

Formulation of Quinoa oil-alginate loaded nanoemulsion and its anticancer efficacy as a therapy for chemically induced breast cancer

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Abstract

Quinoa seeds (*Chenopodium quinoa* Willd.) have grown in interest due to their naturally occurring phytochemicals and antioxidants. It was stated that it has a powerful anticancer effect against human colorectal cancer. Gas chromatography-mass spectrometry was used to analyze the quinoa oil fatty acids. Sodium alginate was used as a loading agent to encapsulating the nanoemulsion, and its acute oral toxicity was assessed in rats. The nanoemulsion's ability to scavenge free radicals was evaluated using the DPPH radical-scavenging assay. The *in vivo* anticancer potential of the quinoa oil nanoemulsion on 7, 12-dimethylbenz (a) anthracene (DMBA)-induced breast cancer in rats was studied. Quinoa oil nanoemulsions were given daily to DMBA-breast cancer models for one month. Through the assessment of ROS, protein carbonyl, gene expression evaluation of anti-oncogenes, and histopathological analysis, the anticancer activity of the nanoemulsion was studied. Quinoa oil nanoemulsion administration significantly decreased the rise in serum ROS and PC levels brought on in breast cancer tissue. The quinoa oil nanoemulsion alleviated the mRNA expression levels of anti-oncogenes in breast cancer tissue. As well, nanoemulsions improved breast tumor tissue architecture.

Conclusion: We can infer from the results that quinoa oil nanoemulsion can obstruct the breast cancer cells of the rats.

Introduction

Natural products may hold the key to the future of breast cancer treatment as a source of new drugs that can interfere with certain processes and eventually be used as adjuvant therapy in clinics [1]. Quinoa (*Chenopodium quinoa* Willd.) is a pseudocereal crop that belongs to the Chenopodiaceae family, originates from the Andean region, and has recently been successfully grown in Egypt [2, 3]. Quinoa was known as the golden grain, and it has long been a valuable source of food. It has a wide range of secondary metabolites with diverse bioactivities. They primarily consist of steroids, phenolic acids, flavonoids, terpenoids, and nitrogen-containing substances. These metabolites have a wide range of biological activities, including antioxidant, cytotoxic, anti-diabetic, and anti-inflammatory properties [4]. Quinoa seeds have a high concentration of vitamins, minerals, proteins, and vital amino acids. They also include high-quality fatty acids as well as other bioactive substances [5]. In addition, due to its antioxidant and anti-inflammatory properties, which have a positive impact on human health and reduce the risk of developing major diseases like cancer, quinoa is considered a naturally functional food [6]. Several studies have confirmed the anticancer properties of quinoa. Mohamed et al. [7] evaluated the quinoa seed powder's *in vitro* anticancer activity against liver cancer, and they indicated that the quinoa possessed cytotoxicity against the hepatocellular carcinoma cell line HEPG2. Stikic et al. [8] showed that quinoa seed extract has a powerful anticancer effect against the human colorectal cancer cell line. HCT-116. Bata et al. [9] studied the cytotoxic potential of the C. quinoa lipoidal components and different fractions against the human lung cancer cell line A549 (ATCC), human hepatocellular carcinoma cells (HepG2), and human colon cancer cell line (Caco-2) and revealed that the A549 human lung cancer cells were the most sensitive to treatment with a single dose of 100 µg/ml. Shen et al. [10] reported that black

quinoa seed oil inhibited the growth of HCT 116 cells by inducing apoptosis in a dose-dependent manner, which may be more important in the chemoprevention of cancer and other oxidative stress-related disorders. Also, quinoa oil nanoencapsulation was found to be an effective way to boost oil antioxidant activity [11]. Oxidative processes are triggered by the interactions of ROS with nitrogenous bases and deoxyribose, resulting in mutations. This molecular modification alters the normal apoptotic signaling process, which includes uncontrolled cell proliferation and tumor formation [12]. Earlier research informed that there is a strong link between an imbalance in antioxidant levels and the onset of cancer [13].

The most typical malignant tumor in women around the world is breast cancer. Up to 36% of patients with cancer are breast cancer patients. Breast cancer was thought to have been discovered in 2.26 million new cases in 2020 [14]. All over the world, the prevalence of this cancerous tumor is rising, but it is most prevalent in developed nations. Around the world, developed nations account for almost half of the cases [15]. Breast cancer is the most common public malignancy among Arab women in the Eastern Mediterranean Region. Breast cancer is the most common cancer affecting women in Egypt [16]. Current therapies used for treatment in the clinic include tyrosine kinase inhibitors, monoclonal antibodies, hormone treatment, and chemotherapy. They do, however, have drawbacks, such as virtual inefficacy in many forms of cancer or severe adverse effects that label them as dangerous medications [17].

Recently, the need to develop efficient delivery systems for lipophilic active ingredients has led to a significant increase in interest in nanoemulsions. It is known that 40% of pharmaceuticals on the market today and 90% of drugs under development are hydrophobic; this means that their low water solubility significantly reduces their bioavailability and absorption efficiency. So, hydrophobic pharmaceutical compounds are the focus of much research and development of oil-in-water nanoemulsion systems [10]. Hydrogel encapsulation is highly applied in manipulative active drug delivery systems because the size of the nanoparticles affects how well therapeutic or diagnostic substances enclosed in them are delivered to various places throughout the human body [18]. The most suitable encapsulation material is alginate, in which the structural constituents of alginate are alginic acid derivatives that exist as an intercellular gel medium in brown algae. This alginate is recommended for medical requests since it is safe for humans [19].

The anti-oncogenes are genes that regulate cell division and replication by blocking cancer progress and contributing to the development of normal cells [20]. Tumor protein p53, which is produced by the TP53 gene, controls cell division by preventing cells from expanding and reproducing in an uncontrolled manner. Mutant p53 proteins lose their capacity to bind DNA and result in aberrant cell division [21]. BRCA 1 and 2 are described as "breast cancer susceptibility proteins" that are normally expressed in breast cells. They play a crucial role in the repair of DNA double-strand breaks in addition to being engaged in the repair of chromosomal damage. Mutations in these genes halt their role in tumor suppression, which is associated with an elevated risk of breast cancer [22]. Therefore, the primary objective of the present study was the formulation and characterization of a quinoa oil nanoemulsion. The second objective was to explore the nanoemulsion's anticancer properties against DMBA-induced breast cancer in female Dawley rats.

Materials and Methods

Chemicals

The 7, 12-dimethylbenz(a) anthracene (DMBA), was bought from Sigma chemical company (St. Louis, MO, USA). Sodium alginate [Manutex FAV, ISP Alginates, (UK)], calcium chloride (Merck, Germany), and Tween 20, (Merck KGaA, Darmstadt, Germany).

Investigation and saponification of quinoa oil petroleum ether extract

The quinoa seeds were obtained from Harraz-Agricultural Seeds, Spices, and Medical Plants Co., Egypt, and were identified in the Egyptian Agricultural Museum. Quinoa oil was extracted using the Soxhlet extraction method, according to Tsuda et al. [23].

Fatty acid methyl ester analysis by GC-MS spectrometry

The quinoa oil fatty acid methyl ester analysis by GC-MS spectrometry was conducted as in El Makawy et al. [24].

Formulation of nanoemulsions

According to Youssef and Abdelmegeed [25], sodium alginate (Sigma-Aldrich) as an aqueous part was used to emulsify nanocapsules and quinoa oil as an oil. Sodium alginate was dissolved in deionized water to make the alginate solution (3% w/v). Then, drop by drop, 30 g of quinoa oil with an emulsifier was added. This mixture was vigorously stirred at room temperature with a mechanical stirrer (Greave Mixer, England) to be emulsified, and seemed creamy. The emulsion was sonicated using an ultrasonic cleaner set (WUC-DO3H 290W and 60 Hz) for 30 minutes, then a high-energy ultra-sonication probe (model VCX750, 750W, 20 kHz) for 3 minutes. For equilibration, calcium chloride solution in a ratio (2:10 CaCl₂ to alginate) was added to the mixture and sonicated as mentioned before until the oil/water nanoemulsion was formed.

Transmission electron microscope

Transmission electron microscopy (TEM) was applied to examine the shape and size of the prepared nanoformulation. A carbon-coated copper grid was covered with the diluted nanoemulsion and stained with 1% phosphotungstic acid, then detected and photographed at a magnification of 20000X.

In vitro assay of nanoemulsion antioxidant activity

The ability of the nanoemulsion to free radical scavenge was examined using the DPPH radical scavenging assay described by Ibrahim et al. [26]. The absorbance of the solution was measured spectrophotometrically at 517 nm, using BHT as a reference. The percentage of DPPH radical scavenging activity was calculated as $[(A_0 - A_1) / A_0] \times 100$. Where, A₀ is the control absorbance and A₁ is the

absorbance of the extractives or standard. The inhibition percent was plotted against concentration, and the IC50 was calculated from the graph.

Nanoemulsion LD50

To detect the acute toxicity of nanoemulsion in rats, thirty female Sprague-Dawley rats were divided into six groups (n = 5). Animals in the control group received normal saline, while the other five groups were orally given different doses of the quinoa oil nanoemulsion (100, 500, 1000, 1500, and 2000 mg/kg). To look for early signs of toxicity or mortality, all groups were monitored daily for 14 days. The LD50 was estimated and used to determine the doses that were tested in the anticancer study. The LD50 of nanoemulsion was 2000 mg/kg, so the chosen dosages were 1/20 and 1/10 of the LD50 (100 and 200 mg/kg b.w.).

Nanoemulsion Anticancer efficiency

Experimental animals

Mature female Sprague-Dawley rats weighing between 150 and 160 g were obtained from the Animal House of the National Research Centre, Egypt. The animals were housed in appropriate settings for a week while being fed a regular pellet diet to aid in their acclimatization. The animals were kept in polycarbonate cages in a proper validation room free from any chemical contamination, at a normal temperature ($25 \pm 1^\circ\text{C}$), with a 12:12-hour cycle of darkness and light. This animal study was performed under the ethical standards as laid down in the Declaration of Helsinki, and the Scientific Ethics Committee of the NRC in Egypt gave its approval to the study's protocol (Registration No. 19164).

Sixty-three female Sprague-Dawley rats were used in this study, along with eighteen control and forty-five breast cancer model rats. Control animals were distributed into three groups, each with six rats, and administered orally with corn oil as a vehicle; quinoa nanoemulsion (100 and 200 mg/kg) daily for 28 days. The breast cancer model was prepared by intra-gastric injection of 7, 12 – dimethylbenz (a) anthracene (DMBA, Sigma-Aldrich) dissolved in corn oil at a single dose of 80 mg/kg body weight. Four months after injection, breast cancer model rats were divided into four groups. The breast cancer group consists of fifteen rats that remain without treatment. The other three groups included ten rats and were treated with 5-Flu 20 mg/kg, 100, 200 mg/kg quinoa oil nanoemulsion daily for 28 days, respectively. The animals have been euthanized after the end of the treatment period using intraperitoneal injections of xylazine (5 mg/kg b.w.) and ketamine (50 mg/kg b.w.).

Blood and tissue collection

Blood samples were collected in heparinized tubes of the inferior vena cava and centrifuged for 10 minutes at 5000 rpm. Before usage, plasma was isolated and kept in aliquots at 80°C . As well, animals were weighed and sacrificed, and then the mammary glands were cut up for the different techniques.

Assessment of oxidative stress markers

The levels of intracellular reactive oxygen species in normal and tumor tissues were determined using a rat reactive oxygen species kit (Cat. No. SL1189Ra, Sunlong Biotech Co., Ltd.), that evaluates the intracellular hydrogen peroxide rate. Protein carbonyl (PC) content was measured using Sunlong Biotech Co., Ltd.'s rat protein carbonyl (PC) Elisa kit (SL 1055Ra).

RT-qPCR for analyzing gene expression

Following the manufacturer's instructions, RNA was isolated from the mammary gland with the Easy Red RNA Extraction Kit (Intron bio, Korea). The NanoDrop™ 1000 Spectrophotometer (Thermo Fisher Scientific, USA) and gel electrophoresis were used to assess the yield and quality of RNA. To remove any genomic DNA contamination, 1 g of RNA was treated with a RNase-free DNase kit (Promega), and cDNA was synthesized with a HiSenScript™ cDNA kit (iNtRON biotechnology). Specific primers for the studied genes (**BRCA1**, F: AGA CTG CTC GCA GAG TGAT, R: AAGC TTC CAG GTG AGC CAT TTC; **BRCA2**, F: TTGAGGACCCCAAGACCTGT, R: CCGGAGAGACAAA GGTGCA; **Tp53**, F: CCACCACGCTCTTCTGTCTAC, R: ACCACCAGTTGGTTGTCTTTG; **GAPDH**, F: AACTTTGGCATTGTGGAAGG, R: ACACATTGGGGGTAGGAACA) were purchased from Macrogen (Korea). A real-time polymerase chain reaction was performed in the Strata Gene Mx3005P Real-Time PCR System (Agilent Technologies) in a 20µL reaction. After each real-time PCR, a melting curve analysis was performed. Gene expression data were normalized to GAPDH and analyzed using the $2^{-\Delta\Delta C_t}$ method [27].

Histopathological Investigations

Mammary gland sections were processed through fixation in 10% neutral buffered formalin, dehydrated using alcohol in graded concentrations, cleaned with xylene, and embedded in paraffin wax. For morphological analysis, sections with a 5-µm thickness were cut using a microtome and stained with hematoxylin and eosin [28].

Statistical analysis

The data analysis was completed using SPSS version 18 software, which stands for Statistical Package for the Social Sciences. The data was examined using Duncan's test following a one-way analysis of variance (ANOVA). When the probability (P) level was less than 0.05, the data was deemed statistically significant.

Results

Fatty acids Profile

The GC/MS analysis of the FAME of quinoa oil is shown in Fig. 1 and Table 1. The GC/MS results indicated that the total identified fatty acid content is 95.65% of the total peak area. The fatty acid analysis of quinoa oil as methyl esters revealed palmitate acid (Hexadecanoic acid, methyl ester; 57.73%) and linoleic acid methyl ester (9,12-Octadecadienoic acid, methyl ester; 31.32%) as the major recognized

fatty acids at R_t 13.05 and 16.88 min respectively (Fig. 1). Linolenic acid (1.78%) and arachidonic acid (1.52%) also existed as minor compounds.

Table 1
GC/MS analysis of fatty acids methyl ester of quinoa

No.	R _t	Compounds	MW	Molecular Formula	Relative Area%
1	8.6	Tetradecanoic acid methyl ester Myristic acid, methyl ester	242	C ₁₅ H ₃₀ O ₂	0.18
2	10.82	Pentadecenoic acid methyl ester	254	C ₁₆ H ₃₀ O ₂	0.36
3	10.91	Pentadecanoic acid methyl ester	256	C ₁₅ H ₃₂ O ₂	0.42
4	13.05	Hexadecanoic acid methyl ester Methyl palmitate	270	C ₁₇ H ₃₄ O ₂	57.73
5	14.58	14-methyl-hexadecanoic acid methyl ester <i>14-methyl Palmitic Acid methyl ester.</i>	270	C ₁₇ H ₃₄ O ₂	0.11
6	16.09	13-Octadecenoic acid methyl ester	296	C ₁₉ H ₃₆ O ₂	0.29
7	16.87	9,12-Octadecdienoic acid methyl ester Linoleic acid methyl ester methyl	294	C ₁₉ H ₃₄ O ₂	31.32
8	17.37	9,12,15-Octadecatrienoic methyl ester α -Methyl linolenate	292	C ₁₉ H ₃₂ O ₂	1.78
9	17.60	14-methyl-Pentadecanoic acid methyl ester	298	C ₁₉ H ₃₈ O ₂	0.3
10	18.00	5,8-Octadeca-dienoic acid methyl ester	294	C ₁₉ H ₃₄ O ₂	0.19
11	18.17	10,13-Octadecadienoic acid methyl ester	294	C ₁₉ H ₃₄ O ₂	0.11
12	21.32	Eicosenoic acid methyl ester	324	C ₂₁ H ₄₀ O ₂	1.52
13	21.84	Eicosanoic acid methyl ester Arachidic Acid methyl ester	326	C ₂₁ H ₄₂ O ₂	0.4
14	123.90	Heneicosanoic acid methyl ester	340	C ₂₂ H ₄₄ O ₂	0.02
15	25.14	20-methyl- Hineicosanoic acid methyl ester	354	C ₂₃ H ₄₆ O ₂	0.07
16	25.39	11-Docosenoic acid methyl ester	352	C ₂₃ H ₄₆ O ₂	0.65
17	25.88	Docosanoic acid methyl ester Behenic Acid methyl ester	354	C ₂₃ H ₄₆ O ₂	0.20
Total identified					95.65

Nanoemulsion particle formulation

The shape and size of the nanoemulsion particles were examined by TEM. The nanoparticles were more or less spherical, and the mean particle sizes ranged between 5–30nm, as seen in Fig. 2.

Antioxidant activity of nanoemulsion

Figure 3 displays the free radical scavenging activity as well as the IC₅₀ of Vit C and BHT as standards and the quinoa oil nanoemulsion. The outcomes demonstrated that nanoemulsion increased the DPPH value in a concentration-dependent manner. Quinoa had an IC₅₀ of 307, while vitamin C and BHT had IC₅₀s of 373.76 and 206.33, respectively. This finding indicated that the free radical scavenging activity of quinoa oil nanoemulsion is less than that of Vitamin C and more than that of BHT.

Effect of quinoa oil nanoemulsion on breast tumor and body weights

Data in Fig. 4 revealed that there was no difference in body weights between DMBA-treated animals and each of the control, 5-FLU, and quinoa oil nanoemulsion-treated animals. While the quinoa oil nanoemulsion at two doses significantly diminished ($P \leq 0.05$) the volume of breast tumor induced by DMBA. There was a significant difference ($P \leq 0.05$) between the effects of 5-Flu and low-dose nanoemulsions against breast cancer, while there was no difference between the effects of a high dose and 5-Flu.

Influence of quinoa oil nanoemulsion on oxidative stress markers

Figure 5A shows the effect of quinoa nanoemulsion and 5-Flu on the level of serum ROS in the DMBA breast cancer rat model. There were high levels of ROS in animals treated with DMBA, while in animals treated with 5-Flu and quinoa oil nanoemulsion, the ROS levels significantly declined ($P \leq 0.05$). There was no significant difference between the effects of 5-Flu and low-dose nanoemulsion, while the high dose was more effective than 5-Flu in reducing the level of ROS.

The protein carbonyl level was elevated significantly in DMBA breast cancer rats when compared with normal rats. The 5-Flu and the two doses of quinoa oil nanoemulsion significantly declined the serum protein carbonyl levels ($P \leq 0.05$), but the quinoa nanoemulsion revealed more reduction than the 5-Flu (Fig. 5B).

Effect of Quinoa nanoemulsion on anti-oncogenes mRNA expression

As seen in Fig. 6, DMBA administration revealed significant upregulation ($p \leq 0.05$) in the expression of the BRCA1 and 2 genes. Meanwhile, there was no detectable change in the expression of the TP53 gene as compared to the control. Similar to 5-FLU, the treatment with quinoa oil nanoemulsion significantly

decreased the mRNA levels of BRCA1 and BRCA2 as compared to the DMBA group. In contrast, there was a significant increase in the expression of the TP53 gene in tumor tissue as compared to the DMBA group.

Histopathological examination

In the control mammary gland, the small ducts looked normal and consisted of two layers of epithelial cells, myoepithelial cells, and basement membrane, which were surrounded by a sum of fibrous connective tissue and much adipose tissue (Fig. 7A). In addition, no pathological alterations were seen in both tissues treated with Quinoa oil nanoemulsion (100 and 200 mg/kg) compared to the normal tissue of the control (Fig. 7B and C). In the DMBA-treated group, some of the mammary ducts showed dysplastic cells with hyperchromatic nuclei in the ductal epithelial lining, focal infiltration, and a mild desmoplastic reaction around these ducts (Fig. 7D). The histopathological examination of the mammary gland of a breast cancer model rat treated with 5-fluorouracil revealed marked degeneration in the ductal cell lining and mild desmoplasia observed (Fig. 7E). While in the tumor tissue of animals treated with quinoa oil nanoemulsion 100mg/kg, the mild degenerative reactions of epithelial cells of the ducts, as well as mild desmoplasia was seen (Fig. 7F). Whereas in tumor tissue of animals treated with quinoa oil nanoemulsion 200 mg/kg, marked improvement manifested in the form of a reduction in epithelial cell proliferation, and ducts appeared nearly normal (Fig. 7G).

Discussions

Cancer is one of the main issues of concern for the global public health system. The abundance of diverse chemicals and pharmacologically active compounds found in nature makes it a rich source of anticancer medications. Some natural products and their synthetic analogues have been studied in recent years as potential cancer treatments (Sharifi-Rad et al., 2019). The use of quinoa oil nanoemulsion as a source for antitumor medications is the main objective of this article. The antitumor activity of quinoa has been identified in many cancer types, including breast [6, 26].

The fatty acid analysis of quinoa oil revealed a high content of unsaturated fatty acids; palmitate (57.73%) and linoleic (31.32%) acids are the major recognized fatty acids. This was in agreement with Bata et al. [9], who reported that linoleic acid (52.7%) is the major identified fatty acid in quinoa seeds. In many cell lines, including those from breast, colon, and prostate cancer, alpha-linolenic acid has been found to possess antitumor properties [29].

The origin and progression of breast cancer are influenced by a variety of mechanisms and factors. Oxidative stress is one of the elements connected to the beginning and development of this illness. Damage to cellular macromolecules, such as lipids, proteins, and DNA, can result from an imbalance between the creation of radical species and the antioxidative defense mechanisms brought on by oxidative stress [12]. According to Krishnamoorthy and Sankaran [30], DMBA-induced oxidative stress has a toxic appearance and is linked to a variety of cellular macromolecular damages. Our data indicated that the tumor breast tissue illuminates a high ROS content that is declined by the quinoa oil

nanoemulsion. Ranjan et al. [31] mentioned that quinoa possesses a huge number of secondary metabolites that play many physiological roles against harmful organisms and exhibit features beneficial to humans, including anticancer.

Cancer development and suppression depend on the irreversible post-translational modification known as protein carbonization, which is brought on by oxidative stress. Levels of ROS may be higher in malignancies than in surrounding healthy tissue. However, it is unclear how exactly the ROS that prompted protein carbonization plays a specific role in the development or suppression of cancer. The result of our study was in agreement with the study of Aryal and Rao [32]. They found that breast cancer tissue has higher amounts of total protein carbonization, which can be ascribed to the tumor tissue's higher levels of ROS and oxidative stress. Meanwhile, quinoa oil nanoemulsion attenuated the levels of protein carbonyl. Bochkov et al. [33] reported that vegetable oils' micronutrients may protect against ROS formation and its impacts via their anti-oxidant activity.

It was confirmed that differential mRNA expression levels of BRCA 1, BRCA 2, and TP53 may serve as a diagnostic indicator with an important prognostic value in breast tumor patients [34]. Since non-functional tumor cells for p53 do not respond to systemic therapy due to an inability to undergo apoptosis, the P53 expression status has been widely employed as a predictor for response to systemic therapy [21]. BRCA 1, BRCA 2, and TP53 function as tumor suppressor genes that control cell cycle checkpoints and repair DNA within a normal cell. When these tumor suppressor genes are mutated, it results in a loss or reduction in their function that may be even more significant in the development of cancer. The result of our study clarified that BRCA1 and 2 expressions in tumor cells were up-regulated when compared to controls, while there was no difference in TP53 expression. BRCA and TP53 share the same downstream pathways used to activate them via phosphorylation and for their respective biological functions. Despite these similarities, the relationship between TP53 and BRCA revealed that TP53 stimulation down-regulates BRCA. The division arrest and senescence of TP53 are additional factors that contribute to the down-regulation of BRCA [35]. Al-Dhaheri et al. [36] show how changes in the expression of the TP53 proteins are related to the morphological changes in the mammary gland brought on by a single gavage of DMBA. The sequence of the morphological changes is followed by an initial downregulation and then an upregulation in the expression of p53. Based on the results of Zghair et al. [34], overexpression of BRCA1 was reported in breast cancer patients. In a recent clinical study by Satyananda et al. [37], they stated that high BRCA2 gene expression was associated with highly proliferative and aggressive breast cancer. Using bioinformatics analyses, Wang et al. [38] showed that the expression of BRCA1 and 2 was elevated in the tissues of breast cancer. Aubrey et al. [39] reported that in addition to TP53's role in tumor suppression, it also has a significant impact on how many anticancer therapies affect malignant cells via cell cycle arrest and many cellular metabolic pathways to directly regulate by forming transcription factors with the target gene. The primary mechanism by which p53 prevents tumor development is the induction of apoptotic death in developing neoplastic cells. Results of this study revealed that the mRNA levels of TP53 were found to be significantly increased in both 5-FLU and quinoa oil nanoemulsion-treated rats when compared with those treated with DMBA, while, BRCA1 and 2 expression significantly decreased. 5-FLU is one of the most commonly used drugs

used to treat different types of cancer, including breast cancer [40]. It was found that the MCF7 breast cancer cell line treated with 5-FLU showed p53 overexpression [41]. Accordingly, it is concluded that quinoa oil nanoemulsion can effectively modulate the expression of anti-oncogenes in DMBA-induced breast cancer rat models to a level that is comparable to the anticancer effects induced by the 5-FLU drug.

Female rats treated with DMBA were used to characterize the initial structural pre-neoplastic changes that take place during breast cancer development. According to Hamza et al. [42], DMBA primarily causes lesions to form in the terminal ducts and causes ductal epithelial cells to become hyperplastic, atypically hyperplastic, and cancerous. DMBA-treated rats' mammary gland examination showed the existence of dysplastic cells with hyperchromatic nuclei in the ductal epithelial lining and duct wall infiltration with desmoplasia of the breast stroma. Quinoa oil nanoemulsion administration showed marked improvement, manifested by a reduction in epithelial cell proliferation. This is in agreement with a recent study that confirms the anti-proliferative role of quinoa peptide in obstructing colon cancer cell proliferation [43]. Also, a study demonstrated that quinoa seed oil inhibits the growth of HCT 116 cells by inducing apoptosis, suggesting that it may be used as a functional food to prevent colon cancer. As a result, the induction of apoptosis in cancer cells is crucial to the success of cancer chemotherapy [10]. Natural bioactive compounds encapsulated in nanocarriers can result in increased *in vivo* stability and water solubility, a longer circulation time of the natural product in the blood, and improved bio-distribution and targeting of BC cells. They are regarded as strong antioxidants with fewer side effects and favorable effects on cancer-specific pathways. Through active or passive targeting of the breast tumor tissue, natural therapeutic drugs can accumulate more effectively and for longer at the tumor site [44, 45]. Several *in vivo* BC model studies were established and validated for the antitumor functions of nano-encapsulated phytochemicals [46–48]

Conclusion

One of the areas where contemporary research is needed is the relationship between micronutrient nanotechnology and the treatment of diseases. Based on the most recent research, we can say that quinoa oil nanoemulsion might be a prospective adjuvant therapy for breast cancer. All of these encouraging results may open the door to the development of quinoa nanoemulsion as a chemoprotective medication to lower the risk of developing breast cancer.

Abbreviations

BRCA (Breast Cancer gene), DMBA (Dimethylbenz (a) anthracene), DPPH (Diphenyl-1-picrylhydrazyl), FAME (Fatty acid methyl esters), GC/MS (Chromatography-Mass Spectrometry), ROS (Reactive oxygen species), TEM (Transmission Electron Microscopy), TP53 (Tumor protein 53), PC (Protein carbonyl).

Declarations

Declaration of competing interests

The authors declare that they have no competing interests.

Submission declaration and verification

The work described has not been published previously (except in the form of an abstract), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or any other language, including electronically without the written consent of the copyright holder.

Ethics approval

This Animal study was performed in accordance with the ethical standards as laid down in the Declaration of Helsinki. The study was approved by the National Research Centre Ethics Committee of Medical research number (19164).

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Availability of data and material

The datasets analyzed during the current study are not publicly available due to privacy but are available from the corresponding author upon reasonable request.

Author contributions

AE formulated the principal research aims. DY prepared the nanoemulsion. SM, SH and FM performed animal experiments; SH, DM, HA, FM, SM performed the experimental methods and analyzed results. AE, DM statistically analyzed the results and wrote the manuscript. All authors read and approved the final manuscript.

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Figures

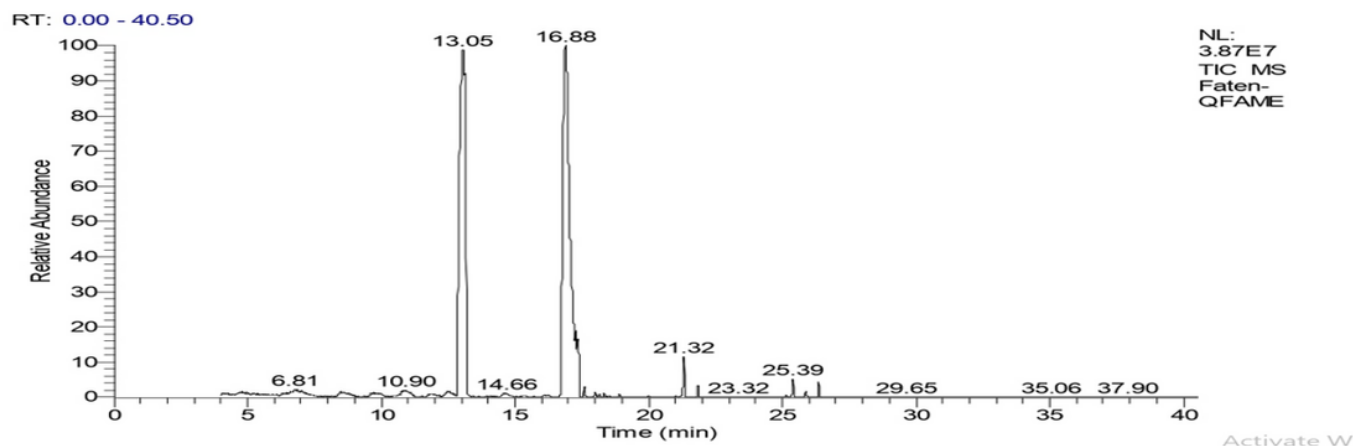


Figure 1

Total ion chromatogram of quinoa fatty acids

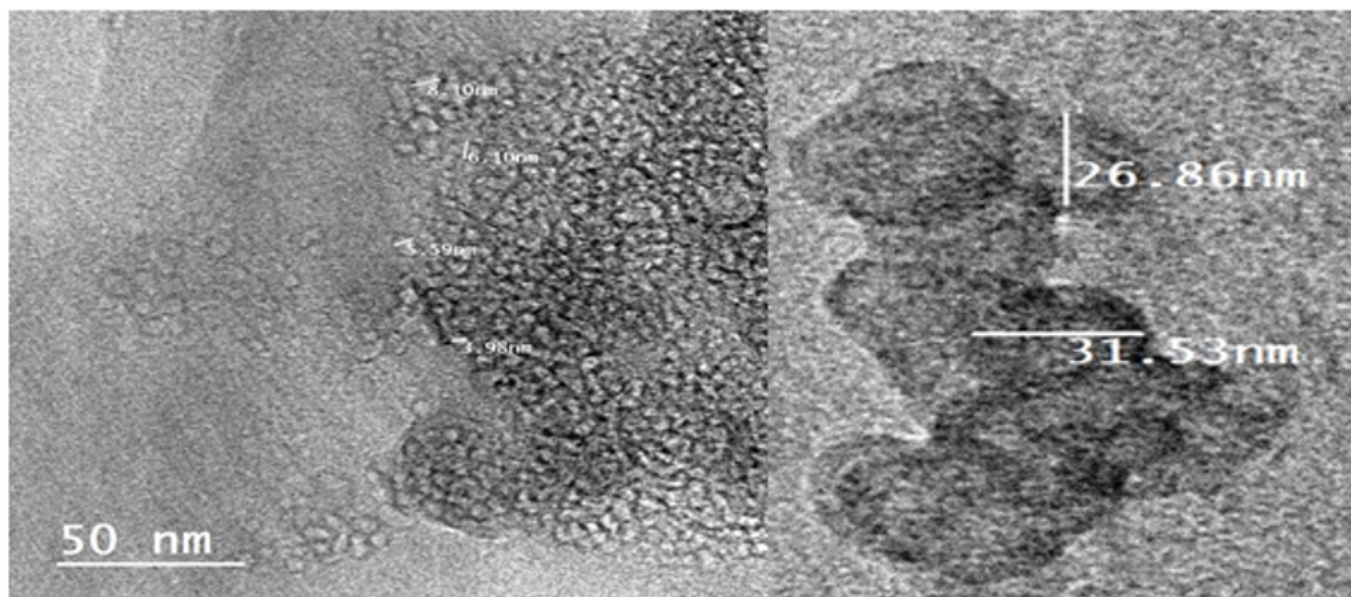


Figure 2

Transmission electron microscope image of quinoa oil nanoemulsion particles.

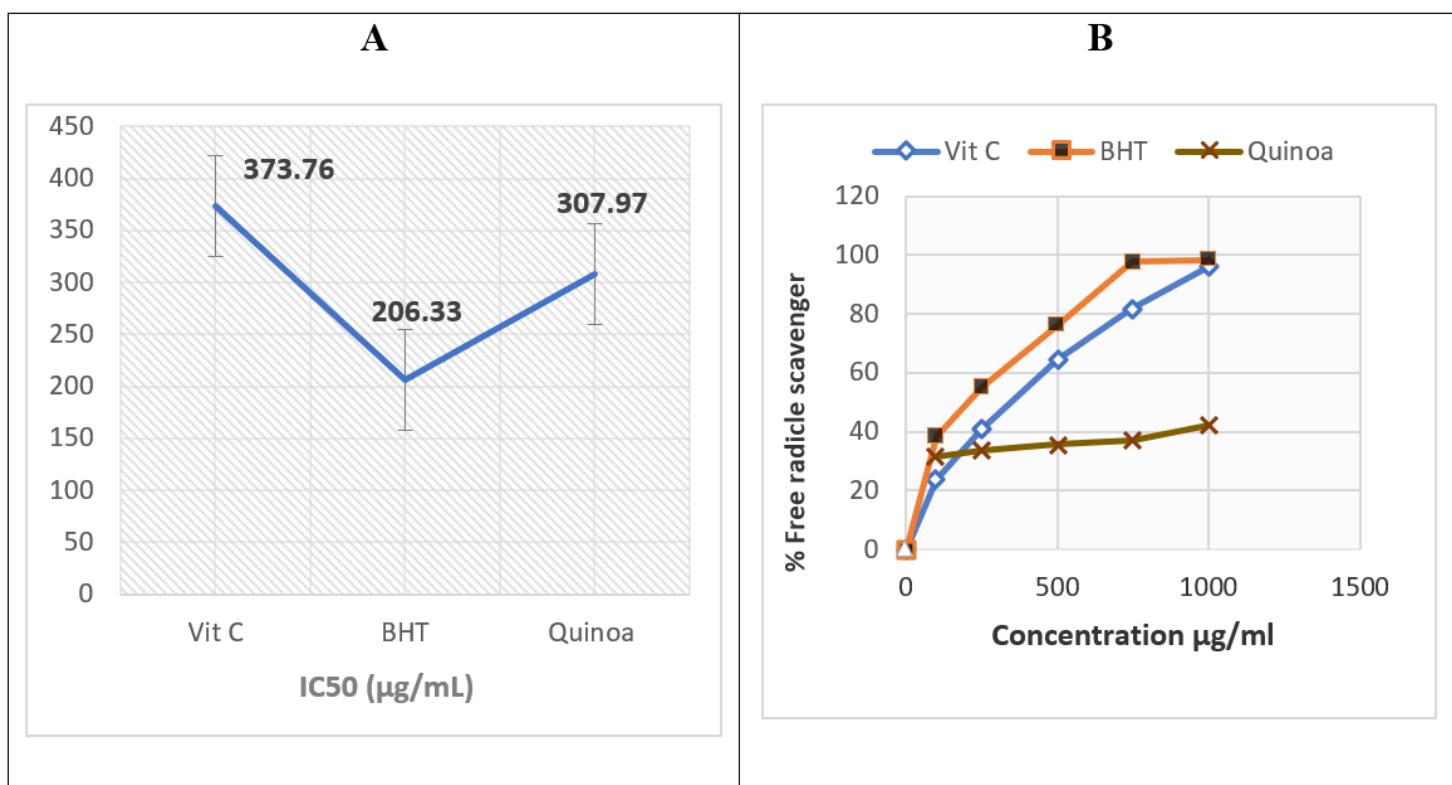
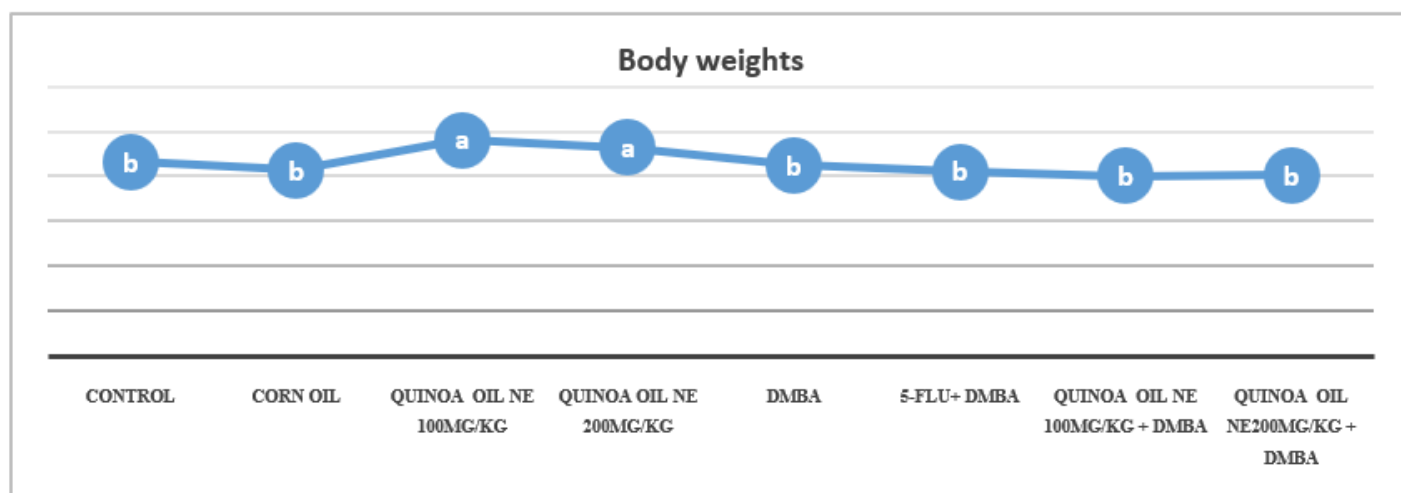


Figure 3

Determination of A) IC₅₀ of quinoa nanoemulsion and B) DPPH radical scavenging activity

A



B

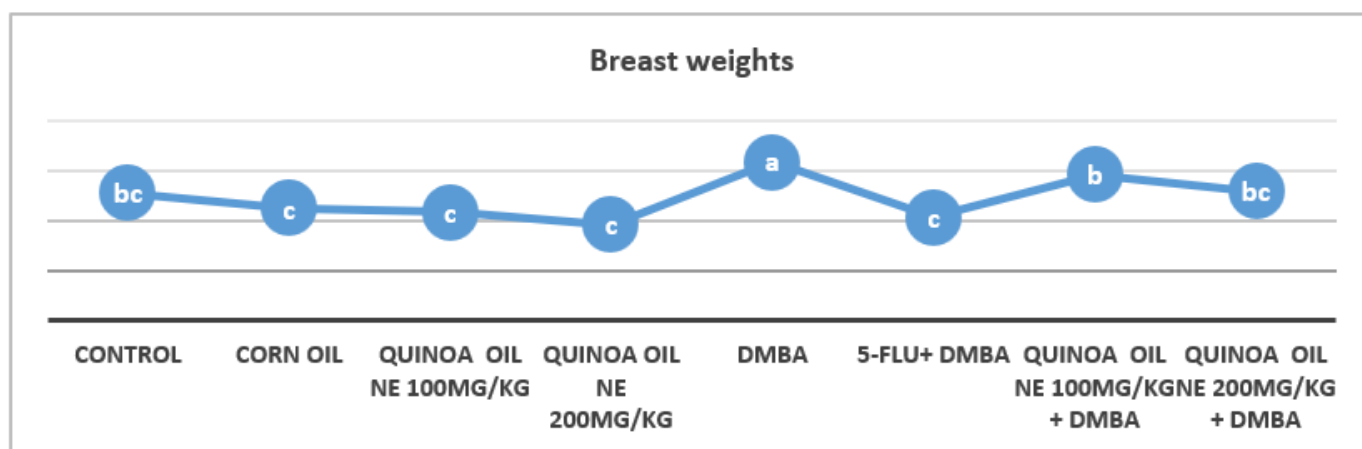
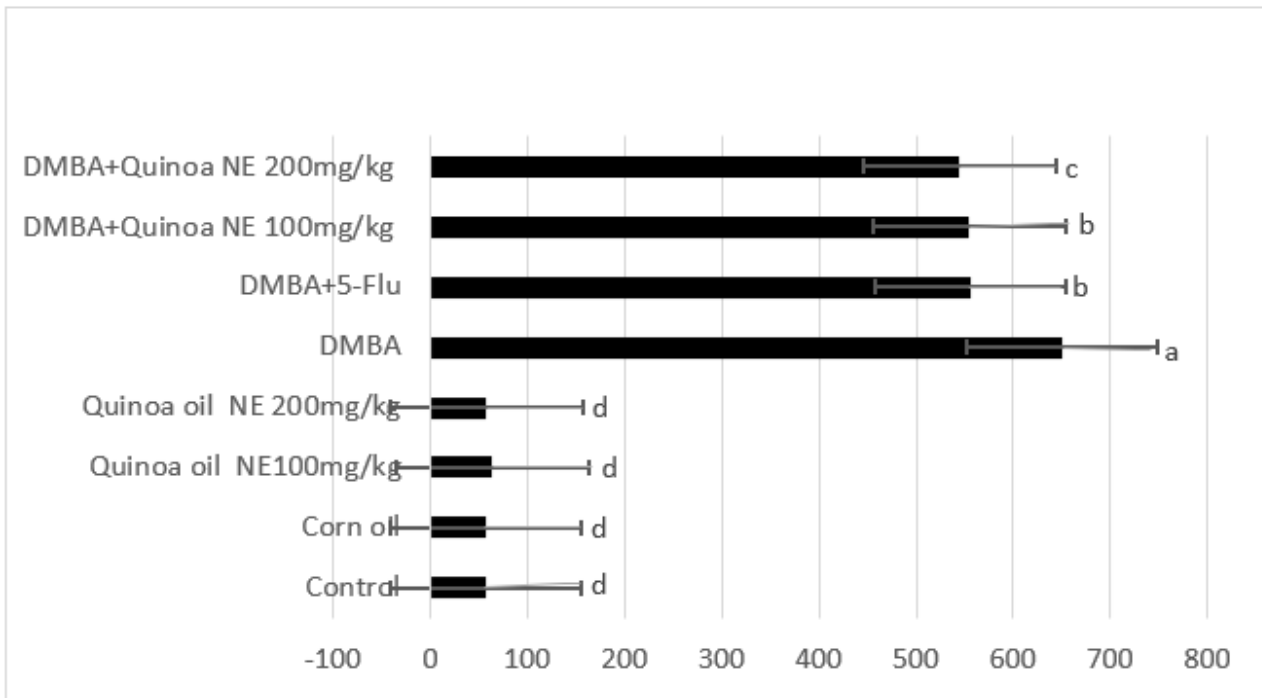


Figure 4

Effect of quinoa oil nanoemulsion on (A) bodyweights (B) breast weights of DMBA treated female rats.

Data expressed in mean+ SE (n=6) for all tested groups and different letters means significant differences ($P \leq 0.05$)

A



B

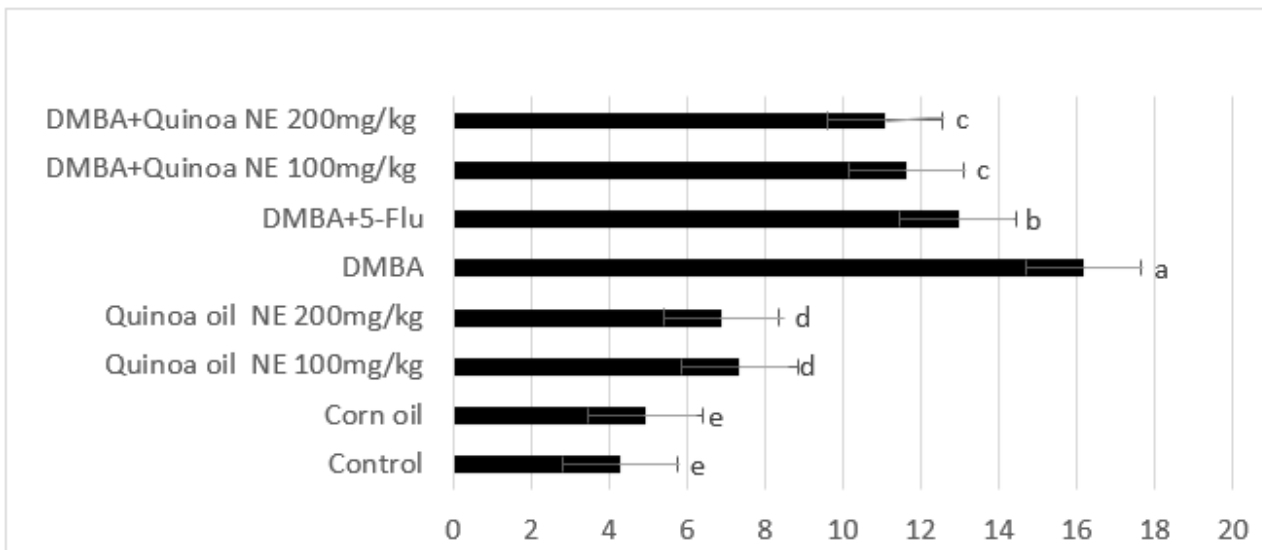


Figure 5

Influence of quinoa oil nanoemulsion on **(A)** ROS and **(B)** PC levels in DMBA treated female rats. Data expressed in mean+ SE (n=6) for all tested groups and different letters means significant differences ($P \leq 0.05$)

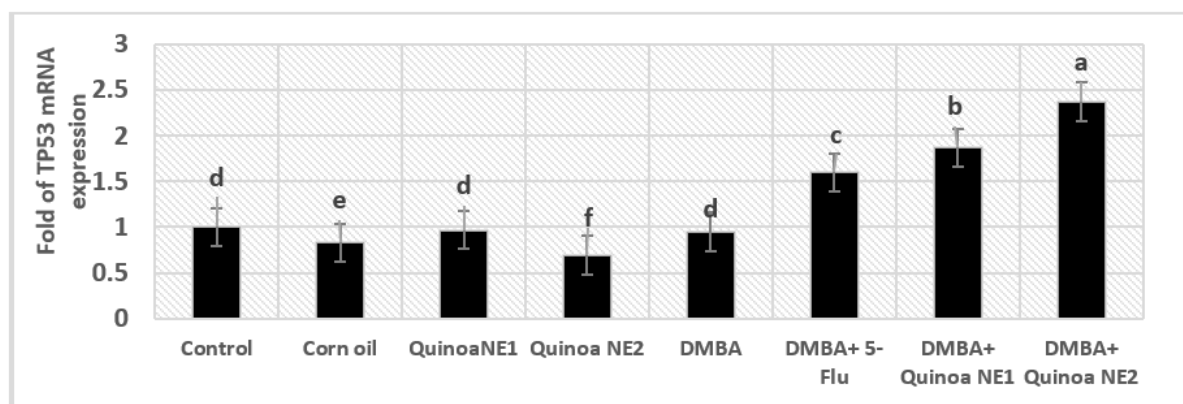
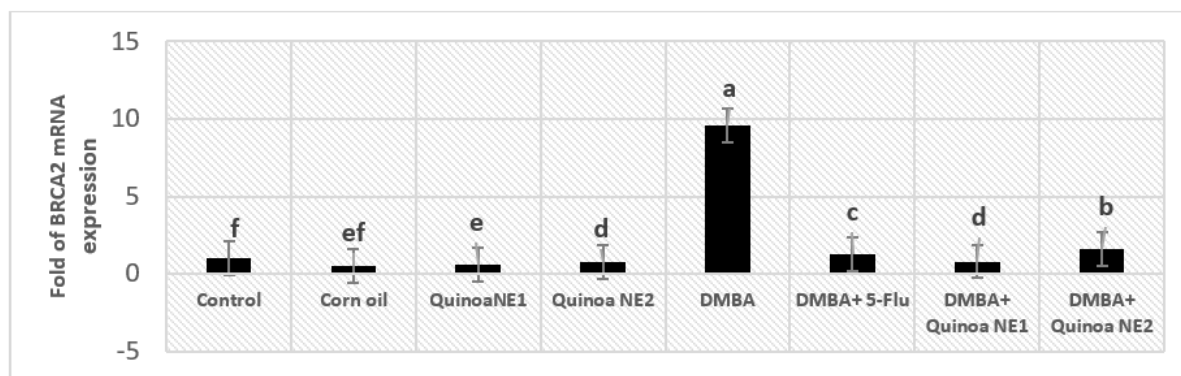
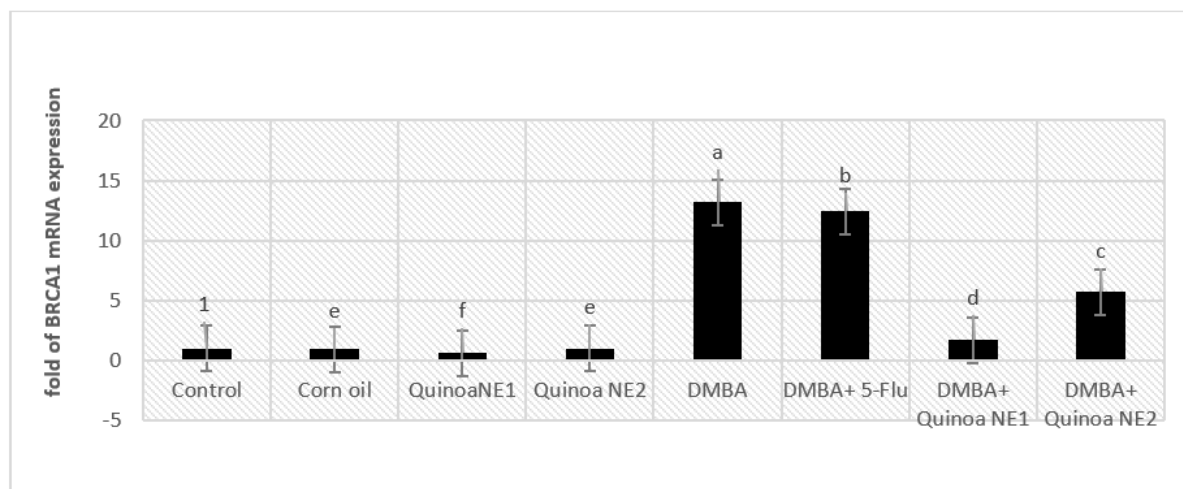


Figure 6

The effect of quinoa oil nanoemulsion on BRCA1 and 2 and TP53 expression in mammary gland tissues of DMBA-treated rats. Data expressed as mean $\pm$ SE (n=6) for all tested groups and significant variances are shown by unlike superscripts ($P \leq 0.0$)

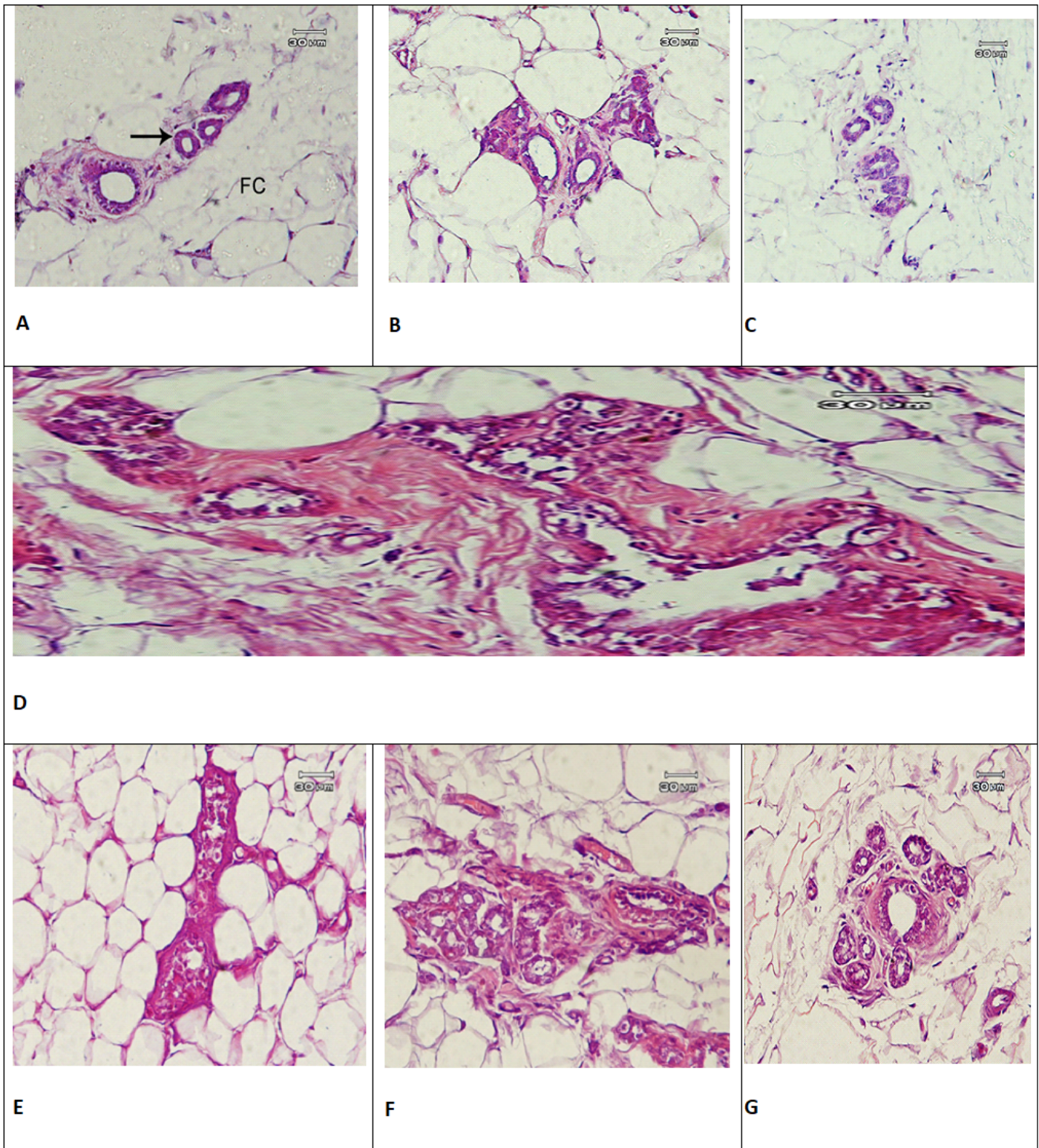


Figure 7

Photomicrograph of mammary tissue of (A) control rat showing normal small ducts (arrow) surrounded by a small amount of fibrous connective tissue and embed in adipose tissue (FC); (B) and (C) rat treated with 100mg/kg and 200mg/kg quinoa oil nanoemulsion showing normal histological structure of mammary gland.;(D) rat treated with DMBA showing dysplastic cells with hyperchromatic nuclei (arrow) in ductal epithelial lining and infiltrating the duct wall (arrow head) with desmoplasia of the breast

stroma.;(E) breast cancer rat treated with 5-fluorouracil showing marked degeneration of the ductal lining epithelial cells and mild desmoplasia still observed.;; (F) breast cancer rat treated with 100mg/kg) quinoa oil nanoemulsion showing few degenerated epithelial cells of ducts, as well as mild desmoplasia.;; (G) breast cancer rat treated with 200mg/kg quinoa oil nanoemulsion showing marked improvement in the form of reduction of epithelial cells proliferation, ducts appeared nearly normal (HxandE).