Bulletin of the National Research Centre

RESEARCH

Open Access

Exploration of tumor growth regression of quinoa and chia oil nanocapsules via the control of PIK3CA and MYC expression, anti-inflammation and cell proliferation inhibition, and their hepatorenal safety in rat breast cancer model

Aida I. El makawy[1*], Sekena H. Abdel-Aziem[1], Shaimaa E. Mohammed[2], Faten M. Ibrahim[3], Heba A. Abd EL-Kader[1], Hafiza A. Sharaf[4], Dalia A. Youssef[5] and Dalia M. Mabrouk[1]

Abstract

Background The second most common cancer in the world is breast cancer. Chemotherapy is used to treat breast cancer, but instances of multidrug resistance, targets that are not selective, and physicochemical issues raise doubts about its efficacy. So, the exploration of chemopreventive agents from efficient natural sources has been required. The chia and quinoa seeds have health-promoting activities that include cardio-protective, antidiabetic, and anticancer effects. Given the paramount importance of their oils and their potential bioactivities, this work aimed to assess the repressive effect of their oil nanocapsules against mammary tumors in rats. Rat models of chemically induced mammary tumors were gavaged with chia and quinoa nanocapsules for one month. The repressive effect of nanocapsules was studied by quantifying TNF-α, assessing the gene expression of proto-oncogenes (PIK3CA and MYC) using qRT-PCR, and analyzing the cell cycle in mammary tissue.

Results The studies clarified that the inhibition of tumors in response to quinoa and chia nanocapsules was associated with a reduction in TNF-α levels, proliferation capability, and motivation for apoptosis. Furthermore, quinoa and chia nanocapsule management repressed the activation of the MYC and PIK3CA genes. As well as nanocapsules modulated the liver enzymes and kidney function alterations induced in mammary tumor animals. Meanwhile, both oils' nanocapsules do not have an impact on the liver and kidneys of healthy rats.

Conclusions The findings indicate that quinoa and chia nanocapsules are safe and can reduce tumor growth, suggesting a potential natural therapeutic target for breast cancer treatment.

Keywords Nanoencapsulation, Anti-inflammation, Proto-oncogenes, Cell proliferation

*Correspondence:
Aida I. El makawy
aelmakawy@yahoo.com
Full list of author information is available at the end of the article

Background

In Egypt, there are over 22,000 new cases diagnosed with cancer annually, accounting for 33% of all female cancer cases. Given the growing population, shifting population pyramid, and adoption of a more Westernized lifestyle, this is predicted to increase exponentially over the coming years. Breast cancer (BC) is one of the most important cancers in Egyptian women (Abdelaziz et al. 2021). According to the last global cancer statistics, it was the second-leading cause of cancer-related deaths in 2018. In 2018, more than two million women were affected by this disease, with a mortality rate of 6.6%, resulting in more than 500 thousand deaths of women (Bray et al. 2020).

Chemotherapy resistance is defined as a medication's low ability to generate a positive response during treatment and is one of the primary causes of chemotherapy failure (Vasan et al. 2019). Chemotherapy resistance affects the choice of chemotherapy in recurrence cases and presents a challenge to the treatment of neoplasms. Multidrug resistance is a characteristic of cells that can endure chemotherapy treatment and decrease drug absorption, which encourages the efflux of anticancer medications from tumor cells. Membrane transporter protein activity is dependent on MDR, which can explain why some cancer cells are resistant to chemotherapy (Nedeljkovic and Damjanovic 2019).

In terms of genetic and biochemical processes, cancer has several characteristics that may cause the deregulation of signaling pathways like p53 and nuclear factor-B (NF-B), accelerating cancer development (Aunan et al. 2017). The immune system's primary building block is inflammation, where chronic inflammation is thought to be a sign of cancer development and has the potential to cause a variety of complex changes at the molecular, cellular, and hampered healing processes. Also, the ROS/RNS random production causes mutations, decreases DNA repair efficiency, and significantly increases cytokine/chemokine release, as well as pathophysiologic protein synthesis and signaling pathways (Kubatka et al. 2021).

The pleiotropic proinflammatory cytokine that is encoded by tumor necrosis factor (TNF) is important for controlling immune cells. The cytokine was implicated in the perception of a broad range of biological processes, such as cell division, proliferation, death, lipid metabolism, and coagulation. TNF was discovered to be an endotoxin-induced serum factor that contributes to several malignancies, including necrosis. TNF-α has been associated with breast cancer developmental stages and metastasis (Rana et al. 2021).

A highly complex process is involved when tumor tissue transforms regularly over the course of years or even decades of life. Strong stresses that have the potential to damage DNA, persistent inflammation, extensive interaction between relevant molecular pathways, and cellular cross talk between adjacent tissues all play a role in this process. Based on modifiable risk factors, which are essential in cancer prevention, the majority of cancer cases are naturally sporadic (Kapinova et al. 2018).

The discovery of chemopreventive agents from efficient natural sources has received a lot of scientific attention in recent years. Through various cellular and molecular methods, a wide range of phytoconstituents have been investigated for their potential to inhibit the development of carcinogenesis both in vitro and in vivo (Kaur et al. 2018).

Due to their superior nutritional value and wide range of bioactive compounds, chia (*Salvia hispanica* L.) and quinoa (*Chenopodium quinoa* Willd.) seeds have regained popularity in developed nations (Pellegrini et al. 2018a, b). Natural antioxidants like tocopherols, phytosterols, carotenoids, and phenolic compounds are abundant in chia and quinoa seeds (Pereira et al. 2019; Marineli et al. 2014). Omega-3 and omega-6 fatty acids can be found naturally in chia (*Salvia hispanica* L.) seeds. A sizeable amount of dietary fiber, proteins with high biological value, tocopherols and polyphenols, vitamins, carotenoids, and minerals are also present in them. Because of their health-promoting properties, which include effects on diabetes prevention, cancer prevention, and cardioprotection, chia seeds have seen a sharp increase in popularity in recent years (Camara et al. 2020). Also, Shaer and Al-Abbas (2022) concluded that chia nanoparticles are a hopeful adjuvant therapy in breast cancer cells. Quinoa (*Chenopodium quinoa* Willd) grains are renowned for their superior nutritional value and potential health advantages. They contain high levels of dietary fiber, unsaturated fatty acids, and minerals, and their protein content ranges from 12 to 20% (Carvalho et al. 2020). In addition, quinoa is rich in polyphenols, phytosterols, flavonoids, and vitamins E, B, and C, which have positive health effects (Carvalho et al. 2019). Quinoa has been identified in numerous studies as a protective agent against a wide range of illnesses, including cancer, allergies, and inflammatory diseases. Additionally, they may lower the risk of cardiovascular diseases (Heck et al. 2019). A few subsequent in vitro investigations have indicated the antitumor properties of quinoa seeds. Quinoa seeds exhibit a promising cytotoxic effect against liver cancer cell lines (Mohamed et al. 2019), and human cervical carcinoma cell lines (Pasko et al. 2019). In addition, Mollaei et al. (2021) proposed quinoa as a latent complementary agent for lung cancer therapy that could induce apoptosis in lung cancer cells (A549) through the increased ratio of BAX/BCL2.

The tumorigenic perspective of an oncogene is well defined by how well it collaborates with other driver alterations to alter a cell's structure in complicated ways (Bell et al. 2019). One of the most frequently altered genes in cancer is phosphatidylinositol-3 kinases (PIK3), which are heterodimeric lipid kinases that encode the catalytic subunit p110 of PI3K. Activated PI3K is essential to tumor development by regulating a variety of cellular processes, including metabolism, genomic stability, cell motility, and proliferation (Venetis et al. 2020). The MYC oncogene family consists of three members, C-MYC, MYCN, and MYCL, which encode c-Myc, N-Myc, and L-Myc, respectively. The Myc oncoproteins are members of the family of super-transcription factors, which may control at least 15% of all genome transcription. A variety of biological functions, such as cell survival, differentiation, and proliferation, as well as immune monitoring, are controlled by the principal downstream effectors of Myc (Chen et al. 2018). MYC deregulation is linked to poor outcomes and contributes to the onset and spread of breast cancer. The loss of tumor suppressors and the activation of oncogenic pathways are associated with MYC deregulation in breast cancer, which is caused by a variety of mechanisms including gene amplification, transcriptional regulators, and protein and mRNA stabilization (Kalkat et al. 2017; Ahmadi et al. 2021).

The phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) gene encodes for the catalytic subunit of PI3K (p110 protein). Numerous forms of human cancer, including colon, breast, brain, liver, stomach, and lung cancers, have been linked to gene insertions, deletions, and somatic missense mutations in this gene. It was suggested that somatic PIK3CA mutations could increase the kinase activity of the protein change the cellular structure. As the most frequently mutated oncogene in breast cancer, PIK3CA mutations have been linked to breast cancer. According to current theories, 20–30% of all cases of human breast cancer have PIK3CA mutations (Alqahtani et al. 2019).

Treatment decisions for breast cancer must take into account the patient's preference for alternative treatments such as CAM in addition to traditional care options like surgery, radiation, chemotherapy, and endocrine therapy. As a means of boosting immune system function, managing cancer symptoms, preventing cancer from returning, and reducing emotional distress. Up to 80% of breast cancer patients reported including complementary and alternative therapies in their treatment plans. In addition, CAM therapy use was 36% higher among breast cancer survivors than in general patients. So, the study aimed to investigate the potential role of quinoa and chia nanocapsules in repressing inflammation, proto-oncogene expression variation, and cell cycle proliferation in rats' breast cancer. In addition, to determine the safety of quinoa and chia nanocapsules by assessing liver and kidney functions.

Materials and Methods

Chemicals

The 7, 12-dimethylbenz(a)anthracene (DMBA) was purchased 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). The seeds of chia and quinoa were purchased from Harraz Agricultural Seeds, Spices, and Medical Plants Co., Egypt, and were characterized in the Egyptian Agricultural Museum.

Oil extraction

Oil was extracted from 500 g of seeds powdered by soaking them in petroleum ether 40–60. The rotary evaporator was used to evaporate the solvent under a vacuum at 35 °C. The extraction was carried out until it was exhausted. To create saponifiable fractions for GC/MS analysis, the dried solvent-free extract was employed (El Makawy et al. 2022).

Unsaponifiable compound isolation

Oil was saponified by heating 5 g of oil at 95 °C for 1 h while being mixed with 50 ml of 1 N ethanolic KOH. 100 cc of distilled water was added when the mixture had cooled, and it was thoroughly mixed. The resultant solution was extracted twice using 100 ml of diethyl ether in a decanter funnel. Every extraction stage involved collecting the top organic layer, washing it two times with 75 ml of distilled water, once with 100 ml of 0.5 N ethanolic KOH, and then neutralizing it with 100 ml of distilled water. After that, the organic layers were dried with Na2SO4, and the solution was filtered and evaporated using a vacuum oven at 45 °C (Tavakoli et al. 2019).

Nanocapsule preparation and characterization

Sodium alginate solution was used as the aqueous phase in the manufacturing process of nanocapsules, according to El Makawy et al. (2022). Transmission electron microscopy (TEM) was applied to assess the nanocapsule's dimensions and morphology.

Animals

The experiment of the study was done on 96 healthy female Wistar rats from the National Research Centre animal house, weighing between 130 and 150 g. They were exposed to a 12 h day/night cycle at an ambient temperature of 22 3 °C and a humidity of 50 ± 10% with free access to food and water. Animals were kept for acclimatization for about 14 days. In all animal

operations, the handling and use of experimental animals were done in accordance with the standards set by the National Institutes of Health (NIH) and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). All experiments were performed in line with the ethical guidelines approved by the Medical Research Ethics Committee of the National Research Centre, Egypt, for Experimental Animals (No.19164).

Experimental design

Firstly, thirty-six rats were distributed to six groups, six rats each, and treated as the follows: distilled water and considered a negative control; 100 mg/kg b.w. corn oil as a vehicle; 100 and 200 mg/kg b.w. Quinoa oil nanocapsules; 100 and 200 mg/kg b.w. chia oil nanocapsules. Secondly, sixteen female rats were injected subcutaneously into the mammary region with a single dose of 80 mg/kg b.w. DMBA dissolved in 0.5 ml corn oil. Then, after four months of treatment, rat breast cancer models were divided into six groups of 10 animals each: DMBA group animals remained without treatment; 5-Flu group animals were managed with a dose of 20 mg/Kg b.w./day 5-fluorouracil; chia oil nanocapsules two groups were treated with chia oil nanocapsules 100 and 200 mg/kg b.w./day; the other two groups were administered with quinoa oil nanocapsules at a dose of 100 and 200 mg/kg b.w./day. For all treatments, the animals were administered orally for one month.

Anesthesia and tissue collection

Once the course of treatment had ended, the rats were anaesthetized through IP injections of xylazine (40 mg/kg b.w.) and ketamine (4 mg/kg b.w.), according to Bhatia et al. (2022). According to Jekl et al. (2005), blood was withdrawn from the inferior vena cava in heparinized tubes and centrifuged for 10 min at 5000 rpm. Then, plasma was collected and retained in aliquots at -80 °C. Besides, rats were killed by cervical dislocation, and afterward, PBS was pumped into them to collect the required tissues.

Determination of the serum biochemical parameters

Activities of enzyme markers in the liver and kidney, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine, uric acid, urea, and albumin in serum, were determined. All the analyses were performed in triplicate for every sample using available commercial kits from Bio Diagnostic (Egypt).

Quantification of TNF-α in mammary gland tissues as measured by ELISA

The level of TNF-α was determined by an enzyme-linked immunosorbent assay (ELISA) test using a rat tumor necrosis factor α (TNF-α) kit (SL 0722Ra, Sunlong Biotech Co., Ltd.) as described by the kit manufacturer.

Assessment of PIK3CA and MYC gene expression by quantitative real-time PCR

Samples of inguinal mammary glands were homogenized in an Easy Red Total RNA Extraction Kit (Intronbio, Korea), then RNA was extracted according to the manufacturer's instructions. The yield and quality of RNA were analyzed using a NanoDrop™ 1000 spectrophotometer (Thermo Fisher Scientific, USA) and gel electrophoresis. RNA (1000 ng) was treated with the RNase-free DNase kit (Promega) to remove any genomic DNA contamination, then cDNA was synthesized using the First Strand cDNA Synthesis Kit (Thermo Scientific). Two oncogenes (PIK3Ca and MYC) were used in the study, and glyceraldehyde-3-phosphate dehydrogenase (GADPH) was used as an internal control (Table 1). qRT-PCR was performed in a 15 µl reaction containing cDNA, TOPreal™qPCR 2X PreMIX (SYBR Green with low ROX) (Enzynomics), forward and reverse primers (10 pmol/µl) (Macrogen), and nuclease-free water. The resulting data were normalized to GAPDH and analyzed using the $2-\Delta\Delta CT$ method (Livak and Schmittgen 2001).

Histopathological tissue preparation and cell cycle analysis:

Inguinal mammary gland samples were fixed for 72 h in 10% neutral-buffered formalin, then dehydrated using alcohols, cleaned with xylene, and embedded in paraffin wax. Using a microtome, 5-m-thick sections were sliced and stained with Feulgen. The image analysis of stained DNA was done using the computerized Leica Qwin 500 Image Analyzer (LEICA Imaging Systems Ltd., Cambridge, UK). Cell cycle analysis was performed on a real-time image from the microscope that we visualized on the video monitor. The normal control samples were analyzed first to establish the reference values. The optical density of the selected nuclei in each microscopic field is measured and automatically converted by the system

Table 1 Sequence of primers used in the study

Gene	Primer	Accession no.	Product size
PIK3CA	CCT TGT TCT AAT CCC AGG TG GGA CAG TGT TCC TCT TTA GC	NM_133399.3	134
MYC	GCT CTC CGT CCT ATG TTG CG TCG GAG ACC AGT TTG GCA G	NM_012603.2	235
GAPDH	AACTTTGGCATTGTGGAAGG ACACATTGGGGGTAGGAACA	NM_017008.4	223

into a cell phase in the life cycle. Up until the appropriate number of nuclei (100–150) was measured, numerous fields were chosen. Percentages of cells in the first growth phase (G1), proliferating cells (S), and second growth phase (G2) were calculated and determined automatically by the system.

Statistical analysis

The statistical analysis was carried out using SPSS software (SPSS, IBM, Chicago, IL, USA). The results were displayed using means and the standard error of means (SEM). An analysis of variance (ANOVA) and a Duncan post hoc descriptive test were used in the statistical analysis, with $P \leq 0.05$ being recorded as statistically significant.

Results

The unsaponifiable fraction of quinoa and chia oil

GC/MS analysis of the unsaponifiable fraction of quinoa and chia oils is presented in Table 2 and 3. The analysis of quinoa oil identified 20 compounds constituting 83.25% of the total peak area, with squalene as the major compound (51.87%). For chia oil, GC/MS analysis of the unsaponifiable fraction revealed the identification of 15 compounds constituting 74.23% of the total peak area of identified compounds. The major compounds were 1,1,3,3-Tetraethoxypropane (25.17%); 3,3-Diethoxy-1, 2-propanediol (22.81%), 2-Ethyl-1-Hexanol (12.72%), butylated hydroxytoluene (8.76%), and many other compounds belonging to different classes as diterpenoid alcohol (phytol), steroids (Spiro[androst-5-ene17,1′cyclobutan]-2′-one,3-hydroxy, (3á,17á). and stigmast-5en-3-ol).

Nanocapsules characterization

The nanocapsule examination by TEM showed that quinoa nanocapsules were spherical, and the mean particle sizes ranged between 5 and30 nm. The chia nanocapsules were polygonal, with sizes ranging between 10 and 35 nm.

The impact of chia and quinoa oil nanocapsules on serum TNF-alpha levels

The data in Fig. 1 represent the values of serum TNF-α in all experimental groups. The results of the serum TNF-α analysis revealed that there were no significant differences between the corn oil and the negative control. Although quinoa and chia nanocapsules induced a significant increase in the levels of TNF-α ($P \leq 0.05$) in healthy animals as compared to the control. In breast

Table 2 GC/MS analysis of the unsaponifiable fraction of quinoa oil

No.	R_t	Compounds	BP	M+	Molecular formula	Area %
1	9.34	1-Octanol	57	130	$C_8H_{18}O$	6.84
2	14.06	Ethyl(ethyl thio)acetate	75	148	$C_6H_{12}O_2S$	3.80
3	14.78	2-Hydroxy-3-methylsuccinic acid	103	148	$C_5H_8O_5$	0.36
4	19.35	1,1,3,3-Tetra ethoxy propane	103	220	$C_{11}H_{24}O_4$	6.84
5	19.95	Trans-Caryophyllene	79	204	$C_{15}H_{24}$	0.58
6	22.24	Butylated hydroxytoluene	205	220	$C_{15}H_{24}O$	3.23
7	24.12	Octadecane	57	254	$C_{16}H_{34}$	0.30
8	24.23	2-Phenyl decane	105	218	$C_{16}H_{26}$	0.33
9	25.19	2 -Phenyl undecane	105	232	$C_{17}H_{28}$	0.30
10	28.62	2-Phenyl dodecane	105	246	$C_{18}H_{30}$	0.26
11	29.38	6,10,14-trimethyl-2-Pentadecanone	58	268	$C_{18}H_{36}O$	1.26
12	35.34	Docosene	55	308	$C_{22}H_{44}$	0.61
13	36.03	Docosane	57	310	$C_{22}H_{46}$	0.78
14	37.34	Ethyl isoallocholate	69	436	$C_{26}H_{44}O_5$	0.36
15	41.07	Pentacosane	57	352	$C_{15}H_{32}$	2.89
16	44.14	Hexacosane	57	366	$C_{16}H_{34}$	1.43
17	45.61	Triacontane	57	420	$C_{30}H_{46}$	0.34
18	46.19	Squalene	59	410	$C_{30}H_{50}$	51.87
19	46.49	Nerolidol	55	222	$C_{15}H_{26}O$	0.28
20	53.67	Stigmast-5-en-3-ol	55	414	$C_{29}H_{50}O$	0.59
		Identified compounds			–	83.25
		Non-identified			–	16.75

R_t: Retention time (min); M^+ molecular weight; BP: base peak

Table 3 GC/MS analysis of the unsaponifiable fraction of chia oil

No.	R_t	Compounds	M^+	BP	Molecular formula	Relative %
1	9.35	2-Ethyl -1-Hexanol	130	57	$C_8H_{18}O$	12.72
2	12.57	Camphor	152	95	$C_{10}H_{16}O$	0.52
3	12.75	1-Dodecene	168	55	$C_{12}H_{24}$	0.45
4	13.02	β-Panasinsen	204	161	$C_{15}H_{24}$	0.27
5	13.29	1,2,4-Butanetriol	106	75	$C_4H_{10}O_3$	0.97
6	14.08	3,3-Diethoxy-1, 2-propanediol	164	103	$C_7H_{16}O_4$	22.81
7	17.48	Pentadecane	212	57	$C_{15}H_{32}$	0.31
8	19.05	1,1,3,3-Tetraethoxypropane	220	103	$C_{11}H_{24}O_4$	25.17
9	21.30	2,6-di-(tbutyl) 4-hydroxy4methyl-2,5-cyclohexadien-1-one	236	165/137	$C_{15}H_{24}O_2$	0.38
10	22.25	Butylated hydroxytoluene	220	205	$C_{15}H_{24}O$	8.76
11	26.50	Hexadecane	226	57	$C_{16}H_{34}$	0.29
12	29.38	6,10,14-trimethyl -2-Pentadecanone,	268	58	$C_{18}H_{36}O$	0.38
13	33.34	Spiro[androst-5 ene17,1′cyclobutan] 2′one,3-hydroxy,(3á,17á)	328	55	$C_{22}H_{32}O_2$	0.41
14	34.62	Phytol	296	71	$C_{20}H_{40}O$	0.31
15	52.98	Stigmast-5en-3-ol	414	55	$C_{29}H_{50}O$	0.48
		Total identified compounds				**74.23%**
		Non-identified				**25.77%**

R_t: Retention time (min); M^+ molecular weight; BP: base peak

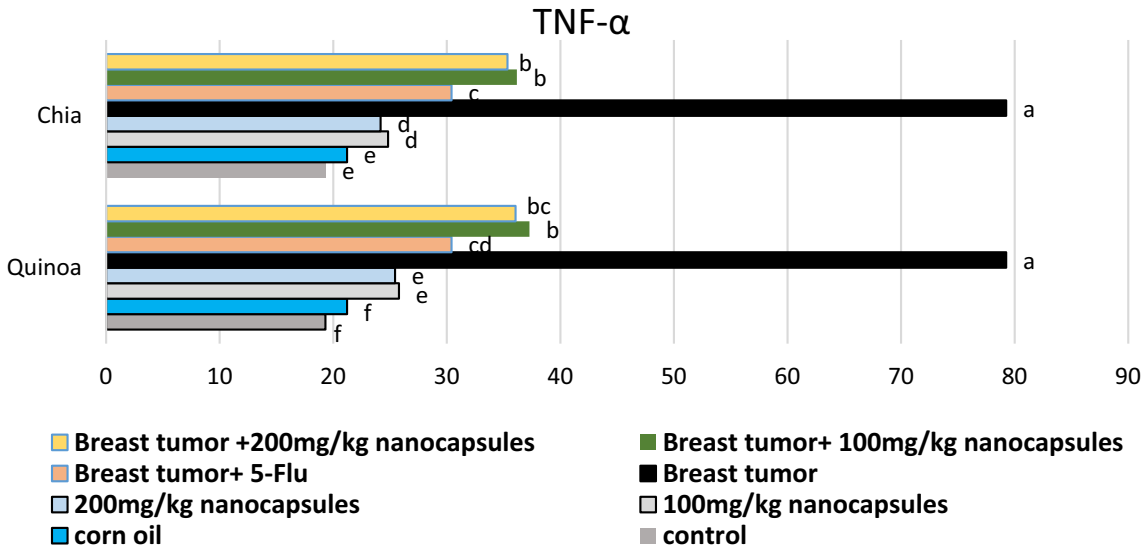

Fig. 1 The levels of serum TNF-α in healthy and tumor rats. Data presented as mean ± SE ($n = 6$) for all tested groups. Different letters show significant difference among groups in a column ($P \leq 0.05$)

tumor animals, the TNF-α content showed a highly significant increase, while treatment with 5-Flu, quinoa, and chia nanocapsules at two doses significantly ($P \leq 0.05$) attenuated the elevation of TNF-α levels in tumor tissue.

Cell cycle analysis

Figure 2 clarifies the analysis of the cell cycle of rat breast tumor cells before and after management with quinoa, chia nanocapsules, and 5-Flu. Quinoa and chia nanocapsules-treated animals with two doses of 100 and 200 mg/kg showed a typical DNA pattern that was

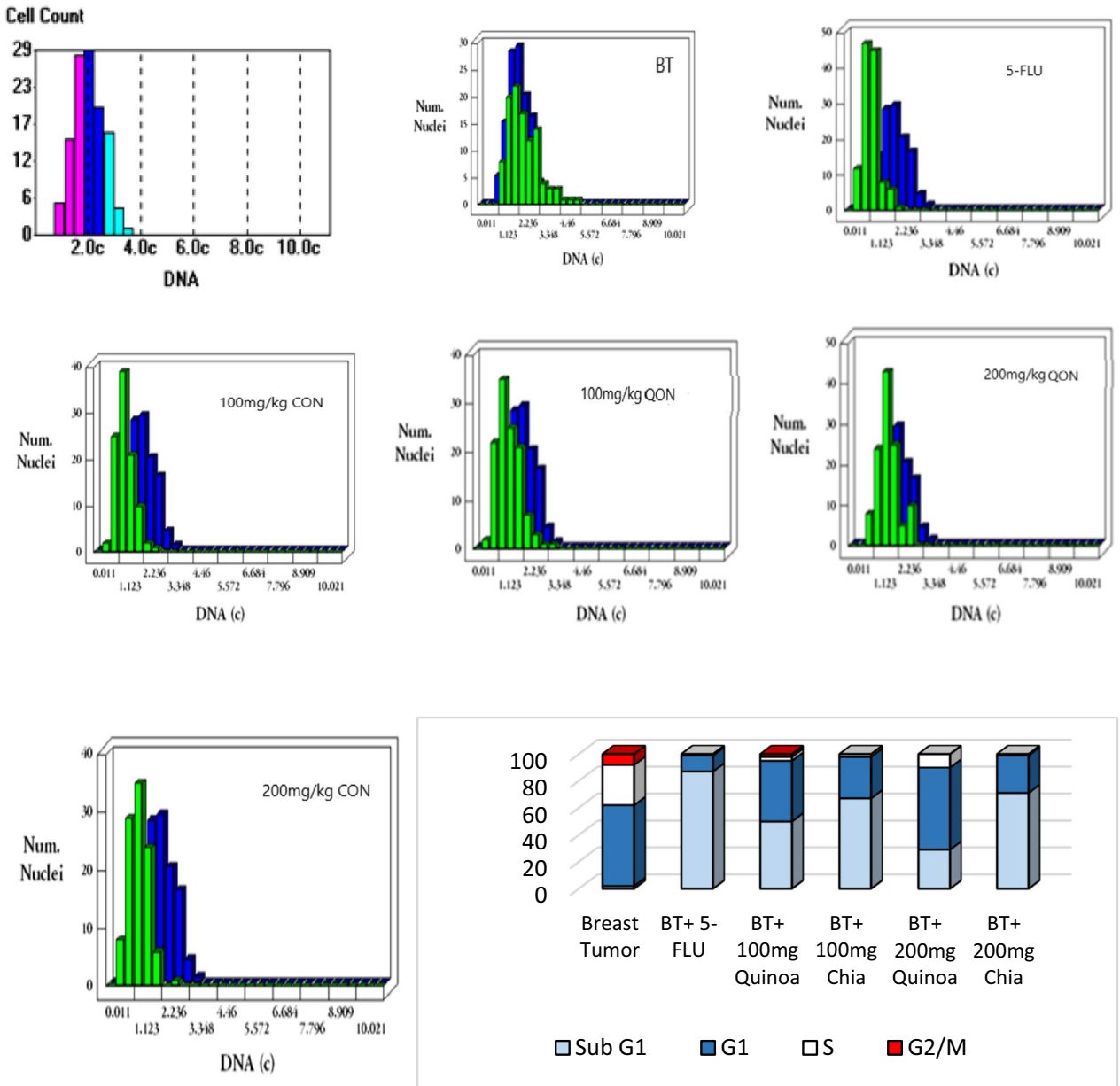

Fig. 2 Effect of quinoa and chia nanocapsules on cell cycle distribution in rats' breast tissue of all experimental groups. BT: Breast tumor; 5-Flu: 5-fluorouracil; QON: quinoa oil nanocapsules; CON: chia oil nanocapsules ($n=6$) for all tested groups

characterized by sub-G1, G1, S, and G2/M cell cycle phases. The population of cell nuclei containing a sub-G1 complement of DNA representing the apoptotic cells was high in all treated groups. In chia nanocapsules, the low-dose treatment was 67% and the high-dose was 71%: in quinoa nanocapsule-treated animals, was 50% for the low dose and 29% for the high-dose, and in 5-Flu-treated animals, was 87%. Quinoa nanocapsules at low dose decreased the percentage of cell population in the G1 phase from 60% in tumor tissue to 45%, and chia capsules to 31% and 28%, as well as 5-Flu, which decreased the cell population to 12%. On the other hand, the percentage of cells in the S phase was very low in the quinoa and chia-treated groups compared to tumor group. In addition, all treatments triggered an associated decrease in the proportion of cells in the cell cycle G2/M phase. These findings proposed that quinoa low dose and chia two doses, as well as 5-Flu, induced G1-phase cell cycle arrest.

Proto-oncogenes expression

Figure 3 illustrates the effect of chia and quinoa nanocapsules on MYC gene expression in the mammary gland tissue of healthy and tumor-model rats. No obvious difference was detected in the MYC gene expression between the control and tumor groups. Compared to the tumor group, quinoa, chia, and 5-Flu significantly reduced MYC expression ($P \leq 0.05$). There was no difference in MYC expression between the breast cells of animals treated with the high dosage of quinoa and those treated with the 5-Flu.

Figure 4 shows the influence of chia and quinoa nanocapsules on PIK3ca expression in the mammary gland tissue of healthy and tumor-model rats. As depicted in Fig. 4, DMBA significantly increased Pik3ca expression as compared with the control group. Interestingly, treatment with 5-Flu significantly increased Pik3ca expression as compared with the DMBA group. On the contrary, treatment with chia and quinoa reduced the expression of PIK3ca more than in DMBA and 5-Flu-treated groups. Both quinoa and chia nanocapsules (100 mg/kg) significantly decreased the PIK3ca expression more than in 200 mg/kg ($P \leq 0.05$).

Effect of chia and quinoa oil nanocapsules on liver functions

Data analyses of the effect of quinoa and chia oils nanocapsules on the liver function of the DMBA-breast cancer rat model are illustrated in Table 4. The administration of chia and quinoa oil nanocapsules to healthy animals did not display a significant alteration in the ALT and AST serum levels, while significantly increasing the serum ALP level ($P \leq 0.05$) as compared to the vehicle. Meanwhile, in the breast tumor model, chia nanocapsules exhibited a dose-dependent reduction in the elevation of ALT, AST, and ALP. However, the quinoa nanocapsules significantly diminished the level of ALT and nonsignificantly decreased the levels of AST and ALP as compared to the DMBA-treated group. Moreover, 5-Flu management in breast cancer models significantly ($P \leq 0.05$) diminished the levels of serum ALT, AST, and ALP as compared to breast tumor animals.

The effect of quinoa and chia oil nanocapsules on kidney function

As shown in Table 5, chia and quinoa nanocapsules did not affect the serum creatinine levels in healthy rats. Low doses of chia and quinoa oil nanocapsules did not induce

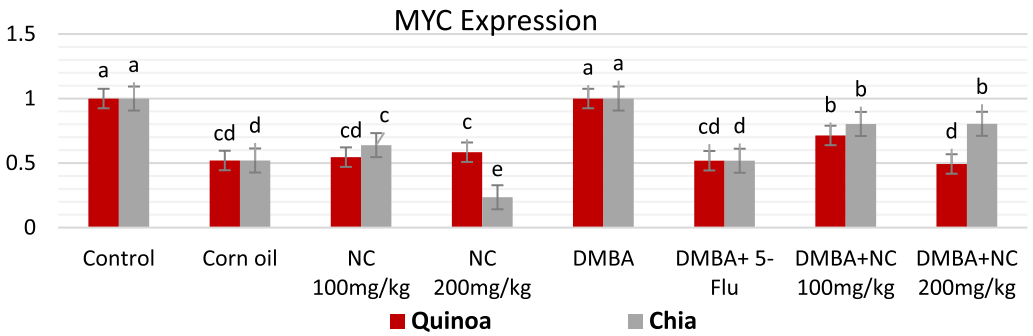

Fig. 3 The effect of chia and quinoa oil nanocapsules and 5-flurouracil on Myc expression in mammary gland tissues of DMBA-treated rats ($n = 6$) for all tested groups

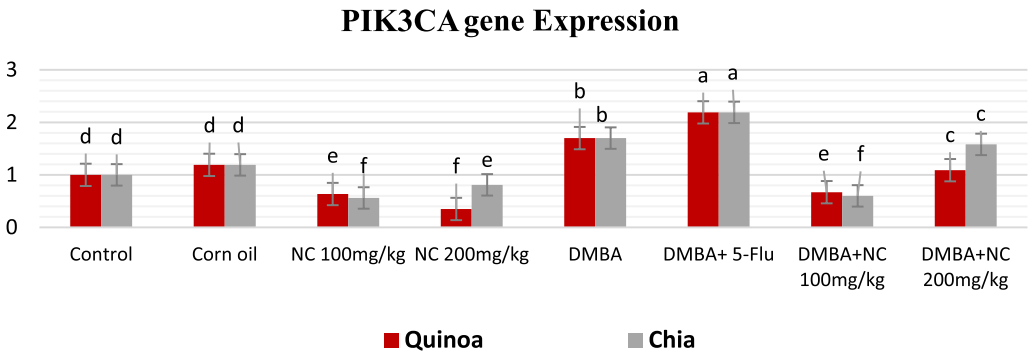

Fig. 4 The effect of chia and quinoa oil nanocapsules and 5-flurouracil on Pik3 expression in mammary gland tissues of DMBA-treated rats ($n = 6$) for all tested groups

Table 4 Effect of chia and quinoa oil nanocapsules on liver functions

	Control	Corn oil	Chia NC 100 mg/kg	Quinoa NC 100 mg/kg	Chia NC 200 mg/kg	Quinoa NC 200 mg/kg	Breast tumor	Breast tumor + 5-Flu	Breast tumor + Chia NC 100 mg/kg	Breast tumor + Quinoa NC 100 mg/kg	Breast tumor + Chia NC 200 mg/kg	Breast tumor + Quinoa NC 200 mg/kg
ALT	47.25 + 2.36d	51.48 + 0.48bc	52.68 + 1.06c	36.21 + 2.47d	54.48 + 0.56bc	36.23 + 3.28d	60.88 + 1.83a	52.59 + 1.13bc	58.32 + 1.71ab	52.16 + 1.92bc	55.42 + 2.57bc	55.05 + 1.22b
AST	120.82 + 12.03a	106.17 + 2.35 cd	106.55 + 2.46 cd	107.21 + 2.51bc	104.66 + 2.16 cd	100.55 + 3.30c	115.25 + 3.03ab	103.18 + 3.53c	112.06 + 1.62bc	113.75 + 1.74ab	105.53 + 3.17 cd	112.94 + 5.10ab
ALP	72.31 + 2.46d	52.72 + 1.66e	121.38 + 3.63bc	131.71 + 2.26b	131.83 + 5.13b	95.67 + 2.16c	174.45 + 7.80a	115.98 + 9.07bc	124.64 + 3.95bc	168.11 + 2.74a	111.38 + 4.41c	160.88 + 17.03a

Data are expressed as mean ± SE ($n = 6$). Groups with unlike superscript letters in each raw were significantly different $P \leq 0.05$)

Table 5 Effect of chia and quinoa oil nanocapsules on kidney functions

	Control	Corn oil	Chia NC100mg/kg	Quinoa NC 100 mg/kg	Chia NC 200 mg/kg	Quinoa NC 200 mg/kg	Breast Tumor	Breast Tumor + 5-Flu	Breast tumor + Chia NC 100 mg/kg	Breast Tumor + quinoa NC 100 mg/kg	Breast Tumor + chia NC 200 mg/kg	Breast Tumor + quinoa NC 200 mg/kg
Creatinine	1.01 + 0.36c	0.79 + 0.07 cd	0.79 + 0.04d	0.84 + 0.05 cd	1.03 + 0.05c	0.81 + 0.06 cd	1.61 + 1.03a	0.92 + 0.08 cd	1.43 + 0.03b	1.17 + 0.17b	1.01 + 0.10c	0.69 + 0.05d
Uric acid	2.04 + 0.19c	0.59 + 0.02e	2.33 + 0.0bc	2.51 + 0.10b	2.37 + 0.03 cd	2.95 + 0.11a	2.57 + 0.10ab	1.59 + 0.12d	2.92 + 0.17ab	2.72 + 0.18ab	2.48 + 0.13b	2.55 + 0.12b
Urea	53.10 + 0.99 cd	48.06 + 1.57d	54.50 + 3.40c	59.45 + 1.99c	83.81 + 2.26a	67.95 + 1.59a	66.09 + 2.53ab	49.76 + 2.12d	42.86 + 2.07e	61.76 + 2.39bc	46.20 + 2.45de	48.30 + 2.2.64d
Albumin	2.91 + 0.10 cd	2.57 + 0.25d	2.77 + 0.14d	2.39 + 0.20d	3.51 + 0.21bc	3.34 + 0.38bc	5.09 + 0.18a	3.51 + 0.25bc	4.06 + 0.16b	4.00 + 0.13b	3.48 + 0.09c	3.77 + 0.14b

Data are stated as mean ± SE ($n = 6$). Groups with unlike superscript letters in each raw were significantly different $P \leq 0.05$)

significant change in the serum levels of uric acid and urea, while the high doses significantly elevated the uric acid, urea, and albumin levels as compared to the control. In breast tumor tissues, levels of serum creatinine, uric acid, urea, and albumin were significantly higher ($P \leq 0.05$) than in normal tissue. Meanwhile, chia and quinoa oil nanocapsule management reduced the rise in creatinine, urea, and albumin levels in a breast cancer rat model but did not diminish the uric acid level.

Discussion

The global effort to prevent and treat breast cancer is being made by scientists. Rapid technological progress produces synthetic medicines, but due to their harmful side effects, phytomedicine has gained great attention in recent years (Laskar et al. 2020). Natural ingredient medications for the treatment of various forms of cancer depend on modifying multiple pathways, including cellular proliferation, differentiation, apoptosis, angiogenesis, and metastasis. These phytochemicals have shown anticarcinogenic capabilities by preventing the onset, development, and progression of cancer (Muhammad et al. 2022). The current study investigates the role of chia and quinoa oil encapsulation in breast cancer repression. The findings showed that rats exposed to either of the two oil nanocapsules had a lower risk of developing breast cancer. Numerous studies have supported the anticancer properties of chia and quinoa. Cardenas et al. (2018) reported that chia seeds are a promising source of protein fractions with anticancer activity because they have a high protein content and a good amino acid profile. Chia seed extract has been shown to be effective in treating cancer (Mutar and Alsadooni 2019). Liu et al. (2020) found that quinoa seed is rich in phenolic components that can be responsible for their superior antioxidant, anti-inflammatory, and antitumor activities.

Our results revealed that squalene is the major component of the unsaponifiable fraction of quinoa oil. Squalene is a metabolite involved in the biosynthesis of cholesterol. It is established that it has in vitro anticancer activities, enhances the action of the immune system against tumors, and protects breast cells against the accumulation of mutagenic lesions in their DNA (Palaniyandi et al. 2018; Sanchez-Quesada et al. 2022).

The presence of inflammatory cells and inflammatory mediators, such as cytokines, in the cancer microenvironment is one aspect of cancer-related inflammation that contributes to the disease's progression. Of all the cytokines, tumor necrosis factor-alpha (TNF-α) is a significant multifunctional inflammatory cytokine that regulates immune responses, survival, and apoptosis through a variety of pleiotropic effects. Numerous cell types, including fibroblasts, Kupffer cells, keratinocytes, infiltrating inflammatory cells, and tumor cells have been reported to be capable of releasing TNF-α. By binding to its receptors, that can increase the production of growth factors, proteases, and cytokines, such as TNF-α receptor 1 (TNFR1) and TNF-α receptor 2 (TNFR2) (Cai et al. 2017). In breast cancer, high proinflammatory cytokine (TNF-α) expression levels are associated with increased cell proliferation, a higher malignancy grade, and a higher incidence of metastasis (Mercogliano et al. 2020). Quinoa and chia seed nanocapsule treatments reduced the serum TNF-α content of breast cancer animals. These results were supported by Ferreira et al. (2018) and Ahmed et al. (2021), who confirmed that chia seeds reduced plasma IL-6 and TNF-α levels as well as ROS levels. Abdel-Wahhab et al. (2021) found that quinoa supplementation inhibits the overproduction of inflammatory mediators, as evidenced by a significant decrease in serum TNFα, IL-1β, IL-6 levels in cyclophosphamide-intoxicated mice. The abundance of antioxidants, such as linolenic acid, may be to blame (Kulczynski et al. 2019). Also, the phytosterol squalene present in quinoa may be responsible for its observed anti-inflammatory activity. It was suggested that squalene has the potential to prevent the over-activation of neutrophils, monocytes, and macrophages via targeting pro- and anti-inflammatory pathways (Ibrahim and Naina Mohamed 2021).

The present study showed a significant increase in Pik3ca expression in tumor tissue as compared to the control. The phosphatidylinositol-3-kinase (PI3K) pathway plays a main role in the development and progression of breast cancer. The Pik3ca gene is one of the most frequently mutated genes in breast cancer, which leads to PI3K activation (Dong et al. 2021). Recently, PI3K inhibitors can be used as a strategy for cancer treatment alone or combined with other strategies of cancer therapy (Alowiri et al. 2019). Herein, treatment with chia and quinoa nanocapsules showed a significant decrease in Pik3ca expression, suggesting their capability to inhibit the PI3K pathway. This was in concurrence with (Wang et al. 2017). They suggested that KIF20A knockdown brought cell cycle arrest in the G0/G1 phase and stimulated apoptosis by deactivating the PI3K/Akt pathway via c-Myc and triggering apoptosis.

Normal biological functions like cell proliferation and apoptosis depend on MYC control and transcriptional activity. MYC plays an important regulatory role in the occurrence of breast cancer, and its amplification can be used as a predictor of diagnosis (Liu et al. 2021). More than half of human malignancies have been proven to be caused by the deregulation of the MYC oncogene (García-Gutierrez et al. 2019). In healthy cells, expression of the endogenous MYC gene is elevated in response to diverse mitogenic and developmental signals. The MYC

protein functions as a transcription factor that integrates these signals into wide changes in gene expression and prompt cell growth and proliferation. Many of the genetic alterations that occur in tumors decouple MYC expression from its normal regulatory constraints, thereby resulting in an elevation of MYC proteins that are less sensitive to normal and extracellular signals (Schaub et al. 2018).

Data from our study showed that there was no obvious change detected in MYC gene expression levels between the control and breast tumor groups. The exact mechanism by which the oncoprotein contributes to the development, maintenance, and progression of cancer is still unknown, despite evidence that MYC dysregulation is causally involved in these processes. It might accomplish this through a variety of pathways, including those that promote angiogenesis, reduce apoptosis, regulate stem cell production, and boost cell proliferation (Beaulieu et al. 2020).

It was suggested that up- or down-regulation of MYC mRNA levels controls the entering and exiting of the cell cycle. MYC stimulates cell cycle mainly through the repression of cell cycle inhibitors. MYC stimulates the cell cycle progression through the regulation of many genes related to cell cycle control (Ahmadi et al. 2021). Our research results revealed that chia and quinoa nanocapsules, as well as 5-Flu treatment, lowered MYC mRNA expression and arrested the cell cycle in G1 phase. The decrease in MYC levels and accumulation of p53 are typical responses to reducing cell damage. MYC promotes apoptosis by indirectly increasing p53 levels, which in turn induces p53 to block MYC production (Ahmadi et al. 2021). Taken together, the results of the current study and of our earlier research (El Makawy et al. 2022, 2023) were in agreement with the above-mentioned mechanism.

One of the physiological alterations in cancer cells is the absence of apoptotic mechanisms, a characteristic associated with enhanced cell proliferation (Hamza et al. 2022). The result of cell cycle analysis clarified that the tumor tissue showed an increase in cell proliferation, while quinoa and chia nanocapsule management displayed a decrease in cell proliferation, clarifying an increase of apoptosis. Cell death is a fundamental biological process necessary for cellular development, and its degeneration is linked to the pathogenesis, etiology, and treatment of many degenerative diseases such as cancer (Zhang et al. 2016). This indicated that the quinoa and chia nanocapsules have the ability to treat breast cancer in rats' models. In agreement with our results, Shaer and Al-Abbas (2022) concluded that chia seeds loaded with PLGA-PEG nanoparticles are a promising adjuvant therapy in breast cancer cells via exerting apoptosis. According to Albadri [Albadri (2020) and Mollaei et al. (2021), quinoa may have lethal effects on the breast cancer cell lines MCF-7 and MDA-MB231 through an apoptotic mechanism. In DMBA-treated animals receiving Salvadora persica fruit, Hamza et al. (2022) found that an increase in cell death and a decrease in cell proliferation would result in enough cell turnover to reduce the number of potentially cancerous cells and prevent tumor growth. In addition, Hashem et al. (2022) concluded that the majority of natural products target intrinsic apoptotic signaling pathways, which result in excessive intracellular signals that set off mitochondrial-initiated processes that kill cancer cells. Luca et al. (2020) reported that the essential oils of different plants have been demonstrated to stop the cell cycle by modulating the expression of cell cycle regulatory proteins. In the present study, the cell cycle analysis of mammary gland tissues indicated that quinoa and chia nanocapsules arrested the cell cycle at the G0/G1 phase. A similar observation regarding cell cycle arrest at different phases was established by Pani et al. (2022). They indicated that individual drugs, curcumin, ellagic acid, quercetin, and resveratrol effectively arrested the cell cycle at the S phase, while the combination of drugs arrested the cycle at the G2/M phase.

Regarding the impact of quinoa and chia nanocapsules on liver and kidney functions, the findings showed that chia oil nanocapsules did not significantly change the ALT and AST blood levels in healthy animals but did lower them in tumor-model rat liver and kidney tissues. According to Mohammed and Basuny (2020), rats fed chia seed oils for eight weeks did not experience any appreciable alterations in the activity of their liver enzymes. In contrast, ALT and AST levels in healthy rats were reduced by quinoa nanocapsules. This result was in line with that of Ng and Wang (2021), who noted that because a high level of these markers is associated with liver damage, a drop in ALT and AST levels caused by quinoa eating is likely to be advantageous to liver health. Quinoa's capacity to protect the liver has also been proven in various studies using animals. Quinoa attenuated the liver function in rats managed with a high-cholesterol diet, CCl4, nicotine, a high-fructose diet, and infected fish (Alghamdi 2018; Halaby et al. 2017; Al-Qabba et al. 2020; Saxena et al. 2017; Ali 2019; Mohamed et al. 2019; Ahmed et al. 2020). The results of the current study also revealed that chia and quinoa nanocapsules did not affect creatinine levels, but their high doses increased the levels of urea, uric acid, and albumin in healthy rats and moderated the hepatotoxicity brought on by tumor models. According to Demir and Bilgic (2019), a chia-rich diet does not affect renal function parameters.

Conclusions

We can infer that quinoa and chia nanocapsules are risk-free and can inhibit tumor growth by lowering TNF-α levels, decreasing the ability of cells to proliferate, and leading to apoptosis. Furthermore, the management of nanocapsules repressed the activation of the MYC and Pik3ca genes. MYC elevation signifies one of the possible mechanisms through which breast tumors progress resistance to PI3K pathway-specific targeted therapies. As products of oncogenes, MYC and PIK3CA are well-established oncoproteins that contribute to breast oncogenesis. Meanwhile, results confirmed that both oils' nanocapsules do not impact healthy rats' livers and kidneys. So we can advise that quinoa and chia nanocapsules are safe and promising adjuvant therapies for breast cancer treatment. However, additional assessments are required to validate our findings and ensure their safety. There was a research limitation due to the paucity of prior research investigating the assessment of these oils in vivo potential anticancer properties.

Abbreviations

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ALP	Alkaline phosphatase
DMBA	Dimethylbenze(a)anthracene
5-Flu	5-Fluorouracil
GADPH	Glyceraldehyde-3-phosphate dehydrogenase
GC/MS	Chromatography–mass spectrometry
ELISA	Enzyme-linked immunosorbent assay
PIK3	Phosphatidylinositol-3 kinases gene
PIK3CA	Phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit alpha gene
qRT-PCR	Real-Time Quantitative Reverse Transcription PCR
TEM	Transmission electron microscopy
TNF	Tumor necrosis factor

Acknowledgements

Not available.

Author contributions

AIE formulated the principal research targets. DAY prepared the oils nanocapsules. SEM, SAH, and FIM performed animal experiments; SEH, DMM, and HAMA performed the gene expression analyses, FIM and SAM performed the biochemical analyses and HAS conducted the cell cycle analysis. AIE and DMM statistically analyzed the results and wrote the manuscript. All authors review and approved the final manuscript.

Funding

This work was supported by the National Research Centre of Egypt (Nos. 12020229).

Availability of data and materials

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

Declarations

Ethics approval and consent to participate

All animal experiments were approved by the National Research Centre Ethics Committee of Medical Research Number (19164; 2/2/2020). All experiments were performed in accordance with relevant guidelines and regulations. All methods are reported in accordance with ARRIVE guidelines for the reporting of animal experiments.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Cell Biology Department, Biotechnology Research Institute, National Research Centre, Giza, Egypt. [2]Food Sciences and Nutrition Department, Food Industries and Nutrition Research Institute, National Research Centre, Giza, Egypt. [3]Medicinal and Aromatic Plants Research Department, Pharmaceutical and Drug Industries Research Institute, National Research Centre, Giza, Egypt. [4]Pathology Department, Medicine and Clinical Studies Research Institute, National Research Centre, Giza, Egypt. [5]Pests and Plant Protection Department, Agricultural and Biology Research Institute, National Research Centre, Giza, Egypt.

Received: 7 July 2023 Accepted: 24 December 2023
Published online: 05 January 2024

References

Abdelaziz AH, Shawki MA, Shaaban AM, Albarouki SK, Rachid AM, Alsalhani OM, Jomaa MK (2021) Breast cancer awareness among Egyptian women and the impact of caring for patients with breast cancer on family caregivers' knowledge and behaviour. Res Oncology 17(1):1–8

*Abdel-Wahhab K G, Mannaa F A, Ashry M, Khaled D M, Hassan L K, Gomaa H F (2021) *Chenopodium quinoa* ethanolic extract ameliorates cyclophosphamide®-induced hepatotoxicity in male rats. Comparat Clin Pathol 1–10

Ahmadi SE, Rahimi S, Zarandi B et al (2021) MYC: a multipurpose oncogene with prognostic and therapeutic implications in blood malignancies. J Hematol Oncol 14:121. https://doi.org/10.1186/s13045-021-01111-4

Ahmed AZ, Mumbrekar KD, Satyam SM et al (2021) Chia Seed Oil Ameliorates Doxorubicin-Induced Cardiotoxicity in Female Wistar Rats: An Electrocardiographic. Biochemical and Histopathological Approach Cardiovasc Toxicol 21:533–542. https://doi.org/10.1007/s12012-021-09644-3

Ahmed S A, Abd El-Rahman G I, Behairy A, Beheiry R R, Hendam B M, Alsubaie F M, Khalil S R (2020) Influence of feeding quinoa (*Chenopodium quinoa*) seeds and prickly pear fruit (*Opuntia ficus indica*) peel on the immune response and resistance to aeromonas sobria infection in Nile Tilapia (*Oreochromis niloticus*). Animals. https://doi.org/10.3390/ani10122266

Albadri AI (2020) The effect of *Chenopodium quinoa* saponins on the proliferation of MCF-7 and MDA-MB-231 breast cancer. MSc thesis, Near East Univ

Alghamdi ES (2018) Protective effect of quinoa (*Chenopodium quinoa* willd.) seeds against hypercholesterolemia in male rats. Pharmacophore 9(6):11–21

Ali OIED (2019) Nutritional value of germinated quinoa seeds and their protective effects on rats' health injected by nicotine. Egypt J Food Sci 47(2):227–241. https://doi.org/10.21608/ejfs.2019.15608.1014

Alowiri NH, Hanafy S M, Haleem R A, Abdellatif A (2019) PIK3CA and PTEN genes expressions in breast cancer. Asian Pac J Cancer Prev 20(9):2841–2846. https://doi.org/10.31557/APJCP.2019.20.9.2841

Al-Qabba MM, El-Mowafy MA, Althwab SA, Alfheeaid HA, Aljutaily T, Barakat H (2020) Phenolic profile, antioxidant activity, and ameliorating efficacy of *Chenopodium quinoa* sprouts against CCl4-induced oxidative stress in rats. Nutrients 12:2904. https://doi.org/10.3390/nu12102904

Alqahtani A, Ayesh HS, Halawani H (2019) PIK3CA gene mutations in solid malignancies: association with clinicopathological parameters and prognosis. Cancers 12(1):93. https://doi.org/10.3390/cancers12010093

Aunan JR, Cho WC, Søreide K (2017) The biology of aging and cancer: a brief overview of shared and divergent molecular hallmarks. Aging Dis 8:628–642

Beaulieu ME, Castillo F, Soucek L (2020) Structural and biophysical insights into the function of the intrinsically disordered Myc oncoprotein. Cell 9(4):1038. https://doi.org/10.3390/cells9041038

Bell CM, Raffeiner P, Hart JR, Vogt PK (2019) PIK3CA cooperates with KRAS to promote MYC activity and tumorigenesis via the bromodomain protein BRD9. Cancer 11(11):1634. https://doi.org/10.3390/cancers11111634

Bhatia A, Saikia PP, Dkhar B, Pyngrope H (2022) Anesthesia protocol for ear surgery in Wistar rats (animal research). Animal Model Exp Med 5(2):183–188. https://doi.org/10.1002/ame2.12198

Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A (2020) Erratum: Global cancer statistics 2018: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA A Cancer J Clin 70:313. https://doi.org/10.3322/caac.21609

Cai X, Cao C, Li J, Chen F, Zhang S, Liu B, Zhang W, Zhang X, Ye L (2017) Inflammatory factor TNF-α promotes the growth of breast cancer via the positive feedback loop of TNFR1/NF-κB (and/or p38)/p-STAT3/HBXIP/TNFR1. Oncotarget 8:58338–58352

Camara A K F I, Vidal VA S, Santos M, Bernardinelli O D, Sabadini E, Pollonio M AR (2020) Reducing phosphate in emulsified meat products by adding chia (*Salvia hispanica* L.) mucilage in powder or gel format: a clean label technological strategy. Meat Sci 163:108085.

Cardenas A, Villalba A, de Juan RC et al (2018) Evolution of cortical neurogenesis in amniotes controlled by robo signaling levels. Cell 174(3):590-606. e21. https://doi.org/10.1016/j.cell.2018.06.007

Carvalho FALD, Lorenzo JM, Pateiro M, Bermúdez R, Purrinos L, Trindade MA (2019) Effect of guarana (*Paullinia cupana*) seed and pitanga (*Eugenia uniflora* L.) leaf extracts on lamb burgers with fat replacement by chia oil emulsion during shelf life storage at 2 C. Food Res Int 125:1–10

Carvalho FALD, Munekata PES, Pateiro M, Campagnol PCB, Domínguez R, Trindade MA, Lorenzo Rodrigues JM (2020) Effect of replacing backfat with vegetable oils during the shelf-life of cooked lamb sausages. LWT Food Sci Technol 122:1–10

Chen H, Liu H, Qing G (2018) Targeting oncogenic Myc as a strategy for cancer treatment. Sig Transduct Target Ther 3:5. https://doi.org/10.1038/s41392-018-0008-7

Demir E. A, Bilgic Y (2019) Chia seed (*Salvia hispanica* L.) supplementation may contribute to raising the levels of vitamin B 12: an option for the vegan diet. Rev Nutr 32

Dong C, Wu J, Chen Y, Nie J, Chen C (2021) Activation of PI3K/AKT/mTOR pathway causes drug resistance in breast cancer. Front Pharmacol 12:628690. https://doi.org/10.3389/fphar.2021.628690

El Makawy AI, Mabrouk DM, Mohammed SE, Abdel-Aziem SH, El-Kader HAA, Sharaf HA, Ibrahim FM (2022) The suppressive role of nanoencapsulated chia oil against DMBA-induced breast cancer through oxidative stress repression and tumor genes expression modulation in rats. Mol Biol Reports 49(11):10217–10228

El Makawy IA, Mabrouk MD, Ibrahim MF, Abdel-Aziem HS, Abd El-Kader MAH, Youssef AD, Sharaf AH, Mohammed ES (2023) Quinoa oil encapsulation mitigating effect on breast cancer via regulation of anti-oncogenes and oxidative stress repression in rats. In: BMC-complementary medicine and therapy. Springer (in press)

Ferreira MR, Alvarez SM, Illesca P, Gimenez MS, Lombardo YB (2018) Dietary Salba (*Salvia hispanica* L.) ameliorates the adipose tissue dysfunction of dyslipemic insulin-resistant rats through mechanisms involving oxidative stress, inflammatory cytokines and peroxisome proliferator-activated receptor γ. Eur J Nutr 57:83–94. https://doi.org/10.1007/s00394-016-1299-5

García-Gutierrez L, Delgado MD, Leon J (2019) MYC oncogene contributions to release of cell cycle brakes. Genes (Basel) 10(3):244. https://doi.org/10.3390/genes10030244

Halaby MS, Abdel-Rahman MK, Hassan RA (2017) Protective influence of quinoa on hypercholesterolemia in male rats. Curr Sci Int 6(01):259–270

Hamza AA, Khasawneh AM, Elwy MH, Hassanin OS, Elhabal FS, Fawzi MN (2022) Salvadora persica attenuates DMBA-induced mammary cancer through downregulation oxidative stress, estrogen receptor expression and proliferation and augmenting apoptosis. Biomed Pharmacoth 147:112666. https://doi.org/10.1016/j.biopha.2022.112666

Hashem S, Al TA, Akhtar S, Nisar S, Sageena G, Ali S, Bhat AA (2022) Targeting cancer signaling pathways by natural products: exploring promising anti-cancer agents. Biomed Pharmacoth 150:113054. https://doi.org/10.1016/j.biopha.2022.113054

Heck RT, Saldana E, Lorenzo JM, Correa LP, Fagundes MB, Cichoski AJ, Campagnol PCB (2019) Hydrogelled emulsion from chia and linseed oils: a promising strategy to produce low-fat burgers with a healthier lipid profile. Meat Sci 156:174–182

Ibrahim N, Naina Mohamed I (2021) Interdependence of anti-Inflammatory and antioxidant properties of squalene-implication for cardiovascular health. Life (Basel) 11(2):103. https://doi.org/10.3390/life11020103

Jekl V, Hauptman K, Jeklová E, Knotek Z (2005) Blood sampling from the cranial vena cava in the Norway rat (*Rattus norvegicus*). Lab Anim 39(2):236–239. https://doi.org/10.1258/0023677053739774

Kalkat M, De Melo J, Hickman KA, Lourenco C, Redel C, Resetca D, Tamachi A, Tu WB, Penn LZ (2017) MYC deregulation in primary human cancers. Genes 8(6):151. https://doi.org/10.3390/genes8060151

Kapinova A, Kubatka P, Golubnitschaja O et al (2018) Dietary phytochemicals in breast cancer research: anticancer effects and potential utility for effective chemoprevention. Environ Health Prev Med 23:36. https://doi.org/10.1186/s12199-018-0724-1

Kaur V, Kumar M, Kumar A, Kaur K, Dhillon SV, Kaur S (2018) Pharmacotherapeutic potential of phytochemicals: implications in cancer chemoprevention and future perspectives. Biomed Pharmacoth 97:564–586

Kubatka P, Mazurakova A, Samec M, Koklesova L, Zhai K, Al-Ishaq R, Kajo K, Biringer K, Vybohova D, Brockmueller A, Pec M, Shakibaei M, Giordano FA, Busselberg D, Golubnitschaja O (2021) Flavonoids against non-physiologic inflammation attributed to cancer initiation, development, and progression-3PM pathways. EPMA J 12(4):559–587. https://doi.org/10.1007/s13167-021-00257-y

Kulczynski B, Kobus-Cisowska J, Taczanowski M, Kmiecik D, Gramza-Michałowska A (2019) The chemical composition and nutritional value of chia seeds—current state of knowledge. Nutrients 11(6):1242. https://doi.org/10.3390/nu11061242

Laskar Y B, Lourembam R M, Mazumder P B (2020) Herbal remedies for breast cancer prevention and treatment. IntechOpen: Medicinal plants-use in prevention and treatment of diseases. https://doi.org/10.5772/intechopen.89669

Liu J, Wang Z, Wang Z, Hao Y, Wang YU, Yang Z, Wang J (2020) Physicochemical and functional properties of soluble dietary fiber from different colored quinoa varieties (*Chenopodium quinoa* Willd). J Cereal Sci 95:103045. https://doi.org/10.1016/j.jcs.2020.103045

Liu Y, Zhu C, Tang L, Chen Q, Guan N, Xu K, Guan X (2021) MYC dysfunction modulates stemness and tumorigenesis in breast cancer. Int J Biol Sci 17(1):178–187. https://doi.org/10.7150/ijbs.51458. https://www.ijbs.com/v17p0178.htm

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression dataus-ing real-time quantitative PCR and the 2 (−Delta Delta C (T)) method. Methods 25(4):402–408

Luca T, Napoli E, Privitera G, Musso N, Ruberto G, Castorina S (2020) Antiproliferative effect and cell cycle alterations induced by *Salvia officinalis* essential oil and its three main components in human colon cancer cell lines. Chem Biodivers 17(8):e2000309. https://doi.org/10.1002/cbdv.202000309

Marineli RS, Moraes EA, Lenquiste SA, Godoy AT, Eberlin MN, Marostica Junior MR (2014) Chemical characterization and antioxidant potential of Chilean chia seeds and oil (*Salvia hispanica* L.). LWT Food Sci Technol 59:1304–1310. https://doi.org/10.1016/j.lwt.2014.04.014

Mercogliano MF, Bruni S, Elizalde PV, Schillaci R (2020) Tumor necrosis factor α blockade: an opportunity to tackle breast cancer. Front Oncol 10:584. https://doi.org/10.3389/fonc.2020.00584

Mohamed AD, Fouda AK, Mohamed SR (2019) In vitro anticancer activity of quinoa and safflower seeds and their preventive effects on non-alcoholic fatty liver. Pak J Biol Sci 22:383–392. https://doi.org/10.3923/pjbs.2019.383.392

Mohammed GM, Basuny AM (2020) Chia seed oil a new source of omega-3. Plant Arch 20:2678–2683

Mollaei H, Karimi F, Ghorbany M, Hosseinzadeh MS, Moudi M, Mousavi-Kouhi SM (2021) Investigating cytotoxic effect and molecular mechanisms of quinoa seed extract against human lung cancer cell line. Jentashapir J Cellul Mol Biol 12(4):121089. https://doi.org/10.5812/jjcmb.121089

Muhammad N, Usmani D, Tarique M, Naz H, Ashraf M, Raliya R, Tabrez S, Zughaibi TA, Alsaieedi A, Hakeem IJ, Suhail M (2022) The role of natural products and their multitargeted approach to treat solid cancer. Cells 11(14):2209. https://doi.org/10.3390/cells11142209

Mutar HA, Alsadooni JFK (2019) Antioxidant and anti-cancer activity of Chia seed extract in breast cancer cell line. Health Environ Res Online 22(8):220818. https://doi.org/10.36295/ASRO.2019.220818
Nedeljkovic M, Damjanovic A (2019) Mechanisms of chemotherapy resistance in triple-negative breast cancer—how we can rise to the challenge. Cells 8:957. https://doi.org/10.3390/cells8090957
Ng CY, Wang M (2021) The functional ingredients of quinoa (*Chenopodium quinoa*) and physiological effects of consuming quinoa: a review. Food Front 2(3):329–356. https://doi.org/10.1002/fft2.109
Palaniyandi T, Sivaji A, Thiruganasambandam R, Natarajan S, Hari R (2018) In vitro antigastric cancer activity of squalene, a triterpenoid compound isolated from Rhizophora Mucronata mangrove plant leaves against AGS cell line. Phcog Mag 14:S369–S376
Pani S, Mohapatra S, Sahoo A, Baral B, Debata PR (2022) Shifting of cell cycle arrest from the S-phase to G2/M phase and downregulation of EGFR expression by phytochemical combinations in HeLa cervical cancer cells. J Biochem Mol Toxicol 36(1):e22947. https://doi.org/10.1002/jbt.22947
Pasko P, Tyszka-Czochara M, Namiesnik J, Jastrzebski Z, Leontowicz H, Drzewiecki J, Martinez-Ayala AL, Nemirovski A, Barasch D, Gorinstein S (2019) Cytotoxic, antioxidant and binding properties of polyphenols from the selected glutenfree pseudo cereals and their byproducts: in vitro model. J Cereal Sci 87:325–333
Pellegrini M, Lucas-Gonzalez R, Ricci A, Fontecha J, Fernandez-Lopez J, Perez-Alvarez JA, Viuda-Martos M (2018a) Chemical, fatty acid, polyphenolic profile, techno-functional and antioxidant properties of flours obtained from quinoa (*Chenopodium quinoa* Willd) seeds. Ind Crops Prod 111:38–46. https://doi.org/10.1016/j.indcrop.2017.10.006
Pellegrini M, Lucas-Gonzalez R, Sayas-Barbera E, Fernandez-Lopez J, Perez-Alvarez JA, Viuda-Martos M (2018b) Bioaccessibility of phenolic compounds and antioxidant capacity of chia seeds. Plant Foods Hum Nutr 73:47–53. https://doi.org/10.1007/s11130-017-0649-7
Pereira E, Encina-Zelada C, Barros L, Gonzales-Barron U, Cadavez V, Ferreira ICFR (2019) Chemical and nutritional characterization of *Chenopodium quinoa* Willd (quinoa) grains: a good alternative to nutritious food. Food Chem 280:110–114. https://doi.org/10.1016/j.foodchem.2018.12.068
Rana S, Dhiman U, Parihar RD, Upadhyay SK (2021) In silico analysis of tumour necrosis factor (TNF) in Homo sapiens. Biointerface Res Appl Chem 12(1):781–794
Sanchez-Quesada C, Gutiérrez-Santiago F, Rodríguez-García C, Gaforio JJ (2022) Synergistic effect of squalene and hydroxytyrosol on highly invasive MDA-MB-231 breast cancer cells. Nutrients 14(2):255. https://doi.org/10.3390/nu14020255
Saxena S, Shahani L, Bhatnagar P (2017) Hepatoprotective effect of *Chenopodium quinoa* seed against CCL4-induced liver toxicity in Swiss albino male mice. Asian J Pharmaceut Clin Res 10(11):273–276. https://doi.org/10.22159/ajpcr.2017.v10i11.20918
Schaub FX, Dhankani V, Berger AC et al (2018) Pan-cancer alterations of the MYC oncogene and its proximal network across the cancer genome atlas. Cell Syst 6(3):282-300.e2. https://doi.org/10.1016/j.cels.2018.03.003
Shaer N, Al-Abbas N (2022) Potential effect of chia seeds crude extract nanoparticles on Mcf-7 breast cancer cell line. Al-Azhar Int Med J 3(2):123–127. https://doi.org/10.21608/aimj.2022.97818.1585
Tavakoli J, Hajpour Soq K, Yousefi A, Estakhr P, Dalvi M, Mousavi Khaneghah A (2019) Antioxidant activity of Pistacia atlantica var mutica kernel oil and it's unsaponifiable matters. J Food Sci Technol 56(12):5336–5345. https://doi.org/10.1007/s13197-019-04004-0
Vasan N, Baselga J, Hyman DM (2019) A view on drug resistance in cancer. Nat Cell Biol 575:299–309. https://doi.org/10.1038/s41586-019-1730-1
Venetis K, Sajjadi E, Haricharan S, Fusco N (2020) Mismatch repair testing in breast cancer: the path to tumor-specific immuno-oncology biomarkers. Transl Cancer Res 9:1852. https://doi.org/10.21037/tcr-20-1852
Wang M, Liu K, Zhou XL, Mei SY, Zhang CJ, Zhang TG (2017) Downregulation of KIF20A induces cell cycle arrest and apoptosis by suppressing PI3K/AKT in human glioblastoma. Int J Clin Exp Med 10(12):16133–16143
Zhang Y, Cai R, Zhou R, Li Y, Liu L (2016) Tousled-like kinase mediated a new type of cell death pathway in Drosophila. Cell Death Differ 23(1):146–157. https://doi.org/10.1038/cdd.2015.77

Publisher's Note