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The Impact of Apple Seed-Derived Nanoemulsion on Cancer Cell Line, Normal Cell Line, and Induced Dermatitis in Rats

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KEY WORDS:
Apple seed oil, cell line, dermatitis, formalin, nanoemulsion

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ABSTRACT

The objective of this study was to prepare a nanoemulsion of apple seed oil and investigate its biological effects on cancerous and normal cell lines, as well as to study its efficacy in curing experimentally induced dermatitis in rats. Apple seed oil nanoemulsion was prepared through a mechanical emulsification method along with ultrasonic waves, employing a formulation containing 5% apple seed oil along with Tween 80 and glycerol as cofactors. The chemical constituents of apple seed oil were identified through gas chromatography-mass spectrometry (GC-MS), wherein 22 chemical compounds were identified, of which 10E,12Z-octadecadienoic acid and oleic acid. Physical properties indicated that the nanoemulsion is stable during storage, as the size of the particles varied between 40-100 nm, along with a zeta potential of -35 mV. Cytotoxicity was assessed using the MTT assay on the A431 melanoma cell line and the Vero normal cell line. The results showed that the nanoemulsion possesses a concentration-dependent inhibitory effect on cancer cell growth, with cancer cell viability 80% significantly reduced at high concentrations, while its effect on normal cells was relatively limited 20%. The therapeutic effect of the nanoemulsion-containing gel was also evaluated in a formalin-induced dermatitis model in rats. Histological examinations revealed a significant improvement in skin structure and a reduction in inflammatory infiltration and hyperkeratosis compared to the untreated group. These results indicate that the apple seed oil nanoemulsion possesses anti-inflammatory and anticancer properties with a good degree of biosafety, making it a promising candidate for future pharmaceutical and dermatological applications.

تأثير المستحلب النانوي المشتق من بذور التفاح على خط الخلايا السرطانية، وخط الخلايا الطبيعية، والتهاب الجلد المستحث في الجرذان

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الخلاصة

هدفت هذه الدراسة إلى تحضير مستحلب نانوي من زيت بذور التفاح ودراسة تأثيراته البيولوجية على الخلايا السرطانية والخلايا الطبيعية، بالإضافة إلى دراسة فعاليته في علاج التهاب الجلد المُستحث تجريبيًا في الجرذان . تم تحضير المستحلب النانوي لزيت بذور التفاح باستخدام طريقة الاستحلاب الميكانيكي مع الموجات فوق الصوتية، وذلك بتوظيف تركيبة تحتوي على 5% من زيت بذور التفاح مع توين 80 والجلسرين كعوامل مساعدة. تم تحديد المكونات الكيميائية لزيت بذور التفاح باستخدام تقنية كروماتوغرافيا الغاز- مطياف الكتلة (GC-MS)، حيث تم تحديد 22 مركبًا كيميائيًا، من بينها حمض 12Z،E10- أوكتاديكادينويك وحمض الأوليك اللذان وُجدا كمركبات سائدة في زيت بذور التفاح. أشارت الخصائص الفيزيائية إلى أن المستحلب النانوي مستقر أثناء التخزين، حيث تراوح حجم الجسيمات بين 40 و100 نانومتر، مع جهد زيتا قدره -35 ملي فولت. تم تقييم السمية الخلوية باستخدام اختبار MTT على خط خلايا سرطان الجلد 431A وخط خلايا فيرو الطبيعية. أظهرت النتائج أن المستحلب النانوي يمتلك تأثيرًا مثبطًا لنمو الخلايا السرطانية يعتمد على التركيز، حيث انخفضت حيوية الخلايا السرطانية بشكل ملحوظ 80% عند التركيزات العالية، بينما كان تأثيره على الخلايا الطبيعية محدودًا نسبيًا 20%. كما تم تقييم التأثير العلاجي للهلام المحتوي على المستحلب النانوي في نموذج التهاب الجلد المُستحث بالفورمالين في الجرذان . كشفت الفحوصات النسيجية عن تحسن ملحوظ في بنية الجلد وانخفاض في التسلل الالتهابي وفرط التقرن مقارنةً بالمجموعة غير المعالجة. تشير هذه النتائج إلى أن مستحلب زيت بذور التفاح النانوي يمتلك خصائص مضادة للالتهابات ومضادة للسرطان مع درجة جيدة من السلامة الحيوية، مما يجعله مرشحًا واعدًا للتطبيقات الصيدلانية والجلدية المستقبلية.

الكلمات المفتاحية: زيت بذور التفاح ؛ خطوط خلوية ؛ التهاب الجلد ؛ الفورمالين ؛ مستحلب نانوي

INTRODUCTION

Seed and peel waste make up about 20% of an apple's weight and include antioxidant, anti-inflammatory, anti-cancer, and neuroprotective compounds, which are due to the presence of polysaccharides, polyphenols, organic acids, pentacyclic triterpenes, and sterols (Alves, *et al*. 2021; Ferrentino, *et al*. 2020). Apple seeds are a rich source of protein, amino acids, lipids, minerals, vitamins, and polyphenols, with scientists exploring their potential as a source of these nutrients. Because of its chemical composition and the study of its in vitro biological activities, apple seed oil was shown to contain fascinating features. These properties emerged from the oil's chemical coating (Abbas, *et al*. 2019).

Apple seed oil was studied after 8-acetyl-7-hydroxy-4-methoxycoumarin and 4,7-dimethoxycoumarin compounds were isolated from it. These compounds showed a toxic effect on cancer cell lines such as MCF-7 (breast cancer), AMN3 (cancer of the mammary gland), and HeLa (cervical cancer) (Suhag, & Gasparini, 2024). In most cases, no high-energy input is required to create a nanoemulsion; it can occur spontaneously. The co-solvent or surfactant is often used in conjunction with the surfactant, oil and water phases (Grady, 2023). The mechanisms of action of nanotechnology-based formulations involve several biological and physicochemical processes. These include enhanced cellular uptake through endocytosis, improved interaction with cellular membranes, and sustained or controlled release of the encapsulated compounds. In cancer therapy, for example, nanoparticles can preferentially accumulate in tumor tissues through the enhanced permeability and retention (EPR) effect, allowing for selective targeting of cancer cells while reducing damage to normal tissues. (Suhag, & Gasparini, 2024)

Using nanotechnology, an oil-in-water concentration may be made into a mini emulsion ideal for reducing transepidermal water loss, protecting the skin better, and allowing the active ingredient to penetrate deeper into the skin. Products for UV protection, moisturizing, and anti-aging might benefit from it. It contributes to the skin-friendly sensation of skin care products; the seed extract of M. pruriens var. Utilis was used for the nanoemulsion preparation. After six hours of skin permeation testing, the retention in the membrane was 44.19%, according to the research.

Thus, there is great promise for the future of cosmetics using nanoemulsions containing seed extract of Pruriens var. Utilis (Chookiat, *et al*. 2024). The skin has a mechanical barrier that keeps water and harmful chemicals out and an active barrier that fights infection (Nestle, *et al*. 2009). Many microbial appendage populations on the skin's surface interact with host epithelium and immune cells, affecting local and systemic immunity. Skin immunological homeostasis depends on finely balanced cellular-microbial interactions. Dysregulation of this equilibrium causes inflammatory skin diseases such as psoriasis (Orsmond, *et al*. 2021). Dermatitis occurs when the immune system overreacts due to an allergy, bacterial, viral, or fungal infection, or exposure to an irritant or foreign substance that cannot be removed by enzymatic breakdown or phagocytosis, such as chemicals, for a period of time. (Pahwa, *et al*. 2023). In recent years, nanotechnology has attracted significant interest in medicine, and nanoemulsions have emerged as a promising method for treating various skin disorders, including dermatitis, which is the focus of the current study.

MATERIALS AND METHODS

Preparation of Nanoemulsion:

Bought oil from the Russian site https://www.aromashka.ru/yabloka- maslo.html specializing in the manufacture of this type of oil (Figure 1). A nanoemulsion containing apple seed oil was made in this research using a modified standard formula. The modified formula is made by stabilizing the concentration of apple seed oil at 5% and changing the concentrations of TWEEN 80 and Glycerin, as in Table 1, since a nanoemulsion was obtained in most concentrations, except for the concentration of 5% apple seeds containing 15% TWEEN 80 and 0% Glycerin, in which several emulsion tests were conducted. A stylish milky monolithic liquid with a smelly, oily alum texture has been obtained. All materials were prepared and weighed. Then, Tween 20 with glycerin was added for 20 minutes on the magnetic stirrer, and at a temperature of 20°C, the apple seed oil was added, and the mixture was mechanically moved for 60 minutes. Then, distilled water was mixed for 10 minutes; in the last step, the mixture was placed in the sonicator for 3 minutes, at a frequency of 20 kh, until a clear semi-nano emulsion was formed. Then, the solution was nominated with a syringe with a filter measurement of 22 µm, and the sample was preserved for testing physical and biological properties.

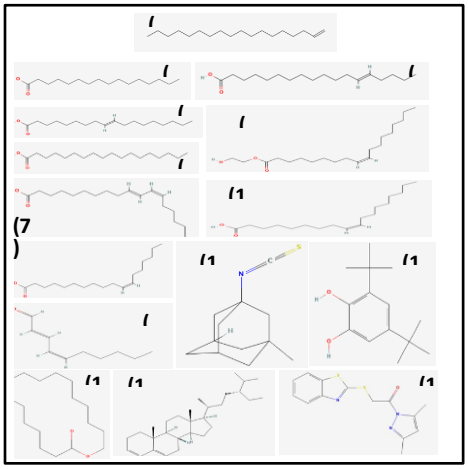

Figure 1: chemical formulas for 2D ligands, https://www.rcsb.org/.

Table 1: Formula of Nano-emulsion Containing apple seed Oil.

Contains	Formula					
Glycerin (%)	0 %	1%	2%	3%	4%	5%
tween 80 (%)	15%	14%	13%	12%	11%	10%
apple seed oil (%)	5%	5%	5%	5%	5%	5%
distilled water (%)	80%	80%	80%	80%	80%	80%

Identification of Active Compounds in Nano Emulsions Using GC-MS Technology:

Phytochemicals are regularly investigated for modern medicine nowadays. These compounds serve as a major factor for the synthesis of various therapeutic agents. Qualitative and quantitative analysis of phytochemical screening and potential chemical components of apple seed oil was performed by GC-MS technique. The GC- MS analysis was carried out at the Basrah oil company laboratories, by using Agilent Technologies, USA.

Physical Tests:

Physical tests were conducted, including storing the preparation at low temperatures, room temperature, and high temperatures for eight weeks. Color changes were then observed visually, as well as solution separation (Ribeiro, *et al*. 2015), homogeneity testing (Jaiswal, *et al*. 2015), pH measurement, viscosity (Leisyah, 2019), centrifugation testing (Estanqueiro, *et al*. 2014), and particle size measurement through a TEM-type electron microscope. For the third concentration of the groups. contining 5% oil, the measurement of oil droplets coated with Tween 20 and glycerin materials, their size ranged from 40 nanometers to ≥ 100 nanometers, measurements of the size of these oil droplets inside the water after 8 weeks of storage. If there is no conglomerate, it is challenging to distinguish droplets from it.

Cell culture source:

We obtained the A431 melanoma cell line and the Vero normal cell line from Rawafed Al-Alam in Hala city. The samples were then cultured in RPMI-1640 medium, supplemented with 10% fetal bovine serum, 50 units/ml penicillin, and 50 mg/ml streptomycin. The cells were harvested when they reached 90% confluence, and the culture medium was changed twice weekly. We used trypsin-EDTA to facilitate the passage process. The cells were then maintained at 37°C with a 5% carbon dioxide concentration (Freshney, 2015).

Cytotoxicity Assays:

We performed the MTT cell viability test using plates with 96 wells to assess the cytotoxic impact. and cultured a cell line at a density of approximately 10^4 cells per well (Dutta, *et al*. 2009). Then treated the cells with different amounts of the nanoemulsion apple seed oil: 1000, 500, 250, 125, 63, 15.5, and 7.8 µg/ml after 48 hours or when a monolayer formed. Next, cell viability was measured by removing the media, adding 100 µL of MTT, and incubating the cells for 2 h at 37°C. Then, we added 100 µL of DMSO (dimethyl sulfoxide), followed by 37°C incubation for 20 min with shaking (Gupta, *et al*. 2023). The absorbency was identified on a microplate reader at 570 nm (test wavelength). The experiment was conducted thrice. The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated using the following equation: Proliferation rate (PR) = B/A*100, where A is the mean optical density of untreated wells and B is the optical density of treated wells.

Preparation of Solutions for Inducing Dermatitis in Laboratory Animals:

The pre-prepared formalin solution is prepared at a 40% concentration diluted with 60% distilled water for experimentation on the animal, which was treated for 2 weeks every 72 hours to induce skin inflammation.

Preparation of gel nanoemulsion for treated dermatitis in laboratory animals:

Use the gel as a topical leather ointment to deliver the nanoemulsion manufactured from apple seed oil. The preparation began with 2 g of genetic sodium dissolved in 50 mL of distilled water in a glass bowl. Heat the mixture in a water bath (70-60°C) with continuous stirring for 20 minutes or more until the material is dissolved using a magnetic blender, then leave the solution to cool before adding the nanoemulsion. Then, add 10 ml of a solution containing 1% nanoemulsion of apple seeds and 99% distilled water, stirring slowly to avoid air bubbles during blending and gutting for 3-5 minutes. To modify the gel's texture, 1 gram of sodium was added. The fetus was constantly stirred until the added quantity dissolved, after which 3-4 drops of glycerin were added.

RESULT AND DISCUSSION:

The results of this study demonstrated clear success in preparing stable nanoemulsions using apple seed oil at a constant concentration of 5%, with varying ratios of both TWEEN 80 and glycerol (Table 1). Optically homogeneous systems with a milky appearance and semi-oily consistency were obtained at most of the prepared concentrations, reflecting the formation of oil-in-water microemulsions with a homogeneous droplet distribution. The failure of one concentration, characterized by the absence of glycerol and a high concentration of the surfactant, is related to the imbalance between oil and water phases. This emphasizes the importance of glycerol, which, as a cosolvent, contributes to improved structural stability and reduced interfacial tension, thus facilitating the formation of nanosystems. This is related to the physicochemical characteristics of emulsions, where co-surfactants enhance stability, thus preventing aggregation.

Gas chromatography-mass spectrometry (GC-MS) results show that apple seed oil has 22 chemical compounds, precisely identified through their exact match in the NIST library. The separation peaks started at an early time, followed by later times as a result of diversity in terms of their polarity and molecular masses. However, it was established that 10E,12Z-octadecadienoic acid dominated as a major component of apple seed oil, forming a high percentage of 45.92% of the total peak area, followed by oleic acid at 29.87% and then n-hexadecanoic acid at 6.49% (Figure 2 and Table 2). The results show that apple seed oil is dominated by unsaturated fatty acids, such as omega-6 fatty acids and omega-9 fatty acids, which are known to have anti-inflammatory properties. Biologically active compounds such as squalene and vitamin E, which are known to enhance the antioxidant properties of apple seed oil to protect cells from oxidative stress, were also identified in this study. Moreover, compounds such as 2,4-decadenaline, which are aldehydes, are known to have antimicrobial and anticancer properties. This diversity of chemical properties explains the high functional potential of apple seed oil, which can be applied in pharmaceutical and cosmetic products, as has been established in other studies on fruit seed oils (Akšić *et al*., 2021).

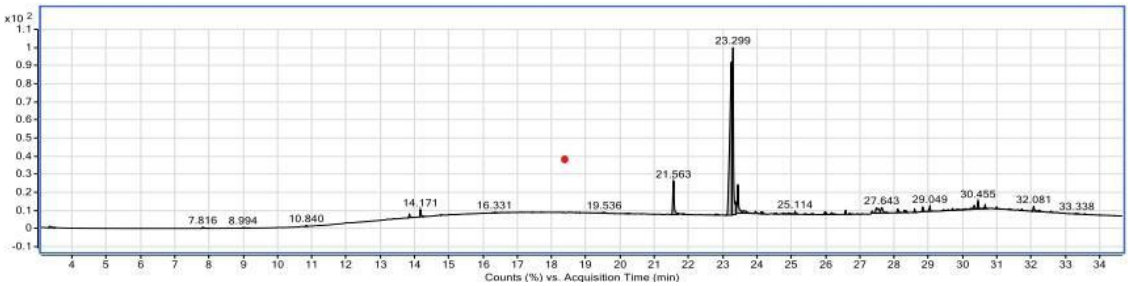

Figure (2): Illustrate phytochemical compounds in AFHO of apple seed analyzed GC-MS measurement in rotation time.

Regarding physical stability, all formulations demonstrated remarkable stability under various storage conditions, including low temperatures, room temperature, and high temperatures, with a clear comparison between samples stored in light and those stored in darkness. A limited color change was observed in one concentration was 2/A, which may be attributed to oxidation reactions or partial decomposition of some photosensitive components, while the remaining concentrations remained optically stable(Figure 3).

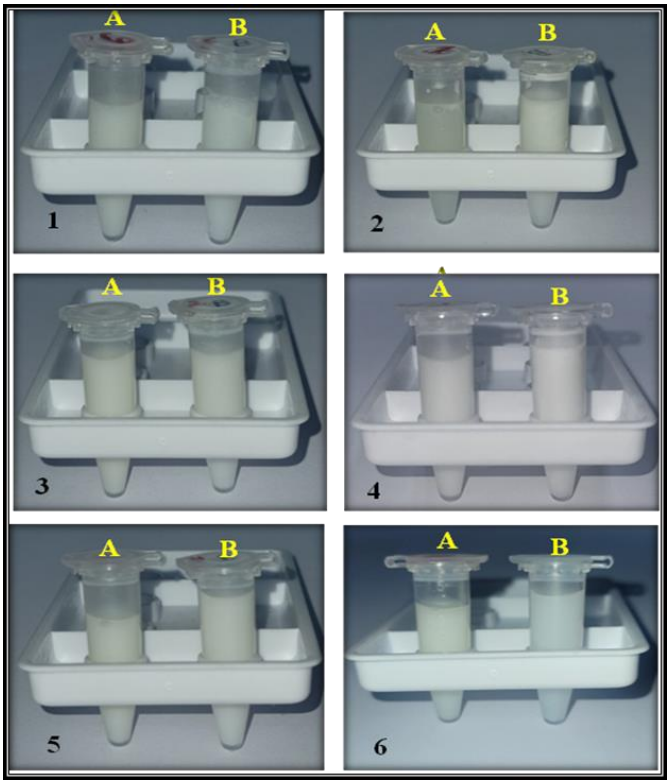

Figure 3: A clear color change is shown when (a) represents the sample at low temperature and is stored in the dark. (b) represents the sample at room temperature and is stored in the light. The acid detection result (pH) of the emulsion showed a marked change during the eight weeks during which it was detected, as it appeared in concentration NO. 3, which is most suitable for dermal pharmaceutical uses. When the emulsion's glycerin content is 2%, it is often the best concentration for skin acid (Table 3).

pH measurements showed a gradual change during the eight-week storage period. However, the 2% glycerol-containing (Table 1) concentration exhibited better skin pH compatibility, making it the most

suitable for topical applications. This is attributed to glycerol's ability to regulate the aqueous environment and maintain acid-base balance

Table (2) Phytochemical compounds in AFHO of apple seed oil

Peak	R.T.	Area	Pct Total	Area Pct	Library/ID	CAS	Qual
1	12.5	459060	0.0	0.05	trans-13-Octadecenoic acid	000693-71-0	87
2	13.2	50439	0.0	0.01	1-Octadecene	000112-88-9	64
3	13.2	276318	0.0	0.03	n-Hexadecanoic acid	000057-10-3	59
4	13.4	874547	0.1	0.09	9-Octadecenoic acid	002027-47-6	70
5	13.9	6874469	0.7	0.73	cis-Vaccenic acid	000506-17-2	55
6	14.2	10237867	1.1	1.09	2,4-Decadienal, (E,Z)-	025152-83-4	70
7	21.6	60917145	6.5	6.49	n-Hexadecanoic acid	000057-10-3	99
8	23.3	4.31E+08	45.9	45.92	10E,12Z-Octadecadienoic acid	002420-56-6	99
9	23.3	2.8E+08	29.9	29.87	Oleic Acid	000112-80-1	99
10	23.4	42363476	4.5	4.52	Octadecanoic acid	000057-11-4	96
11	26.6	4864919	0.5	0.52	Adamantane, 1-isothiocyanato-3-methyl-	136860-48-5	50
12	27.5	14104441	1.5	1.50	9-Octadecenoic acid, (E)-	000112-79-8	91
13	27.6	8182571	0.9	0.87	9-Octadecenoic acid	002027-47-6	90
14	27.6	14555330	1.6	1.55	9-Octadecenoic acid (Z)-, 2-hydroxyethyl ester	004500-01-0	95
15	28.1	7507649	0.8	0.80	10E,12Z-Octadecadienoic acid	002420-56-6	91
16	28.6	3769651	0.4	0.40	Squalene	000111-02-4	92
17	28.8	8746576	0.9	0.93	Heptadecanolide	005637-97-8	44
18	29.1	8862363	0.9	0.94	Nonacosane	000630-03-5	96
19	30.3	8594516	0.9	0.92	1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-	001020-31-1	35
20	30.5	11727458	1.3	1.25	Stigmasta-3,5-diene	004970-37-0	93
21	30.7	4823257	0.5	0.51	Vitamin E	000059-02-9	95
22	32.1	9303448	1.0	0.99	Ethanone, 2-(2-benzothiazolylthio)- 1-(3,5-dimethylpyrazolyl)-	155670-84-1	43

Table 3: pH Result of Nano-Emulsion Containing Apple Oil at Room Temperature for 8 Weeks.

No.	1 day	3 week	8 week	24 week
1	4.2	4.2	4.2	4.0
2	4.9	4.9	4.7	4.7
3	4.7	4.7	4.5	4.4
4	4.9	4.9	4.5	4.0
5	4.9	4.9	4.3	4.0
6	4.6	4.4	4.2	3.9

With regard to the rheological properties, the analysis of the emulsions' viscosity indicated that the emulsions with 5% oil had similar values of viscosity at all concentrations except for the initial concentrations. This could be attributed to the higher glycerol content or the distribution of the droplets. Increasing the viscosity limits the movement of the droplets in the medium and minimizes the aggregation and settling of the droplets, thus increasing the stability (Table 4). The centrifugation test also proved the stability of the emulsions. There was slight separation in the form of a thin white film for some concentrations. This gradually diminished and completely disappeared for all concentrations (Figure 4).

Table 4: Nanoemulsion and Emulsion Viscosity Test Result.

Fluid No.	Viscosity (Ƞ) Poise in second week	Viscosity (Ƞ) Poise at 24week
Water	0.01002	0.01002
1	0.02947	0.02947
2	0.02089	0.02850
3	0.01638	0.02934
4	0.01480	0.02506
5	0.01997	0.02518
6	0.02150	0.02429

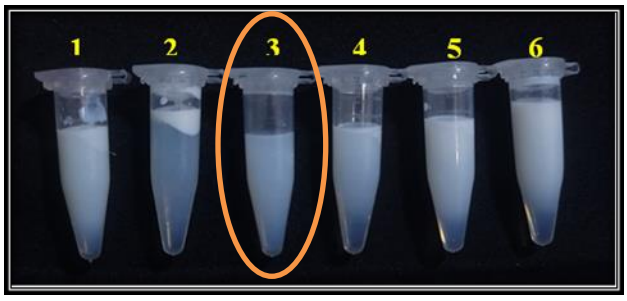

Figure 4: Centrifugation test to determine the stability of the nanoemulsion preparation.

On the other hand, at the nanoscale, it has been clearly shown through transmission electron microscopy (TEM) images that the droplets' diameters ranged between 40-100 nm or a little more than that, which indicates good volumetric stability as well as a lack of agglomeration (Figure 5). This can be explained by many factors, including the formation of a stable monolayer film of TWEEN 80 on the surface of the oil droplets, which prevents their amalgamation, as well as the impact of the ultrasound waves, which break down the droplets into small sizes at the nanometer scale due to their high shear force. This is consistent with Marwa & Jufri (2023). This also results in an increase in Brownian motion, which prevents their settling as well as maintains homogeneity in the system; this is consistent with what was mentioned (Choi, 2022).

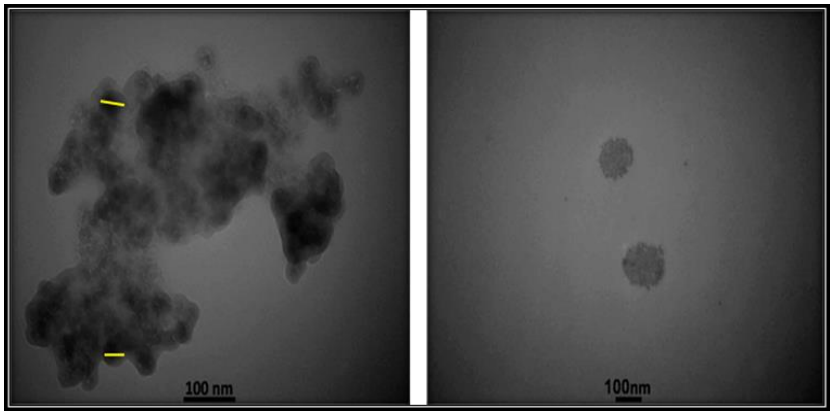

Figure 5: Nanomaterial size, which is ≥ 100 nm in the concentration of model No. 2

Moreover, it has also been shown through zeta potential tests that it has a value of -35 mV (Figure 6) along with a low standard deviation, which indicates a strong negative charge on the surface of the particles, resulting in electrostatic repulsion between them, which prevents their agglomeration as well as ensures their long-term stability. This value also indicates a high level of homogeneity in terms of charges as well as size in this system, agree with (Elimelech, 1994).

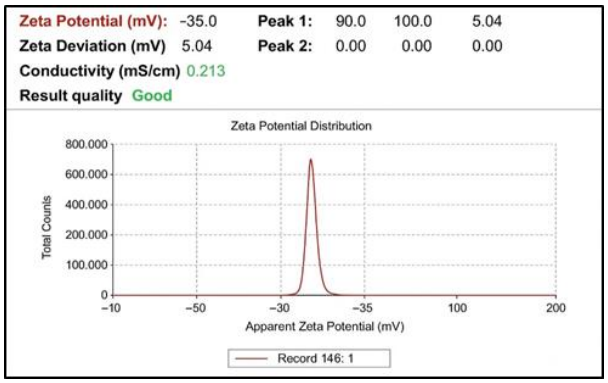

Figure 6: The image shows the surface voltage distribution report (Zeta Potential).

This is a reflection of the fact that the preparation process was successful in producing an emulsion that was highly homogeneous and stable. Nanoemulsified material is more effective, soluble, and absorbable in tissues compared to other non-nanoemulsified materials, in which the range of doses used is 1–10 mg/ml, depending on the effectiveness and purity of the substance. Nanoemulsions are often used to improve the solubility and absorption of active ingredients, which may allow lower doses to be used compared to conventional formulations. This is what was observed in the following study (Wilson *et al*., 2023). In an anti-cancer experiment using nanoemulsion of apple seed oil on cancer and normal cell lines, a difference in vitality was found between the concentrations used on the skin cancer cell line. If the concentrations were found to be high, the vitality of the cancer cells decreased to more than 80%, while it may have reached less than 20%.In Vero normal line cells, we can obtain the result, which confirms the effect of this substance in killing cancer cells and not affecting normal cells at the same concentrations. This result can be explained by the fact that the absorption process in cancer cells of the nanoemulsion has a difference in the composition of the cell membrane, which allows the entry of nanoparticles more efficiently, and this is what I agree with in his study Varlamova *et al*. (2021) if he attributes the reason why cancer cells are affected by the cell membrane to the stress caused by nanoparticles when cells are treated with them. Cancer cell membranes often contain higher levels of trans fats or different membrane proteins than normal cells. These differences may contribute to increased interaction with the nanoemulsion, leading to the emulsion fusing with the

cell membrane and affecting its structural composition, facilitating drug entry or directly killing the cell (Chu *et al*., 2022), causing structural integrity disruption. This is consistent with the study when Linum usitatissimum seed essential oil nanoemulsion caused more cell death in A2780 cells as the dose increased, confirming that the cells died by apoptosis (Keykhasalar *et al*. 2021).

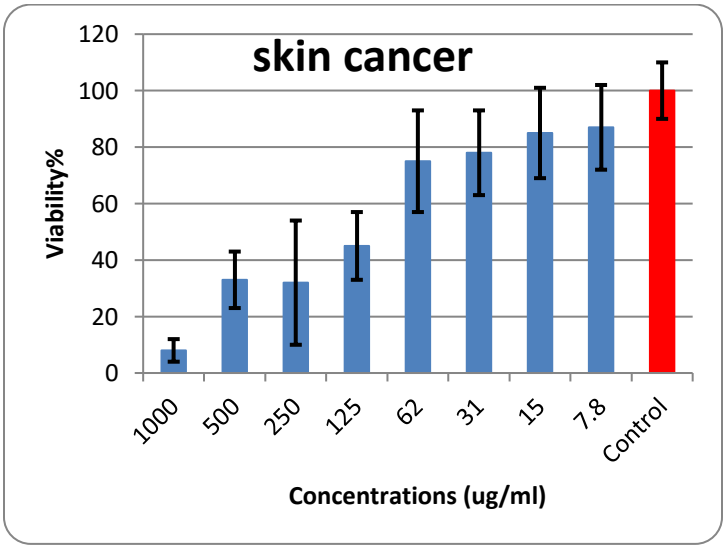

Figure 7: The MTT assay reveals a cancer cell line (A431) with a mean ± SD, n = 5.

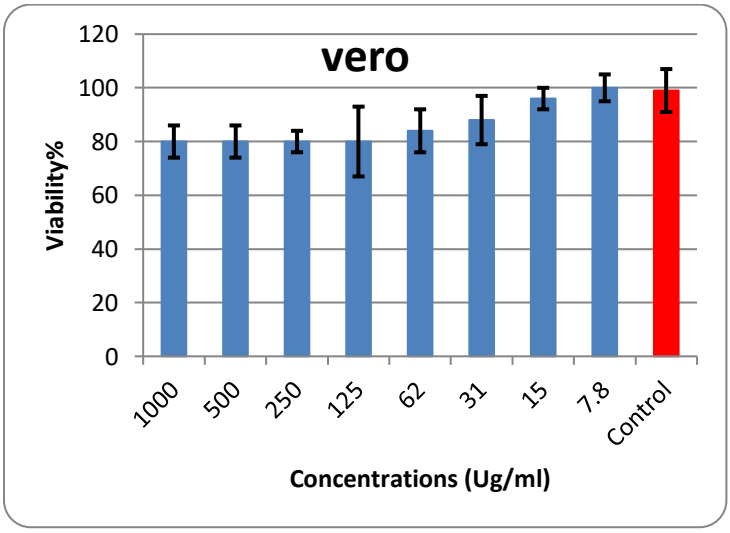

Figure 8: The MTT assay reveals a normal cell line (Vero) with a mean ± SD, n = 5.

The small size of the nanoparticles also enhances their permeability and interaction with intracellular material. These findings were also supported by histopathological analysis, which revealed a significant improvement in the groups that were subjected to treatment with the nanoemulsion compared to the untreated groups. The results of histological examination of the rat's skin, following induction of dermatitis using a 2% formalin solution, revealed clear differences between the experimental groups depending on the duration of treatment. The animals were divided into groups treated for two different time periods: one week (negative control group

A, positive control group B1, treated skin with a gel containing nanoemulsions C1 and intraperitoneal injection nanoemulsion D1) and two weeks (A, B2, C2, D2), following the induction of inflammation. This experimental design enabled the assessment of the time-dependent effect of treatment, with the results showing varying histological changes reflecting the tissue response to treatment depending on the duration of exposure, while a gradual improvement in some histological parameters was observed in the groups that underwent a longer treatment period compared to their counterparts with a shorter treatment period. Group C1 was treated with a gel containing a nanoemulsion of apple seed oil, while group D1 also underwent intraperitoneal injection of this emulsion to assess its systemic effect and its impact on the severity of skin inflammation for one week following the induction of inflammation. During the treatment period, it was observed that groups D1 and D2 exhibited histological changes similar to those recorded in the untreated groups B1 and B2, which retained their pathological features without any significant improvement. When comparing the histological changes between the treated groups, qualitative differences were noted, most notably hyperplasia, which can be interpreted as a compensatory mechanism or a protective response aimed at limiting the effect of formalin and promoting the repair of damaged tissue (Kumar *et al*., 2021). One of the histological symptoms that have been seen repeatedly is fibrosis an increase in fibrocystic tissue was found, which led to a significant appearance of fibrosis in the three groups, which was evident in Groups B1, D1, and C1 which was less obvious in fibrosis Group C2, treated for two weeks on the one hand compared to control group A on the other hand. Although fibrosis is one of the preventive methods to avoid continued damage to the tissue, the presence of hyperplasia in the keratin layer in the inflammation-inducing groups treated with the nanoemulsion, but with a difference in the percentage of hyperplasia if groups C1 and C2, the lowest groups present in this layer, are present (Figures 9 and 10), is what a study agreed upon (Singh *et al*., 2024)

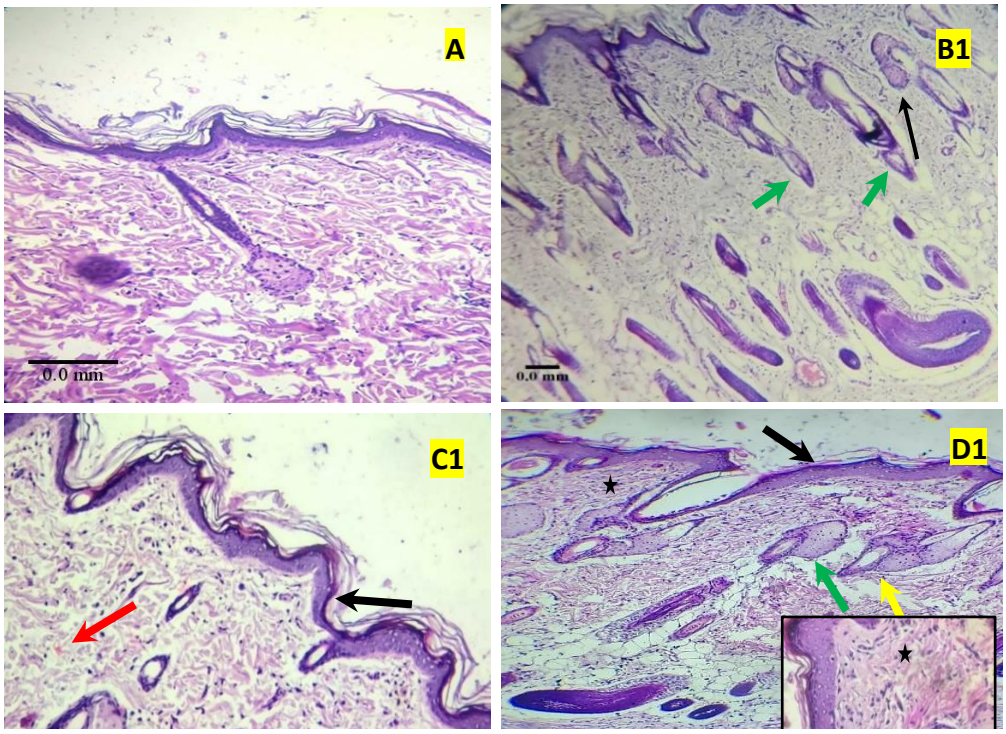

Figure 9: shows a section of skin after one week of treatment. A, histopathological section of rats' normal skin shows normal structure and cells of the skin. B1, histopathological section of rats suffering from dermatitis shows hyperplasia of hair follicles (black arrow) and sebaceous glands (green arrow). C1, skin of rats treaed with apple seed nanoemulsion gel locally show hyperplasia of sebaceous gland (green arrow), edema in epidermis (red arrow), htyperkeratosis (black arrow) (20X); D1, treated with intraperitoneal injection of apple seed nanoemulsion, shows hyperplasia of sebaceous gland (green arrow) and hair follicle (yellow arrow) and filtration of inflammatory cells (star) and hyperkeratosis (black arrow) (H&E, 40X).

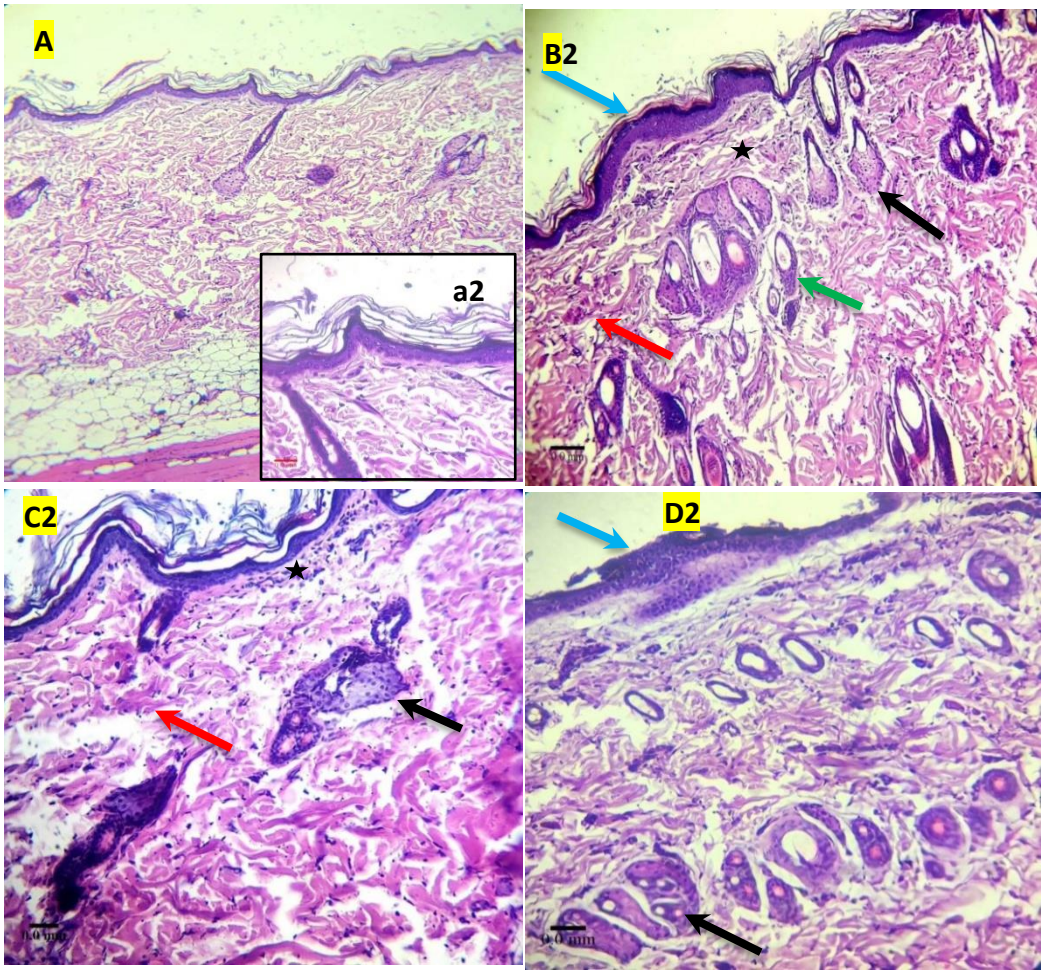

Figure 10: section of skin after two weeks of treatment. A, histopathological section of rats' normal skin shows the normal structure and cells of the skin. B2, histopathological section of rats suffering from dermatitis shows hyperplasia of hair follicles (black arrow), sebaceous glands (green arrow), edema (red arrow), and hyperkeratosis (blue arrow); C2, skin of rats treated with apple seed nanoemulsion gel locally show hyperplasia of sebaceous glands (black arrow), edema in epidermis (red arrow), and infiltration cells (star) (20X) ; D2, treated with intraperitoneal injection of apple seed nanoemulsion, shows atrophy in hair follicle (black arrow) and hyperkeratosis (blue arrow) (H&E, 40X).

CONCLUSION:

The current investigation came to the conclusion that the technique and the proper quantities of the materials employed in the preparation were crucial to the conversion of apple seed oil into a nanoemulsion and its stability. Compared to normal cells, the resultant nanoemulsion was more efficient in killing cancer cells. Additionally, it significantly decreased the chemical's toxicity (formalin). In comparison to the positive control group, the results were visible on the skin of laboratory rats, where it decreased the rate of inflammation when administered directly to the site of inflammation.

DATA AVAILABILITY STATEMENT:

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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ETHICS STATEMENTS:
This study was approved by the Institutional Review Board of Basrah University/ College of Veterinary Medicine. under approval number [74/37/2025].

CONFLICTS OF INTEREST:
The authors declare no competing interests.

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