ab}	569.19 ± 68.27^{b}
Linoleic Acid (LA) 90	84.57 ± 9.37^{bc}	734.32 ± 69.27^{c}
OA + LA (45)	65.74 ± 9.23^{ab}	575.55 ± 60.45^{bc}
OA + LA (90)	70.50 ± 10.23^{ab}	676.33 ± 62.45^{bc}
p value	p<0.001	p<0.001

Data are presented as Mean ± SEM (n = 6 per group). Intergroup differences were analyzed using One-way ANOVA and Duncan's multiple comparison test. There was no statistically significant difference between means sharing the same letter ($p>0.05$). Statistical significance was set at $p < 0.001$. TNF-α: $p<0.001$ across groups; IL-1β: $p<0.001$ across groups. SEM: standard error of the mean; ANOVA: analysis of variance.

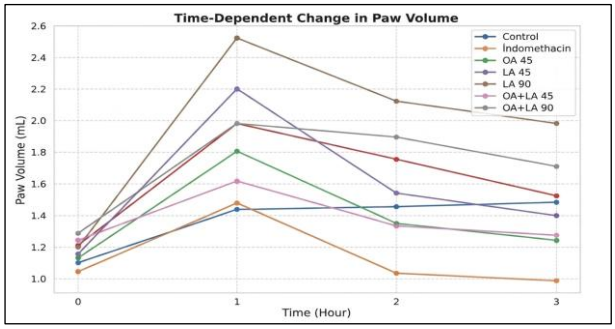

Figure 4: Changes in TNF-α and IL-1β levels in Anti-inflammatory efficacy with Oleic and Linoleic acid application.

DISCUSSION AND CONCLUSION

This study is a significant investigation that comparatively examined the effects of oleic acid (OA) and linoleic acid (LA) on experimentally induced pain and inflammation in rats, alongside reference drugs and cytokine levels. The findings support the potent antinociceptive and anti-inflammatory potential of OA, while indicating that the effect of LA alone is limited, and combined application reveals notable cytokine modulations.

OA's demonstration of efficacy equivalent to the reference drug ASA in both central (Tail Flick, Hot Plate) and peripheral (Acetic Acid Writhing) pain models highlights the therapeutic potential of this monounsaturated fatty acid. The literature reports that OA can inhibit nociceptive signaling by acting through free fatty acid receptor 1 (FFAR1/GPR40) and reduce the sensitivity of the TRPV1 (Transient Receptor Potential Vanilloid 1) channel (Pessina et al. 2024; Sendetski et al. 2024). These mechanisms may explain the potent antinociceptive effect observed in our study at the molecular level.

In the carrageenan-induced paw edema model, OA and combined applications reduced edema to a level similar to indomethacin, indicating that OA is effective in the early phases of acute inflammation. This effect can be associated with OA's ability to modulate cyclooxygenase (COX) and lipoxygenase (LOX) pathways or inhibit nuclear factor kappa B (NF-κB) activation (Santa-María et al. 2023).

In our study, the weak antinociceptive effect of LA alone and its slight reduction of OA's effect in combined application is a critical finding consistent with current literature. LA (n-6 PUFA) serves as a precursor to arachidonic acid (AA) and subsequently to pro-inflammatory and pro-nociceptive lipid mediators (e.g. PGE2, LTB4) in the body (Ramsden et al. 2016; Santin et al. 2023). Evidence suggesting that high dietary LA intake can increase pain susceptibility by increasing plasma accumulation of pro-nociceptive lipid mediators (Birkic et al. 2022) supports the limited efficacy of LA and the potential antagonistic effect in combined application observed in our study.

Inflammation can be described as interactions between soluble factors and cells that may occur in tissues in response to trauma, infection, ischemia, toxic, or autoimmune injury. If the destructive and repair-supporting stages do not proceed smoothly, leukocytes, lymphocytes, or collagen can cause permanent damage to the tissue. The aim here is to eliminate the damaging agent and its products, to keep the damage within limits, and to enable the repair and regeneration of damaged tissue (Guyton and Hall 2001).

Cytokines are known as the first indicators of the body's immune response against microbial attacks in bacterial, viral, fungal, and parasitic infections in many animal models, and TNF-α and IL-1β are stimulated first as cytokines in inflammation (van der Poll et al. 1999). Cytokines such as IL-1β and IL-6 lead to the formation of pro-inflammatory and inflammatory changes and the emergence of a rapid immune response that ensures the elimination of pathogens (Bethea et al. 1992). TNF-α and IL-1β are known to act through similar common signaling molecules that emerge in the early period of inflammation.

In our study, it was observed that oleic acid-linoleic acid and aspirin, used as NSAID preparations, increased TNF-α levels in terms of analgesic effect. Compared to the control group, a statistically significant increase in serum TNF-α levels was observed in oleic and linoleic acid applications ($p<0.001$). In combined applications, serum TNF-α levels were found to be higher than in acetylsalicylic acid, and it was determined that there was no statistically significant difference in low-dose combined application ($p>0.05$), but there was a difference in high-dose application ($p<0.001$). When IL-1β levels were examined, no statistically significant difference was found between the control, acetylsalicylic acid, linoleic acid (45), and combined (45) groups; this indicates that these applications provided a value close to the control ($p>0.05$). There was a significant difference between the groups in which oleic acid applications were given alone and in combination at high doses, and it can be said that they were not effective in treatment compared to the control ($p<0.001$). Although there was a statistically significant difference between the LA (90) group and the control and ASA groups, it can be said that it was not effective in treatment. From an anti-inflammatory perspective, when examining the increase and decrease in paw edema volume, it was observed that paw edema, which increased in the first hour due to the carrageenan effect, decreased with the applications compared to indomethacin. However, linoleic acid application alone was less effective than oleic acid and combined applications. In terms of cytokine levels, it was observed that the applications caused an increase in TNF-α levels after indomethacin-induced inflammation and created a statistically significant difference ($p<0.001$). For TNF-α levels, linoleic acid, combined 45 and combined 90 applications were not statistically different compared to indomethacin and the control group ($p>0.05$), while the other applications were statistically different ($p<0.001$). In terms of IL-1β levels, it was observed that the applications did not decrease IL-1β levels and were statistically different from the control group and the indomethacin group ($p<0.001$).

One of the most striking findings of the study is that OA and LA applications caused a statistically significant increase in serum TNF-α levels after the antinociceptive experiment. This finding contradicts the widespread acceptance that OA generally has anti-inflammatory effects (Santa-María et al. 2023). However, this contradiction should be evaluated in the context of dose-response relationships and duration of application. The literature indicates that OA may exhibit anti-inflammatory effects at low doses, while at high doses or in different cellular contexts, it may trigger the release of pro-inflammatory cytokines (Hidalgo et al. 2021). It is thought that the doses used in our study (45 and 90 mg/kg) might have exceeded this pro-inflammatory threshold. Cytokine levels are indicative of an acute response. While antinociceptive tests measure acute effects, the increase in cytokines may be due to the acute modulatory effect of fatty acids on immune cells. LA being a precursor to pro-inflammatory lipid mediators may have contributed to the increase in TNF-α in combined application and LA alone groups.

In this study, after the anti-inflammatory experiment, LA and combined groups were observed to manage TNF-α levels more successfully compared to control and indomethacin. This situation suggests that in the carrageenan-induced inflammation model, the anti-inflammatory mechanisms of fatty acids (e.g., FFAR1 activation or NF-κB inhibition) become more pronounced. The inability to achieve a general reduction in IL-1β levels indicates that the modulation of this cytokine is more complex or requires different signaling pathways.

Several limitations of the present study should be acknowledged. First, the sample size of six animals per group is relatively small, which may limit the statistical power and generalizability of the findings. Second, only two pro-inflammatory cytokines (TNF-α and IL-1β) were measured; a broader cytokine panel including IL-6, IL-10, and PGE2 would provide a more comprehensive picture of the inflammatory response. Third, histopathological analysis of target tissues was not performed, which would have provided direct morphological evidence of inflammatory changes. Fourth, key mechanistic markers such as cyclooxygenase-2 (COX-2), nuclear factor kappa B (NF-κB) and prostaglandin E2 (PGE2) were not assessed, leaving the molecular pathways underlying the observed effects incompletely characterized. Finally, only acute (single-dose) administration was evaluated; the effects of repeated or long-term treatment with OA and LA remain unknown and warrant investigation in future studies.

In conclusion, it was concluded that oleic acid application, both alone and in combination with linoleic acid, may be effective in reducing inflammation. It is thought that it may be beneficial to examine efficacy in more detail in future antinociceptive or different pain model studies. However, compared to acetylsalicylic acid, whose efficacy was determined and compared at the antinociceptive efficacy level, the efficacy of oleic and linoleic acid was found to be considerable. However, due to the increase in serum cytokine levels, it was thought that oleic acid and linoleic acid stimulated inflammatory markers, and although they were effective in acute pain relief, it would be more appropriate to investigate these effects with different studies and different doses.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest for this study.

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AUTHOR CONTRIBUTIONS

Idea/Concept: ACÖ
Supervision/Consultancy: ACÖ, EAO
Data Collection and/or Processing: ACÖ, EAO
Analysis and/or Interpretation: ACÖ
Writing the Article: ACÖ, EAO
Critical Review: ACÖ

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