Anti-Inflammatory and Immunoregulatory Effects of Sunflower, Pumpkin, and Flaxseed Oils: Association of Fatty Acid Profiles with Modulation of TNF-α, IL-10, and IL-6

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Abstract

Background: Seed oils include bioactive fatty acids that may affect immunological and inflammatory processes. The immunomodulatory effects of different seed oils are mainly caused by their fatty acid composition.

Objective: This study aimed to evaluate the immunomodulatory and anti-inflammatory potential of sunflower, flaxseed, and pumpkin seed oils by comparing their fatty acid profiles as determined by Gas Chromatography–Mass Spectrometry (GC–MS) with serum levels of inflammatory and anti-inflammatory cytokines.

Materials and Methods: Rat-specific sandwich ELISA kits (Elabscience Biotechnology Inc.) were used to quantify serum levels of interleukin-6 (IL-6), interleukin-10 (IL-10), and TNF-α. One Way ANOVA was used for statistical analysis. The Pearson technique was used to calculate the correlation between treatment groups. ROC curve analysis was used to evaluate the cytokine markers' discriminating potential.

Results: The biggest immunomodulatory impact was induced by flaxseed oil, as evidenced by a considerable increase ($p < 0.05$) in IL-10 concentration and a significant decrease ($p \leq 0.05$) in TNF-α and IL-6 levels in treatment groups relative to control groups. While sunflower oil had a lesser cytokine modulation profile, pumpkin seed oil had a modest effect. The correlation analysis revealed that IL-6 and TNF-α had a positive significant association.

Conclusion: The data show that the fatty acid content of seed oils affects their immunomodulatory function. Flaxseed oil's high α-linolenic acid content was linked to better cytokine balance and increased anti-inflammatory activity, indicating that it may be a natural dietary regulator of immune function. as at-risk on zinc level might provide clinically relevant information and indicates that resolving of zinc deficiency may be focused within the panoply of management complexities diabetes with its accompanying endocrine co-morbidities.

Keywords: Immunomodulation; Inflammation; IL-6; TNF-α; IL-10; Fatty Acids; GC–MS.

1. Introduction

Uncontrolled, excessive, or unresolved inflammation is harmful to the host and can lead to disease since the immune system protects the host against infections and is thought to be a natural component of host defense (Calder, 2024). Linoleic acid's effects on health probably go beyond cardiometabolic disease to include inflammation, immunology, and the microbiota (Calder, 2026). Maresins Resolvins and protectins are families of chemically different mediators with powerful roles in the resolution of acute and chronic inflammation in the respiratory system. These mediators are derived from omega-3 fatty acids (e.g., EPA and DHA) (Serhan & Levy, 2025). Fatty acids (FAs) generated from food are vital energy sources and basic cell structure elements. Additionally, they are crucial in controlling immunological responses in both health and illness. By altering the fluidity and composition of membranes and by interacting with certain receptors, saturated and unsaturated FAs affect the effector and regulatory activities of innate and adaptive immune cells (Hu et al., 2024). GC-MS is the method of choice for life and plant scientists due to its high potential in the research of low molecular weight metabolites involved in vital cellular functions (energy production, metabolic adjustment, signaling) (Frolova et al., 2026; Vodolazska & Lauridsen, 2020). shown how dietary hemp seed oil, as opposed to soybean oil, affected the immunological and nutritional health of piglets as well as the transfer of fatty acids to the offspring in nursing sows. Polyunsaturated fatty acids (PUFAs), especially omega-3 and omega-6 fatty acids, have a major impact on signal transduction, cellular membrane integrity, and the regulation of inflammatory mediators (Patted et al., 2024). Fatty acids derived from plant oils may promote immunological regulation and inflammatory homeostasis. The regulation of immune-cell activity and anti-inflammatory characteristics have been linked to omega-3 fatty acids, such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and α-linolenic acid (ALA) (Banaszak et al., 2024). Additionally, cytokine production, oxidative stress responses, macrophage activation, and epithelial barrier integrity can all be impacted by short-chain and unsaturated fatty acids (Mann et al., 2024). Numerous physiologic mechanisms, including alterations in membrane fluidity, modulation of inflammatory signaling pathways, cytokine generation, oxidative stress balance, and immune cell activation, are how dietary fatty acids impact immune responses. Omega-3 fatty acids, particularly α-linolenic acid, have well-known anti-inflammatory and immunomodulatory qualities. Certain pro-resolving mediators (SPMs), like protective and resolving, which support tissue repair and inflammation resolution, are occurred with the help of these fatty acids (Maliha et al., 2024). Soybean oils, sunflower, and corn are rich sources of linoleic acid, a crucial omega-6 fatty acid. It was originally believed that omega-6 fatty acids could be pro-inflammatory because they could serve as precursors for inflammatory mediators made from arachidonic acid. However, recent studies show that modest dietary consumption of seed oils does not always result in an increase in systemic inflammation (Fornari Laurindo et al., 2025). Oxidative stress is closely associated with metabolic issues, chronic inflammatory disorders, and immune dysregulation (Jayaprakash et al., 2024). Edible plant oils may affect the structure of intestinal bacteria and the integrity of the intestinal barrier. Dietary fatty acids may indirectly influence systemic inflammatory and immunological responses through microbiota-mediated pathways since the gut microbiome is crucial for immune (Zou et al., 2024). By lowering systemic inflammation, circulating cytokines (including IL-6 and TNF-α), and pro-tumorigenic adipokines, as well as promoting a gut microbiome rich in short-chain fatty acids, reducing visceral adiposity can enhance intestinal homeostasis (Ma et al., 2024). The current study will use GC-MS to investigate the association between fatty acid content and cytokine modulation in order to assess the potential immunomodulatory and anti-inflammatory effects of flaxseed, pumpkin seed, and sunflower oils on serum levels of IL-6, TNF-α, and IL-10.

2. Materials and Methods

2.1. Animals of the study

The present study was conducted from October 2025 to May 2026 at the College of Veterinary Medicine at the University of Kerbala. From the medical research and care institution in Baghdad, Iraq, we purchased forty adult female (Rattus norvegicus) rats. They were between 14 and 16 weeks old, and their average weight was between 200 and 250 grams. Throughout the research, the animals had unrestricted access to food and water, were kept in hygienic circumstances, and were clinically healthy. The animals were housed in the lab for two weeks before the beginning of the study. The light system has a 12-hour light/dark cycle.

2.2. The design of the experiment

The duration of the experiment is thirty days. The forty female albino rats were split up into the following four major groups, each with ten animals:

1- The general control (GC) group consisted of ten female rats given just oral normal saline (0.5 ml/kg B.W.) as a vehicle.

2- Treatment 1(T1) group: Ten female rats were given oral sunflower oil (0.5 ml/kg B.W.).

3-Treatment 2(T2) group: Ten female rats were given oral flaxseed oil (0.5ml/kg B.W.).

4-Treatment 3(T3) group: Ten female rats were given oral pumpkin seed oil (0.5ml/kg B.W.).

Based on earlier experimental animal research describing the biological effects of dietary oils following oral administration, a dose of 0.5 ml/kg body weight of sunflower oil, flaxseed oil, and pumpkin seed oil was chosen. Rats given oral flaxseed oil at dosages of 0.5 ml/kg showed protective and antioxidant effects. Additionally, the anti-inflammatory and molecular effects of pumpkin seed oil have been tested in rat models at 0.5 ml/kg/day. As a result, this dosage was thought to be suitable for achieving biological activity without administering too much fat. G*Power software was used to conduct an a priori power study based on earlier research. A significance level of ($p \leq 0.01$) and statistical power of 80% were used in the analysis. Ten animals per group were found to be adequate to identify significant changes between groups, according to the computed sample size.

2.3. Blood sample collection

After 30 days, ten female rats from each group were sacrificed. Prior to sacrifice, the rats were put in a closed jar and animals were anesthetized using chloroform inhalation before blood collection. Heart punctures were used to draw blood samples, which were then placed in gel tubes and centrifuged at 5000 rpm for 3 minutes to extract the serum. Following the transfer of the serum to epndrofe tubes for immunological evaluation, all tubes were stored at -20°C until analysis.

2.4. Immunological parameters

According to manufacturer instructions, the ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technique for measuring tumor necrotic factor (TNF-α), interleukin (IL-10), and interleukin (IL-6). Rat-specific sandwich ELISA kits from Elabscience Biotechnology Inc. were used to measure serum quantities of IL-6, TNF-α, and IL-10. Serum samples were evaluated in accordance with the manufacturer's instructions; the catalog numbers were E-EL-R0015 for IL-6, E-EL-R0016 for IL-10, and E-EL-R0019 for TNF-α. Picograms per milliliter (pg/mL) was used to express cytokine concentrations. The IL-6 detection range was 12.5–800 pg/mL, whereas the IL-10 and TNF-α detection ranges were 15.63–1000 pg/mL.

2.5. Preparation of seed oils

The chosen seed materials were acquired from reliable vendors, including pumpkin (Cucurbita pepo), sunflower (Helianthus annuus), and flaxseed (Linum usitatissimum). Before beginning the extraction process, the authors cleaned and dried the seeds. Two distinct methods were used in the oil extraction procedure.

1. Soxhlet extraction (n-hexane solvent, 65°C extraction temperature, 6 hours)

2. Cold pressing, which keeps the temperature below 40°C. The extracted oils were kept in amber glass vials at 4°C.The following analytical equipment was utilized throughout the study.

3.Fatty acid analysis using gas chromatography-mass spectrometry: Agilent 7890B in conjunction with a 5977A MSD; HP-88 column (60 m × 0.25 mm × 0.20 µm)

4.Soxhlet apparatus: Cellulose extraction thimbles in a standard laboratory glassware arrangement

5.Cold Press Machine: A hydraulic press made of stainless steel that runs below 40°C

Brookfield DV-E digital viscometer, accuracy ±1.0%.6

Digital refractive index measurement using an ATAGO RX-5000α refractometer7.

8.The Shimadzu UV-1800 UV-Vis Spectrophotometer is used to evaluate color intensity.

The Soxhlet extraction procedure with n-hexane at 65°C for six hours was used to extract the oils given to the animals. For use in further research, the concentrated extracted oils were kept at 4°C in amber glass vials.

Inclusion and Exclusion Criteria

Inclusion Criteria

1.Adult female albino rats in good health (Rattus norvegicus).

2.14–16 weeks of age.

3.200–250 g is the range of body weight.

4.animals that are clinically healthy and show no symptoms of illness or damage.

5.Prior to the trial, the animals were acclimated for a minimum of two weeks.

Exclusion Criteria

1.Rats showing signs of illness, infection, or aberrant behavior throughout the acclimation phase.

2.Animals whose body weights fall outside of the authorized range.

3.Animals that are injured or pregnant.

4.Animals whose biological samples were either incomplete or died during the experiment.

2.6. Ethical Approval

The Ethical Committee of the University of Kerbala's College of Applied Medical Science authorized the study protocol before to its start. On 1\10\ 2025, reference number PAAMSKU/34. No data collection or sample handling was performed prior to ethical approval

2.7. Statistical analysis

The Statistical Package for Social Sciences (SPSS, IBM, version 21.0) was used to conduct the statistical analysis. The Shapiro-Wilk and Kolmogorov-Smirnov tests were used to assess the normality of the data distribution. Continuous data with a normal distribution is represented by the mean with standard deviation (Mean ± SD). The means of each group were compared using One Way ANOVA. Relationships between variables were ascertained using Pearson correlation. $P < 0.01$ is regarded as significant. To improve statistical rigor and reduce false-positive results, especially when numerous immunological parameters are present, instead of the usual $p < 0.05$, the significance level was set at $p < 0.01$.

3. Results

3.1. Distribution of study parameters (IL-10, TNF-α and IL-6) in control group sunflower oil group, pumpkin seed oil, and flaxseed oil group

Each experimental group's cytokine data distribution was evaluated for normalcy using the Shapiro-Wilk test. The control group, sunflower oil group, pumpkin seed oil group, and flaxseed oil group all exhibited a normal distribution of all evaluated values (IL-10, TNF-α and IL-6). The cytokine values in the control group were found to be normally distributed by the Shapiro-Wilk test, with non-significant results for IL-6 ($p = 0.77$), TNF-α ($p = 0.89$), and IL-10 ($p = 0.83$). Similarly, IL-6 ($p = 0.77$), TNF-α ($p = 0.97$), and IL-10 ($p = 0.65$) showed a normal distribution in the sunflower oil-treated group. Furthermore, TNF-α ($p = 0.78$), IL-10 ($p = 0.52$), and IL-6 ($p = 0.48$) showed regularly distributed results in the pumpkin seed oil group. The flaxseed oil group showed a normal distribution for all cytokine markers, including IL-6 ($p = 0.89$), TNF-α ($p=0.89$), and IL-10 ($p = 0.98$). The null hypothesis of normality was accepted because every p-value was higher than 0.05, indicating that the data satisfied the normal distribution assumption. For the purpose of comparing cytokine levels between the four experimental groups, parametric statistical tests—specifically, one-way ANOVA followed by Tukey's post hoc test—are suitable.

Table 1. Distribution of study parameters (IL-10, TNF-α and IL-6) in control group, sunflower oil group, pumpkin seed oil, and flaxseed oil group.

Groups **Parameters**	**control group** **Statistic**	**df**	**Sig.**
IL-6	0.96	10	0.77
TNF-α	0.97	10	0.89
IL-10	0.96	10	0.83
	sunflower oil group		
IL-6	0.96	10	0.77
TNF-α	0.98	10	0.97
IL-10	0.95	10	0.65
	pumpkin seed oil group		
IL-6	0.93	10	0.48

Groups Parameters	control group Statistic	df	Sig.
TNF-α	0.96	10	0.78
IL-10	0.94	10	0.52
	flaxseed oil group		
IL-6	0.97	10	0.89
TNF-α	0.97	10	0.89
IL-10	0.98	10	0.98

3.2. Effect of sunflower oil, flaxseed oil, and pumpkin seed oil on serum levels of TNF-α ,IL-6 and IL-10 in Mature Female Rats

The administration of (0.5ml/kg B.W.) of sunflower oil, flaxseed oil, and pumpkin seed oil demonstrated a significant decrease ($p \leq 0.01$) in serum of IL-6 and TNF-α concentrations while noted significantly increase ($p \leq 0.01$) in IL-10 concentration of female rats in flaxseed oil group compared with control group. On the other hand, A moderate significant decrease ($p \leq 0.01$) was observed in serum of IL-6 and TNF-α concentrations while noted moderate significant increase ($p \leq 0.01$) in IL-10 concentration of female rats in pumpkin seed oil group compared with control group. Lastly, it was observed that female rats in the sunflower oil group had a mildly notably rise ($p \leq 0.01$) in the concentration of IL-10 and a mildly significant drop ($p \leq 0.01$) in the concentrations of TNF-α and IL-6 in their serum.

Table 2. Effect of sunflower oil, flaxseed oil, and pumpkin seed oil on serum levels of TNF-α , IL-6 and IL-10 and in Mature Female Rats (Means ± SD) .

Parameters Groups	IL-6 pg/ml	TNF-α pg/ml	IL-10 pg/ml
Control group (0.5ml/kg/B.W)	A 41.80±5.09	A 36.00±6.06	A 30.20±5.77
Sunflower oil group (0.5ml/kg/B.W)	B 38.10±5.89	B 30.80±6.41	B 37.30±5.62
Pumpkin seed oil (0.5ml/kg/B.W)	C 27.90±6.44	C 20.50±5.40	C 52.10±8.27
Flaxseed oil group (0.5ml/kg/B.W)	D 16.00±6.06	D 11.00±6.O6	D 69.70±9.96

A significant difference is shown by different letters at ($p \leq 0.01$)

3.3. Correlation coefficient between study parameters (IL-10, TNF-α and IL-6) in treatment groups (sunflower oil, flaxseed oil, and pumpkin seed oil)

The results showed a positive significant correlation ($p \leq 0.01$) between TNF-α and IL-6 (r=0.995) and a negative significant correlation ($p \leq 0.05$) between IL-10 and IL-6 (r=-0.462) and TNF-α and IL-10 (r=-0.429), according to Table 3's correlation results between study parameters in treatment groups (sunflower oil, flaxseed oil, and pumpkin seed oil).

Table 3. Correlation coefficient between study parameters (IL-10, TNF-α and IL-6 and) in treatment groups (sunflower oil, flaxseed oil, and pumpkin seed oil).

Parameters		Parameters IL-6	TNF-α	IL-10
IL-6	r	1	0.995**	-0.462*
	p-value	30	0.0001	0.010
TNF-α	r	0.995**	1	-0.429*
	p-value	0.0001	30	0.018
IL-10	r	-0.462*	-0.429*	1
	p-value	0.010	0.018	30

Note:** Correlation is significant at($p \leq 0.01$), * Correlation is significant at ($p \leq 0.05$), r: correlation coefficient

3.4. Discriminatory performance of IL-6, TNF-α and IL-10 depend on ROC curve analysis in control group and treatment groups

ROC curve analysis demonstrated that IL-10 had the highest discriminatory ability (AUC=0.94), followed by TNF-α (AUC=0.90) and IL-6 (AUC=0.88). Decreased IL-6 and TNF-α and increased IL-10 were considered indicators of improved anti-inflammatory immunomodulation.

Table 4. Discriminatory performance of IL-6, TNF-α and IL-10 based on ROC curve analysis in control group and treatment groups.

Parameters	AUC	Sensitivity (%)	Specificity (%)	Cut-off
IL-6	0.88	80%	80%	37.50 pg/mL
TNF-α	0.90	80%	80%	29.50 pg/mL
IL-10	0.94	80%	100%	40.50 pg/mL

3.5. Fatty Acid Analysis by GC-MS

Following the preparation of fatty acid methyl esters (FAMEs), fatty acid contents were ascertained using GC-MS. The fatty acid makeup of the three oils clearly varied, according to GC-MS analysis. The highest anti-inflammatory and immunoregulatory benefits of flaxseed oil are explained by its high α-Linolenic acid (ω-3) content (55.2%), which increases IL-10 and decreases inflammatory cytokines including TNF-α and IL-6. Due to the presence of unsaturated fatty acids and antioxidant substances, pumpkin seed oil, which had a high percentage of oleic acid (34.1%) and linoleic acid (47.5%), had a modest immunological effect. Sunflower oil had a lesser immunological response than flaxseed oil because of its lower ω-3 acid concentration, even though it had the highest amount of linoleic acid (ω-6) at 58.9%.. Detailed profiles are shown in Table 5

Table 5. Fatty acid composition (%) identified by GC-MS**.**

Fatty Acid	Sunflower Oil	Flaxseed Oil	Pumpkin Seed Oil
Palmitic Acid (C16:0)	6.5	5.8	11.5
Stearic Acid (C18:0)	3.8	3.5	5.2
Oleic Acid (C18:1)	28.5	19.3	34.1

Fatty Acid	Sunflower Oil	Flaxseed Oil	Pumpkin Seed Oil
Linoleic Acid (C18:2 ω-6)	58.9	16.2	47.5
α-Linolenic Acid (C18:3 ω-3)	0.3	55.2	0.7
Others	2	0	1

3.6. ROC curve showing the Discriminatory performance of IL-6, TNF-α and IL-10 in control group and treatment groups

Results of the ROC curve appeared the discriminatory performance of IL-6, TNF-α and IL-10 in treatment groups and control group showed that IL-6 and TNF-α had a sensitivity of 80% and specificity of 80% for distinguishing treatment groups from control group. IL-10 is extremely specific for immunomodulatory effects induced by seed oil supplementation, as evidenced by its apparent sensitivity of 80% and specificity of 100%.

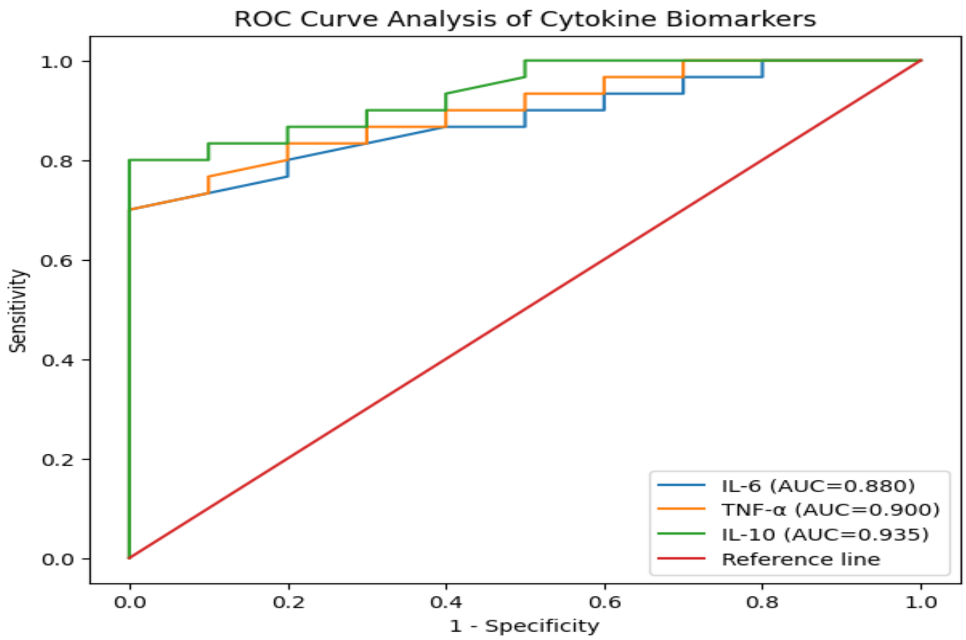

Figure 1. ROC curve showing the Discriminatory performance of IL-6, TNF-α and IL-10 in control group and treatment groups.

3.7. Fatty acid composition of Sunflower Oil

The circular diagram chart illustrates the fatty acid composition of sunflower oil based on GC–MS analysis .Linoleic acid (C18:2 ω-6) was the predominant fatty acid at 58.9%, indicating that sunflower oil is rich in polyunsaturated fatty acids of the ω-6 type.Oleic acid (C18:1) constituted 28.5% of the composition, and it is a monounsaturated fatty acid that may contribute to supporting the balance of the inflammatory response Palmitic Acid (C16:0) 6.5% and Stearic Acid (C18:0) 3.8%α-Linolenic acid (C18:3 ω-3) was very low at 0.3%. Sunflower oil is characterized by a high content of linoleic acid (ω-6) and a very low content of α-linolenic acid (ω-3), so its effect on regulating inflammation is expected to be less compared to flaxseed oil, which is rich in ω-3. However, its content of unsaturated fatty acids may grant it a moderate immune effect by influencing cell membrane composition and inflammation pathways.

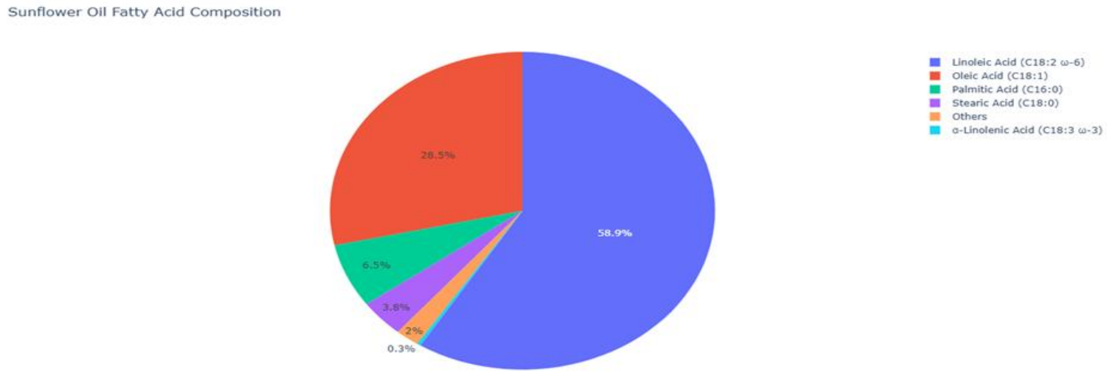

Figure 2. Fatty acid composition of Sunflower Oil.

3.8. Fatty acid composition of Pumpkin Seed Oil

Based on GC-MS research, the circular diagram shows the fatty acid composition of pumpkin seed oil. At 47.5%, linoleic acid (C18:2 ω-6), a polyunsaturated fatty acid implicated in cell membrane composition and immune system modulation, was the predominant fatty acid. Oleic acid (C18:1), a monounsaturated fatty acid linked to better cell function and supporting effects on inflammatory homeostasis, reached 34.1%. Stearic acid (C18:0) is 5.2% and palmitic acid (C16:0) is 11.5%. In comparison to flaxseed oil, the amount of α-Linolenic acid (C18:3 ω-3) was extremely low (0.7%). Therefore, in comparison to flaxseed oil, it is anticipated to have a mild immunological effect. Unsaturated fatty acids and related substances like phytosterols and antioxidants may be responsible for its action, which may lead to a slight decrease in TNF-α and IL-6 and an increase in IL-10.

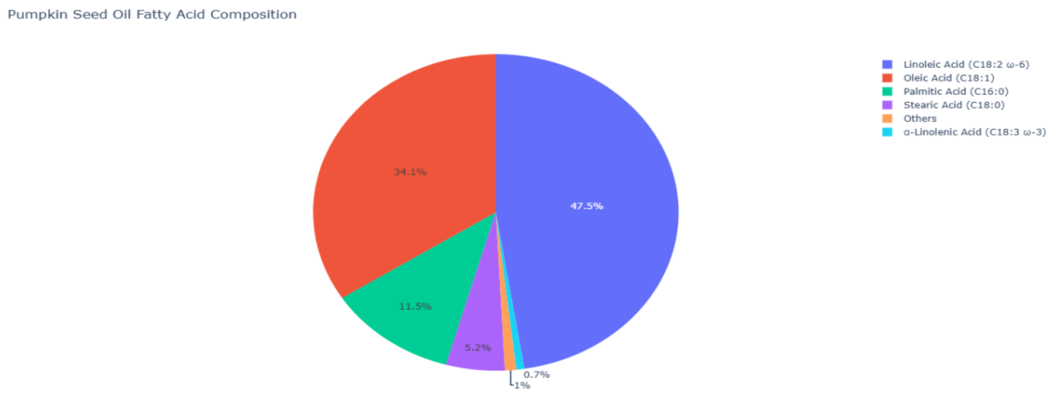

Figure 3. Fatty acid composition of Pumpkin Seed Oil.

3.9. Fatty acid composition of Flaxseed Oil

The circular diagram illustrates the fatty acid composition of flaxseed oil based on GC–MS analysis. α-Linolenic acid (C18:3 ω-3) was the main component at 55.2%, and it is an omega-3 fatty acid associated with anti-inflammatory properties and immune response regulation. Oleic acid (C18:1) constituted 19.3% of the composition, and it is a monounsaturated fatty acid that may contribute to improving the balance of inflammation. Linoleic acid (C18:2 ω-6) reached 16.2%, and it is an essential acid that participates in the composition of cell membranes and immune pathways. The saturated fatty acids were low are palmitic Acid (C16:0) 5.8 % and stearic Acid (C18:0) 3.5 %, supporting the hypothesis that it has anti-inflammatory and immunomodulatory effects, which aligns with the expected decrease in IL-6 and TNF-α and the increase in IL-10 in the rat model.

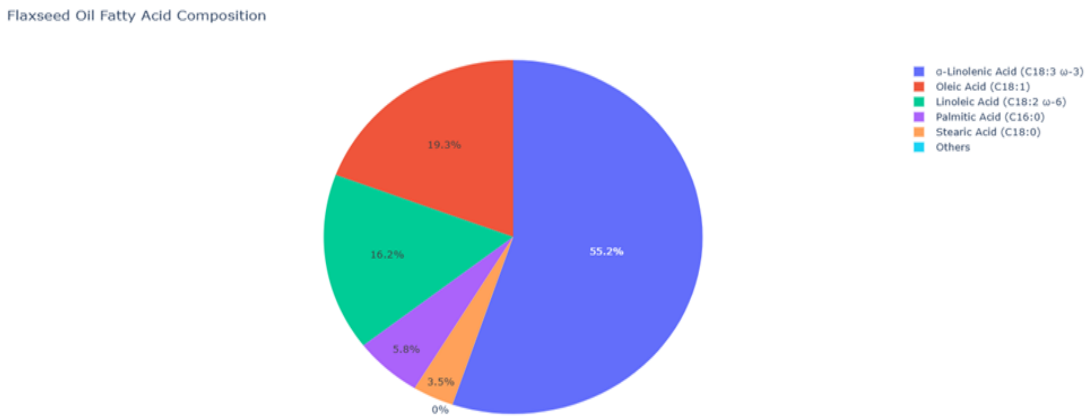

figure 4. Fatty acid composition of Flaxseed Oil.

4. Discussion

The current study's results showed that female rats in the flaxseed oil group had significantly lower levels of IL-6 and TNF-α and significantly higher levels of IL-10 when compared to the control group. Alongside these findings, Martins et al. (2018) showed that the inflammatory marker was significantly lower in the flaxseed group. TNF-α, supporting the notion that components of flaxseed can lower anti-inflammatory and antiatherogenic activity. According to a different study, administering flaxseed powder for four weeks lowers TNF-a, IL-1, and IL-6 (Dali et al., 2019). Tumor necrosis factor-alpha (TNF-α), one of the primary pro-inflammatory cytokines, is elevated in numerous inflammatory diseases and is crucial for regulating inflammatory reactions. Two more inflammatory mediators that actively contribute to the onset and development of inflammatory illnesses are interleukin-6 (IL-6) and interleukin-1 (IL-1). Therefore, controlling or suppressing these immune factors has been considered a potential therapeutic strategy for lowering inflammation. Numerous studies have demonstrated that bioactive dietary ingredients and nutritional supplements can improve immunological homeostasis and reduce the generation of pro-inflammatory cytokines. In addition to its inflammatory functions, TNF-α has been shown to impact lipid metabolism by reducing the absorption of free fatty acids (FFA) and promoting modifications in lipogenesis and lipolysis, which may result in hyperlipidemia and atherogenesis (Chen et al., 2009). Similarly, IL-6 has a major role in the pathogenesis of coronary heart disease (CHD Therefore, down-regulating these cytokines may prevent atherosclerosis from developing. Furthermore, flaxseed was discovered to have analgesic activity that was relatively equal to morphine due to its high antioxidant content (Rafieian-Kopaei et al., 2017). A-linolenic acid, which has anti-inflammatory properties, is abundant in flaxseed oil (Kaithwas et al., 2011). Furthermore, when several proteases degraded the proteins in flaxseeds, bioactive peptides with anti-inflammatory and antioxidant properties were produced (Saini et al., 2010). Inflammatory markers (IL-6 and CRP) significantly decreased after three months of SDG supplementation at 600 mg/day in another research of obese women (Barre et al., 2012) .

Results of the present study showed that moderate significant decrease in IL-6 and TNF-α concentrations while noted moderate significant increase in IL-10 concentration of female rats in pumpkin seed oil group compared with control group These findings, along with (Saleh et al., 2024) , demonstrated that the pumpkin decoction enhanced the immune response, as demonstrated by a significant increase in the expression of interleukin-17 (IL-17) and transforming growth factor (TGF-1β) and a significant decrease in nitric oxide (NO) and tumor necrosis factor (TNF-α). (Kar et al., 2023). focuses on the pleiotropic effects and possible cytotoxic therapeutic significance of n-3 polyunsaturated fatty acids (PUFAs) against cancer cell growth, retinal disorders, cardiovascular illnesses, chronic inflammation, and platelet aggregation in the human body. The production of derivatives in the cytosol, such as Omega-3 FAs, EPA, and DHA, depends on PUFAs, which are linked to inflammation, platelet aggregation, immunological response, and cyclooxygenase (COX) activation, according to other physiological responses. Recent research has demonstrated the potential advantages of providing COVID-19 patients with omega-3 FAs. Omega-3 FAs can lower inflammation when used as a co-therapy for COVID-19, according to clinical research (Rogero et al., 2020). Through the Mitogen-Activated Protein Kinases (MAPKs) pathway, Activator protein-1 (AP-1) activities are downregulated by the therapeutic intracellular activity of omega-3 and EPA, it stops the growth of tumor cells in people by raising different cancer-related cachexia and lowering proinflammatory cytokine levels in chronic inflammatory syndrome (Pan et al., 2009). Omega-3 FAs' anti-inflammatory qualities are utilized to treat inflammatory conditions such psoriasis, rheumatoid arthritis, and inflammatory bowel illness (Fetterman Jr & Zdanowicz, 2009). According to myocardial research, PUFAs can reduce the production of pro-inflammatory cytokines in epicardial adipose tissue, such as monocyte chemoattractant protein-1 (MCP-1), interleukin 6 (IL-6), and tumor necrosis factor-alpha (TNF), thereby controlling chronic pro-inflammatory and thrombogenesis disorders (Puglisi et al., 2011).

Results of the present study showed that mild significant decrease in TNF-α and IL-6 levels while noted mild significant increase in IL-10 concentration of female rats in sunflower oil group compared with control group These findings, along with (Moustafa, 2021). indicated that the intratesticular levels of irisin were considerably raised by the administration of either omega-3 or sunflower oil, which may contribute to testicular protection through its possible anti-inflammatory effects, it markedly reduced the intratesticular concentrations of NF-κB, TNF-α, IL-1β, IL-6, and resistin. It has been demonstrated that proinflammatory mediators including elastase and IL-6 positively associated with seminal resistin levels [Morettiet al., 2014], and TNF-α (Kratzsch et al., 2008) . Resistin has been shown to be an inflammation marker. Moreover, irisin has demonstrated anti-inflammatory properties by lowering NF-κB activity and proinflammatory cytokines such TNF-α, IL-1β, and IL-6. Additionally, via stimulating proinflammatory and anti-inflammatory cytokines, visfatin mediates inflammatory responses in monocytes (Dedoussis et al., 2009). Since visfatin has been primarily described as a proinflammatory marker by increasing the levels of IL-1β, IL-6, and TNF-α, the reduced intratesticular levels of visfatin may have contributed to the changes seen in proinflammatory cytokines after the administration of sunflower oil and omega-3 (Luk et al., 2008). Intratesticular levels of vaspin were suppressed following the injection of either sunflower oil or omega-3 fatty acids (Zieger et al., 2018). Vaspin's anti-inflammatory actions are achieved through the reduction of IL-1β and TNF-α-induced activation of NF-κB. Because omega-3 fatty acids are associated with reductions in pro-inflammatory cytokines, acute-phase proteins, and endothelial activation indicators in humans, they may be used to treat chronic inflammatory diseases (Calder, 2010). Even in populations with elevated oxidative stress, omega-3 supplements have been demonstrated to lower circulating levels of pro-inflammatory cytokines like IL-6, TNF-α, and IL-1β. These results imply that omega-3s may change the immune balance toward a less inflammatory phenotype. In an open-label randomized crossover study with heavy smokers, (Elisia et al., 2022). found improvements in the granulocyte-to-lymphocyte ratio along with decreases in TNF-α and IL-6 after EPA and DHA supplementation. Furthermore, several studies have demonstrated increases in the anti-inflammatory cytokine interleukin-10 (IL-10), suggesting that omega-3 fatty acids may enhance immunoregulatory pathways and reduce pro-inflammatory signaling. (Borja-Magno et al., 2023) found that high-dose EPA and DHA supplementation over a 12-week period significantly reduced TNF-α and IL-6 concentrations in patients with obesity, demonstrating the potent immunomodulatory effects of long-chain omega-3s on metabolic inflammation. Omega-3 supplementation reduces TNF-α and boosts antioxidant capacity in type 2 diabetes mellitus, according to meta-analyses; however, effects on IL-6 appear to vary according on baseline inflammation and supplement dosage (Natto et al., 2019). In obese people, marine omega-3 supplementation decreased IL-6 and MCP-1 while raising pro-resolving mediators and anti-inflammatory cytokines (IL-10) (Torres-Vanegas et al., 2025). The GC-MS study provided detailed information about the oils' potential applications in industrial and food items. Sunflower oil contained more than 58.9% linoleic acid, which is noteworthy because this ω-6 fatty acid promotes cardiovascular health benefits (Calder, 2024). α-linolenic acid (ω-3, 55.2%), the primary fatty acid found in flaxseed oil, has both beneficial cardiovascular and anti-inflammatory properties (Bertoni et al., 2023). According to data, pumpkin seed oil had good nutritional qualities since it had high oxidative stability and balanced amounts of linoleic acid (47.5%) and oleic acid (34.1%) (Nagabhushanam et al., 2022). Flaxseed oil contained remarkably high levels of α-linolenic acid, an omega-3 fatty acid with anti-inflammatory and immunomodulatory properties. Previous research suggests that omega-3 fatty acids may alter immune-cell signaling, regulate inflammatory cytokines, and impact oxidative stress pathways (Patted et al., 2024). Furthermore, several pro-resolving mediators that promote immunological homeostasis and the resolution of inflammation are produced by these fatty acids (Tian et al., 2024) .

While earlier research has shown that dietary seed oils have biological activities linked to metabolic, anti-inflammatory, and antioxidant benefits, there are still a number of unanswered questions about their relative immunomodulatory pathways. While few studies have directly compared sunflower oil, pumpkin seed oil, and flaxseed oil under identical experimental conditions, the majority of prior research has concentrated on the unique health benefits of a particular oil. Furthermore, it is still unclear how these oils' fatty acid composition—specifically, the ratio of omega-3 to omega-6 fatty acids—relates to the control of inflammatory and anti-inflammatory cytokines. While linoleic acid in sunflower and pumpkin seed oils and α-linolenic acid in flaxseed oil have been proposed to affect immune responses, more research is needed to determine how much these fatty acid profiles affect important cytokines including TNF-α, IL-6, and IL-10. In addition, few animal studies have integrated GC–MS fatty acid profiling with serum cytokine assessment to explain the molecular basis of seed oil-induced immunomodulation. Therefore, the current study addresses this gap by comparatively evaluating the effects of sunflower, pumpkin seed, and flaxseed oils on inflammatory and anti-inflammatory cytokines and correlating these effects with their fatty acid composition in a rat model.

6. Conclusion

The current study showed that the fatty acid content of seed oils affects their immunomodulatory capabilities. According to GC–MS analysis, flaxseed oil had the strongest anti-inflammatory efficacy and the largest concentration of α-linolenic acid (C18:3 ω-3). Supplementing with flaxseed oil resulted in the greatest increase in the anti-inflammatory cytokine IL-10 and the most noticeable decrease in the pro-inflammatory cytokines TNF-α and IL-6, suggesting improved immune modulation. Due to its high concentration of oleic and linoleic acids as well as bioactive antioxidant substances, pumpkin seed oil demonstrated a mild immunomodulatory effect. Despite being high in unsaturated fatty acids, sunflower oil had a reduced effect because of its low α-linolenic acid level and high linoleic acid (ω-6) content. The assessed cytokines, especially IL-10 (AUC = 0.94), demonstrated a good ability to distinguish between the control and oil-treated groups, according to ROC curve analysis. Overall, the results indicate that the content of fatty acids is crucial in controlling inflammatory reactions, and flaxseed oil is the most promising oil for enhancing immunological balance and lowering inflammatory activity in the experimental rat model.

7. References

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