Antitumor, antioxidant, and anti-inflammatory activity of spirulina against 7,12-dimethylbenzanthracene-induced mammary cancer

Soha M. Hamdy[a], Asmaa A. Mostafa[b], Doaa G. El-Sahra[c], Marwa H. El-Azma[d], Khaled G. Abdel-Wahhab[d]

[a]Department of Chemistry, Faculty of Science, Fayoum University, Fayoum, [b]Nematology and Biotechnology Research Lab, Faculty of Agriculture, Fayoum University, Fayoum, [c]Medical surgical department, Faculty of nursing, Modern University for Technology and Information, Cairo, [d]Department of Medical Physiology, National Research Centre, Giza, Egypt

Correspondence to Khaled G. Abdel-Wahhab, PhD, Department of Medical Physiology, National Research Centre (60014618), Dokki, Giza 12622, Egypt.
E-mail: kgm194@gmail.com, kgm194@yahoo.com

Received: 14 November 2023
Revised: 9 December 2023
Accepted: 11 December 2023
Published: 15 July 2024

Egyptian Pharmaceutical Journal 2024, 23:459–471

Background
Breast cancer is the most abundant malignancies worldwide; however, its current therapies encounter drug resistance or exhibit numerous side effects. Marine and freshwater algal biomasses, such as spirulina, are rich with many biological active components.
Objective
The main objective of the current study was to investigate the therapeutic, antioxidant, and immune-modulating efficiency of spirulina on breast tumor modelled female rats, especially through the inhibition of the phosphoinositide 3-kinases/Akt/mammalian target of rapamycin pathway.
Materials and methods
7,12-dimethylbenzanthracene (DMBA)-induced mammary cancer rats were ingested with spirulina (500 mg/kg/day) for 6 weeks, then blood and tissue samples of normal and spirulina-treated cancer rats were obtained and tested for biochemical, immunological, and histopathological assessments.
Cancer model is used in this experiment.
Results
The results showed that spirulina is rich in phenolic compounds that have high scavenger activity and reducing power reflecting the antioxidant potential of spirulina. Treatment of DMBA-induced mammary cancer rats with spirulina resulted in improvement in mammary oxidative stress status that was distorted due to DMBA administration; meanwhile, superoxide dismutase, glutathione peroxidase, and reduced glutathione values were elevated significantly coupled with a marked drop in nitric oxide and malondialdehyde levels. In addition, spirulina boosts the immune-modulating response against tumor as the serum proinflammatory cytokines (tumor necrosis factor alpha, interlukin-1 beta, and interlukin-6) were markedly downregulated, and associated with inhibition of Akt and mammalian target of rapamycin pathway; this in turn suppress the tumor proliferation and progression. Furthermore, the prognosis of the treatment was indicated by the clear reduction of serum cancer antigen 15.3 level accompanied by elevation in serum level of the apoptotic biomarkers (caspase-3 and CD4) inferring the upregulation of tumor suppressor genes. Similarly, spirulina ameliorated lipid profile and the biochemical markers of hepatorenal functions (alanine transaminase, aspartate transaminase, urea, and creatinine) that were disturbed by DMBA; therefore, it has a positive impact on the body health. These biochemical improvements were associated with a notable improvement in the histological architecture of the mammary tissue.
Conclusion
In conclusion, spirulina has proved considerable antitumor, antioxidant, and anti-inflammatory activities against DMBA-induced mammary cancer.

Keywords:
Cancer antigen 15.3, cancer, 7,12-dimethylbenzanthracene, inflammation, mammalian target of rapamycin, spirulina

Egypt Pharmaceut J 23:459–471

1687-4315

Introduction

Breast cancer (BC) is the most abundant malignancies worldwide and leads to death. The breast is the most prevalent site affected by cancer. Among Egyptians, a ratio 32% for women and 15.4% for men; these ratios are estimated to be triple by 2050 [1,2]. BC is invasive tumor that has three subtypes characterized by the presence or absence of the expression of either estrogen or progesterone hormone receptor and epidermal growth factor 2 (ERBB2): hormone receptor positive and ERBB2 negative (nearly 70%

of the cases), hormone receptor negative or positive and ERBB2 positive (15–20% of the cases), and triple-negative BC (15% of the cases) [3].

For screening and diagnosis, mammography is conducted, then followed by an ultrasound examination, and at last biopsy for the suspected patients. According to the histopathological examination of the specimens, there are three types of breast carcinoma around 50–75% ductal carcinoma, 5–15% lobular carcinoma, and the least common type is mixed type [4].

The treatment of this disease requires mastectomy in 80% of the cases and followed by endocrine therapy for patients who were positive for the hormone receptor and the treatment may also include chemotherapy and/or radiation depending on the subtype of BC. Although the regular drugs are efficient in diminishing the tumor growth, but they may trigger themselves the traumatizing necrosis causing the tumor recurrence and resistance to the treatment [2,5].

The process of carcinogenesis is a sequential process that involves initiation, promotion, and progression stages. During the whole process, the oxidative stress and inflammation are key players. The free radicals, produced at the initiation process, cause the mutation, the progression stages characterized with the proliferation and uncontrolled growth of the malignancy, and the progression stages involves the invasion, resistance to chemotherapy, and metastasis [6].

Rodent's BC models are the best fit to mimic the pathophysiology of the breast carcinoma. Some chemicals were used to accelerate the tumorigenesis in these models such as 7,12-dimethylbenzanthracene (DMBA) or N-methyl-N-nitrosourea. DMBA-induced mammary cancer models can be utilized to study the carcinoma progression and to anticipate the efficiency and safety of the new potential therapies. It acts as immunosuppressive substance causing the elevation of the inflammatory biomarkers [e.g. tumor necrosis factor alpha (TNF-α)] and consequently the long-term inflammation exerts oxidative stress causing the damage of DNA and promotion of the carcinogenesis [7].

There are several anticancer therapeutics available in the market, for instance, trastuzumab, palbociclib, abemaciclib, and neratinib. They are usually alone or in combination especially with trastuzumab [3]. The natural resources enrich drug industry with effective potential therapies for breast carcinoma as some substances with anticancer activity are extracted from living organisms such as plants (e.g. etoposide, irinotecan, and paclitaxel), and microbes (e.g. mitomycin C and bleomycin). The current therapies are costly, and some patients encounter drug resistance or suffer from numerous side effects. Therefore, there are always a never-ending need to discover new drugs. More efforts were done during the past decades to found new therapies then they are extensively tested for their efficiency, safety, and mechanism of action using cell lines, and animal models before preclinical studies [8].

Marine and freshwater algal biomasses are rich with many biological active components such as carotenoids, polyphenol, fatty acids, and chlorophyll that possess antioxidant, anti-inflammatory, and antitumor activities [9]. *Spirulina sp.* (spirulina), a filamentous blue-green alga from genus cyanobacteria, it represents one-third of the total amount of microalgae worldwide. *Spirulina platensis* is the most common species among spirulina. It is used as a nutraceutical as it has high nutritional value due to its elevated protein content nearly around 60%, moreover it has many bioactive ingredients such as phenolic phytochemicals, polysaccharides, vitamins, minerals, and phycocyanins that help in amelioration of many diseases through exerting antioxidant, anti-inflammatory, antihypertensive, and antidiabetic functions [10].

The antioxidant activity of the spirulina was proved by the alleviation of the hepatotoxicity caused by the administration of carbon tetrachloride in rats [11]. Another study reported that spirulina attenuated the oxidative stress and the inflammation resulted from monosodium glutamate-induced hepatic dysfunction in rats [12]. It was reported that 8 weeks supplementation of spirulina was successful in alleviating oxidative stress, muscle damage, and inflammation produced due to strength training in experimental rats [13]. Another study confirmed these results and suggested a mechanism of action that the spirulina affect the phosphoinositide 3-kinases (PI3K)/Akt/mammalian target of rapamycin (mTOR)/p70S6k pathway in rat groups with resistance training [14]. Spirulina chemopreventive and antiproliferative properties was detected against rat C6 and human U87 glioblastoma cell lines through the up-regulation of miR-34a/miR-125B expression and the suppression of PI3K/Akt/mTOR pathway [15]. The PI3K/Akt/mTOR pathway is intracellular pathway within the cell that modulates the cell cycle,

proliferation, and it is over activation is detected in many types of cancers. PI3K are enzymes modulating the phosphorylation of Akt. Another key player is a serine/threonine protein kinase which is called mTOR that is triggered by Akt. It is called so because the rapamycin was the initial inhibitor to mTOR, and it was used as immunosuppressant in organ transplantation operations. Furthermore, it is implicated in the treatment of renal cell carcinoma after some modification to its pharmacological properties. Akt molecule is agonist to estrogen receptor leading to endocrine resistance to chemotherapy. Many preclinical studies target the downregulation of the PI3K/Akt/mTOR pathway to overcome this dilemma [16]. Therefore, the purpose of the present study was to investigate the therapeutic, antioxidant, and immune-modulating efficiency of spirulina on breast tumor modelled female rats especially through the inhibition of the PI3K/Akt/mTOR pathway.

Materials and methods

Chemicals

DMBA was purchased from Sigma Aldrich (St Louis, Missouri, USA). Dry microalgae spirulina (*S. platensis*, belonging to *Cyanophyta*) used in the current study was obtained from Algal Biotechnology Unit, National Research Center, Giza, Egypt.

In-vitro measurements

Total phenolic content

The total phenolic content of Spirulina was analyzed spectrophotometrically by the modified Folin–Ciocalteu colorimetric method of Jayaprakasha and Rao [17]. Estimation of total phenolic content as catechin equivalents (CE) was carried out using catechin standard curve.

Radical scavenging activity

The capacity of Spirulina antioxidants to quench DPPH radical was determined using the method of Nogala-Kalucka *et al.* [18]; the radical scavenging activity of the extract was calculated according to the following equation:

$$\text{RSA\%} = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control}\ x100$$

Reducing power

The ferric-reducing power of Spirulina was determined according to the method described by Sethiya *et al.* [19]. The reducing power of the extract was calculated as equivalent to ascorbic acid from the standard curve of ascorbic acid

Animals

This study was performed using adult female Wistar albino rats (160–200 g) purchased from Animal Colony, National Research Centre, Cairo, Egypt. The animals were maintained in suitable plastic cages 1 week for acclimation. Tap water and standard rodent pellets were always available. All animals were handled according to the standard criteria for the care and use of laboratory animals, and the protocols were revised and approved by the Institutional Ethical Committee of A-Azhar University with number (AZHAR 7/2021 Feb. 2021).

Induction of breast cancer

Mammary gland tumor was initiated as described by Anwar *et al.* [20] by a single dose (20 mg) of DMBA (obtained from Sigma Aldrich).

Experimental design

After induction of BC, both normal and BC-modelled rats were divided randomly in four groups (10 rats/group) as follows: (a) normal healthy rats were administrated orally with 2 ml distilled water and served as control; (b) normal rats orally ingested with spirulina (500 mg/kg/day) dissolved in distilled water (13); (c) untreated BC-modelled rats; (d) BC-modelled rats treated with spirulina (same dose). All animals were administrated for 6 weeks.

Body weight gain

The rats of each group were weighed at start and end of the experimental period, then the body weight gain was calculated according to the formula:

$$\text{BWG (g/100g b.w)} = [\ \frac{\text{End Body Weight} - \text{StarBody weight}}{\text{Start Body Weight}} \text{x100}\ ]$$

Blood and tissue sampling

At the end of the experimental period, rats were fasted overnight. Under anesthesia, blood samples were withdrawn and cool-centrifuged at 3000 rpm. The clear supernatants (sera) were separated, divided into aliquots, and stored at −80°C till biochemical and biomarkers measurements could be conducted as fast as possible. After blood collection, the animals were sacrificed soon by sudden decapitation; then the mammary glands of each animals group were dissected out; one part was washed in saline, dried, rolled in pieces of plastic sheets and stored at −80°C for determination of either oxidative status or mTOR-related markers, another part was soaked in formalin-saline (10%) buffer for histopathological processing and microscopic examination.

Determination of tumor, immune, and apoptotic markers

Using ELISA instrument (Dynatech Microplate Reader Model MR 5000), CA15.3, TNF-α, interlukin-1 beta (IL-1β), interlukin-6 (IL-6), caspase-3, and CD4 levels were assessed using rats' reagent ELISA-kits bought from SinoGeneClon Biotech Co. (Hang Zhou, China).

Biochemical determinations

Serum values of glucose, urea, creatinine, aspartate transaminase, alanine transaminase, gamma-glutamyl transferase (GGT), albumin, protein, total cholesterol, triglycerides, low-density lipoprotein (LDL)-cholesterol, and high-density lipoprotein (HDL)-cholesterol were evaluated spectrophotometrically using reagent kits obtained from Biodiagnostic (Giza, Egypt).

Assessment of oxidative stress markers

Specimens of mammary tissues were ultrasonically homogenized in ice-cold phosphate buffer (50 mM, pH 7.4) to give 10% homogenate (w/v); the homogenate was cool-centrifuged at 5000 rpm for 20 min to remove the nuclear and mitochondrial fractions; the supernatant was stored at −80°C till its use in the measurements of oxidative stress markers. Mammary reduced glutathione (GSH) and nitric oxide (NO) levels along with superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were assessed spectrophotometrically using reagent kits obtained from Biodiagnostic; however, lipid peroxidation product, [malondialdehyde (MDA)], level was determined chemically as described by Ruiz-Larnea *et al.* [21].

Assessment of mTOR in mammary tissues

The mTOR pathway (p-Akt and mTOR) was measured in the breast tissues of all groups after their lysing in RIPA lysis buffer; however, p-Akt and mTOR level and the total protein concentration were estimated according to the method of Bradford [22] using ELISA reagent kits purchased from RayBiotech Inc. (USA).

Histopathology

Using the formalin-saline immersed mammary tissues, paraffin sections (5 μm thick) were prepared and stained with hematoxylin and eosin [23] and examined by a light microscope.

Statistical analysis

The resulted data were subjected to one way analysis of variance then Duncan or Tukey multiple post-hoc tests were executed at level of *P* value less than or equal to 0.05 according to Steel and Torrie [24] using a statistical analysis system (SAS) program software; copyright (c) 1998 by SAS Institute Inc. (Cary, North Carolina, USA).

Results

In-vitro study

The obtained results revealed that spirulina has a considerable amount of total phenolic content and exhibited valuable radical scavenging activity and reducing power (Fig. 1).

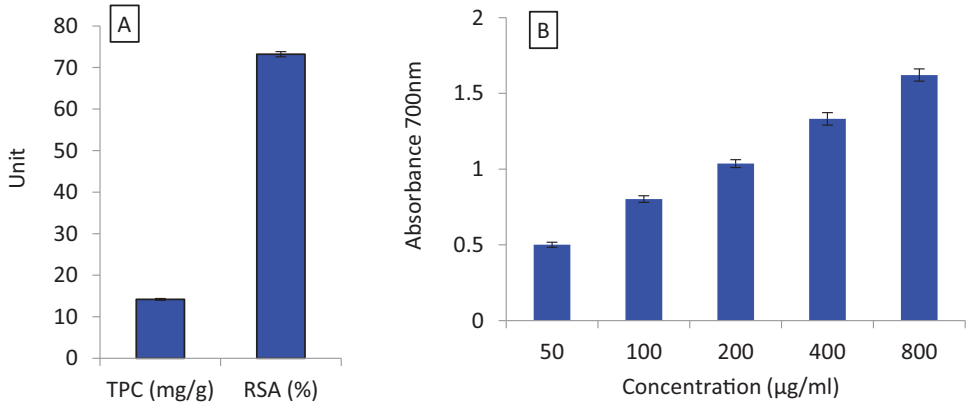
In-vivo study

The animals' study showed important findings; BC-modelled animals showed a marked reduction in the rate of gained body weight which was restored significantly posttreating the modelled animals with spirulina (Fig. 2).

Tumor and apoptotic markers

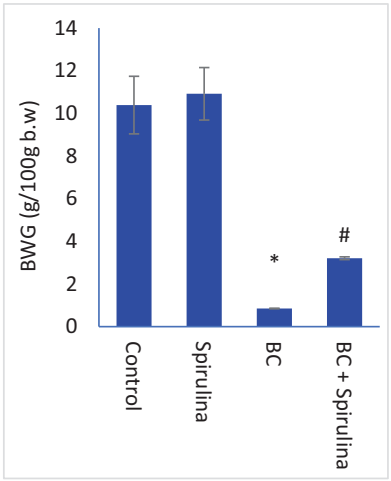
In comparison with control group, BC modelled rats showed a marked elevation in the serum level of tumor marker [cancer antigen 15.3 (CA 15.3)] coupled with

Figure 1



(a) Total phenolic content and radical scavenging activity of spirulina; and (b) reducing power results of spirulina.

Figure 2



Effect of spirulina on body weight gain (BWG) of breast cancer (BC) modelled rats. *Significant versus control group and #significant versus BC group at *P* value less than or equal to 0.05.

sharp reduction in the serum values of apoptotic markers (caspase-3 and CD4). Favorably, treatment of BC-modelled rats with spirulina resulted in a marked anti-tumor effect that was achieved though the decrement in CA 15.3 level that associated with increment in caspase-3 and CD4 values in compare to untreated BC-modelled animals' group (Fig. 3).

Immune-inflammatory cytokines

DMB-induced BC resulted in significant elevation in the immune-inflammatory cytokines (IL-1β, IL-6, and TNF-α) in compare to normal control group. In contrast and in compare to untreated BC group, treatment of BC-rats with spirulina performed a marked anti-inflammatory effect that was monitored from the detectable reduction in the mentioned cytokines (Fig. 4).

Lipid profile

A detectable elevation in serum total cholesterol, triglycerides, and LDL-cholesterol levels alongside with a marked drop in HDL-cholesterol level was noticed in BC animals when compared with normal animals; while a valuable improvement in the level of lipid profile indices was determined after treating BC rats with spirulina in when compared with the untreated ones (Table 1).

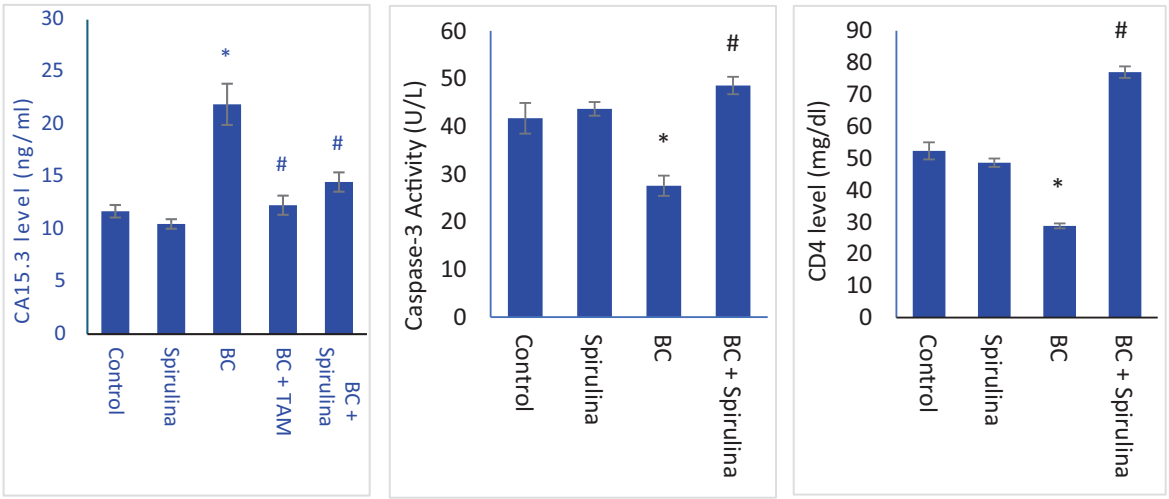
Liver and kidney functions

Regarding serum biochemical markers of hepatorenal function, the BC rats showed a marked increase in hepatic function markers (activities alanine transaminase, aspartate transaminase, alkaline phosphate, and GGT activities, and total proteins and albumin levels) and renal markers (urea and creatinine) in compare to control group. In a promising manner and regarding untreated BC rats, spirulina-treated cancerous rats exhibited hepatorenal ameliorative efficiency that achieved from the significant improvement in the mentioned biochemical markers of hepatorenal functions (Table 2).

Mammary oxidative stress markers

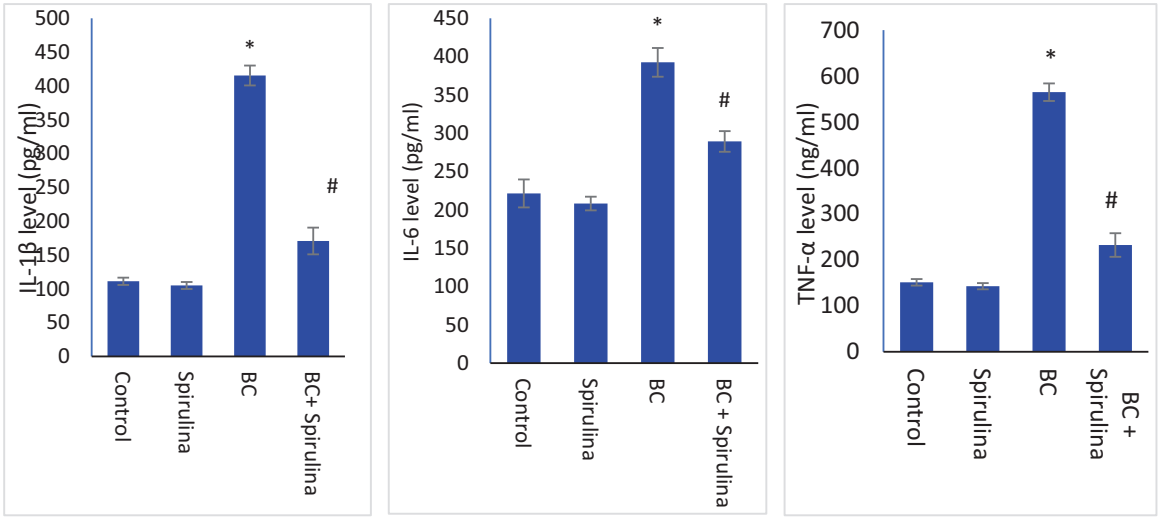
The current study revealed that cancerous rats showed a significant elevation in the mammary level of oxidative markers (MDA and NO) associated with a severe drop in the values of antioxidant battery (SOD, GPx, and GSH) in compare to control group reflecting the strengthen oxidative stress occurred in the mammary tissue. Favorably and in compare to untreated BC

Figure 3



Effect of spirulina on tumor and apoptotic markers of breast cancer (BC) modelled rats. *Significant versus control group and #significant versus BC group at *P* value less than or equal to 0.05.

Figure 4



Effect of spirulina on serum immune-inflammatory cytokines levels of breast cancer (BC) modelled rats. *Significant versus control group and #significant versus BC group at *P* value less than or equal to 0.05.

group, treatment of BC-rats with spirulina succeeded to improve the mentioned oxidative stress markers towards the corresponding values of control group; this reflects spirulina's antioxidant effect (Table 3).

Effect of spirulina on breast cancer invasion

The obtained results showed that treatment of BC-modelled rats with spirulina efficiently reduced the expression of both mTOR and p-Akt (these were greatly expressed in cancerous rats) in their mammary tissues, as the relative protein expression levels of mTOR and p-Akt were markedly inhibited (Fig. 5).

Histopathological findings

The histo-pathological findings of the current study are illustrated in (Figs. 6–9) and explained with footnote below each figure.

Table 1 Effect of *Spirulina* on serum levels of lipid profile of breast cancer modelled rats

	Groups			
Parameters	Control	Spirulina	BC	BC+spirulina
Cholesterol (mg/dl)	100±2.14	94.5±3.26	246±4.67*	167±6.8#
Triglycerides (mg/dl)	71.8±3.93	73.5±2.54	141±7.5*	108±6.9#
LDL-C (mg/dl)	72.5±1.54	69.3±3.2	101±7.59*	86.2±5.3#
HDL-C (mg/dl)	43.9±1.59	41.9±2.77	29.9±1.6*	35.6±1.27#

Data are presented as mean±SEM and subjected to one-way analysis of variance followed by post hoc (Duncan) test at *P* value less than or equal to 0.05. BC, breast cancer; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol. Within the same row, *significant versus control group, and #significant versus BC group.

Table 2 Effect of spirulina on the liver and kidney functions on breast cancer-modelled animals

	Groups			
Parameters	Control	Spirulina	BC	BC+spirulina
ALAT (U/l)	54.2±3.4	52.6±2.79	123±6.2*	67.6±1.89#
ASAT (U/l)	172±6.66	167±9.7	274±8.2*	189±8.5#
ALP (U/l)	214±9.74	208±10.1	324±15.4*	225±8.7#
GGT (U/l)	9.58±0.36	9.37±0.38	17.8±0.42*	12.2±0.52#
Protein (g/dl)	6.36±0.23	6.7±0.28	4.2±0.23*	5.6±0.16#
Albumin (g/dl)	4.59±0.25	4.82±0.31	3.22±0.15*	4.16±0.85#
Urea (mg/dl)	56.4±4.9	54.8±2.5	76.6±3.6*	63.8±2.12#
Creatinine (mg/dl)	0.92±0.05	0.89±0.066	1.33±0.04*	1.15±0.03#

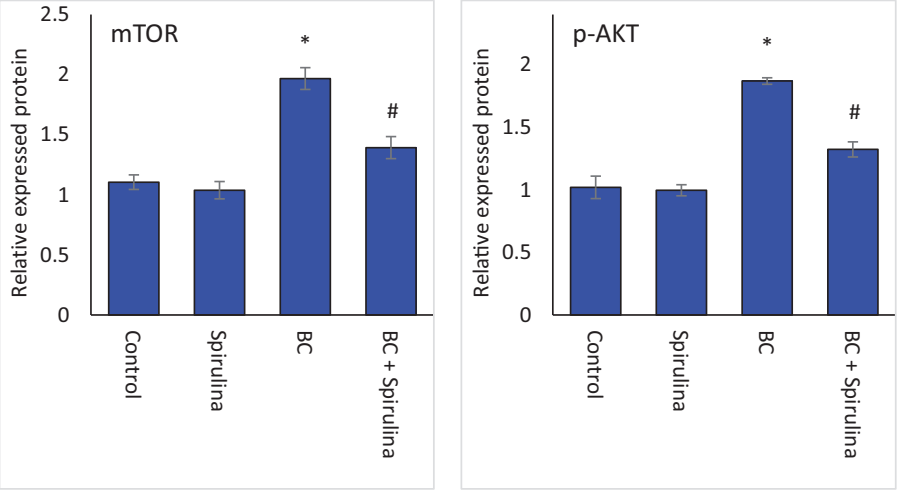
Data are presented as mean±SEM and subjected to one-way analysis of variance followed by post hoc (Duncan) test at *P* value less than or equal to 0.05. ALAT, alanine transaminase; ALP, alkaline phosphate; ASAT, aspartate transaminase; BC, (breast cancer; GGT, gamma-glutamyl transferase. Within the same row, *significant versus control group and #significant versus BC group.

Table 3 Effect of spirulina on mammary oxidative stress markers of breast cancer modelled animals

	Groups			
Parameters	Control	Spirulina	BC	BC+spirulina
MDA (nmol/g tissue)	138±3.32	131.5±5.93	367±25.6*	194±7.4#
NO (μmol/g tissue)	1182±37	1128±83	1805±68*	1359±31.6#
SOD (U/g tissue)	2150±41	2147±32	1530±58*	1845±47#
GPx (U/g tissue)	223±12.95	226±13.5	146±7.7*	190.5±2.8#
GSH (mmol/g tissue)	26.5±1.2	29.4±1.99	17.2±0.53*	23.7±0.99#

Data are presented as mean±SEM and subjected to one-way analysis of variance followed by post hoc (Duncan) test at *P* value less than or equal to 0.05. BC, breast cancer; GPx, glutathione peroxidase; GSH, reduced glutathione; MDA, malondialdehyde; NO, nitric oxide; SOD, superoxide dismutase. Within the same row, *significant versus control group, and #significant versus BC group.

Figure 5



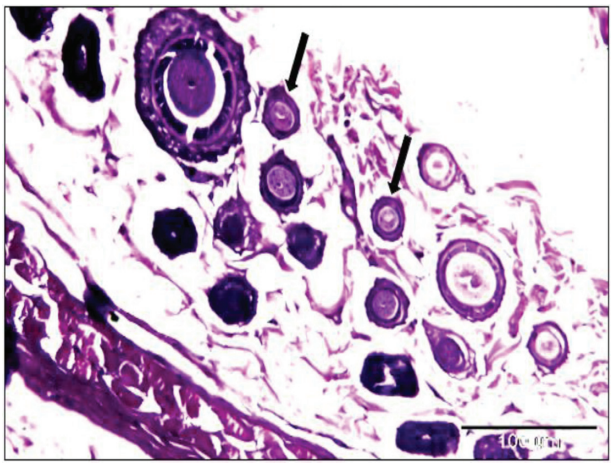
Effect of spirulina on mTOR and p-Akt relative expression levels in the breast tissue of breast cancer (BC) modelled rats. *Significant versus control group and #significant versus BC group at *P* value less than or equal to 0.05.

Discussion

BC is the most common invasive cancer in women worldwide. Unfortunately, chemotherapeutic drugs performing many adverse effects [25]. Natural products provide a vast resource of bioactive compounds with therapeutic benefits. Meanwhile, â 25% of all recently authorized anticancer drugs were derived from natural resources [26]. Spirulina is greatly utilized for the treatment of various diseases; its medical attributes have been reported to include anti-inflammatory, antimicrobial, antidiabetic, anticancer, antihistaminic, and hypotensive effects [27]; therefore, the purpose of the present study was to investigate the therapeutic, antioxidant, and immune-modulating efficiency of spirulina on breast tumor modelled female rats.

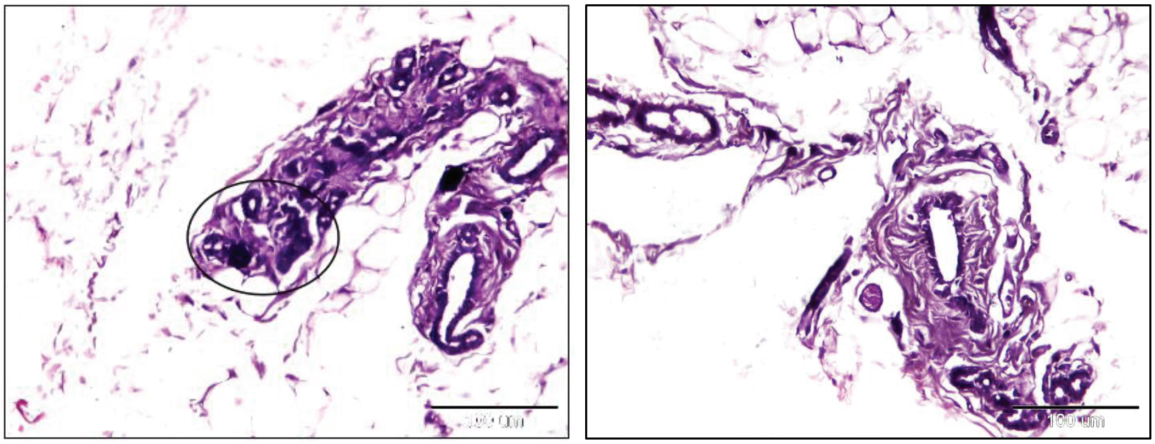
DMBA is a carcinogenic agent broadly utilized to induce estrogenic-dependent cancer model in rodents; however, the liver is the primary organ normally deals with such xenobiotic compound.

Figure 6



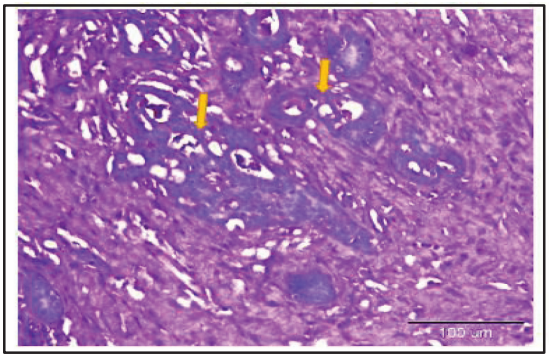
A photomicrograph of a section in the mammary tissue of normal female albino rats (control group). Examination of this group showed a dense collagenous tissue, blood vessels, and islands of glandular tissue surrounded by dense fibrous and adipose tissue. The breast acini and tubules (arrows) are lined by inner epithelial layer (with dense cytoplasm) and outer myoepithelial layer (with clearer cytoplasm) (hematoxylin and eosin, ×200).

Figure 7

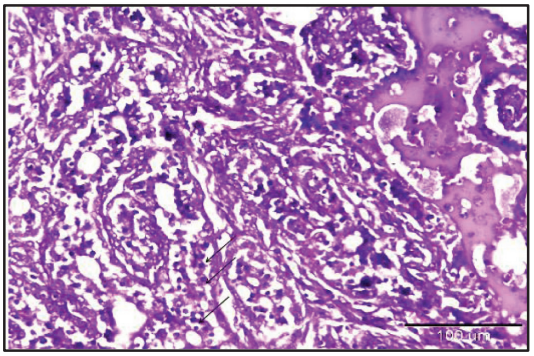


A photomicrograph of a section in the mammary tissue of healthy female albino rats administrated with spirulina (*Spirulina platensis*) at a dose of (500 mg/kg/day) for 4 weeks (group 2). Similarly, normal distribution of the mammary glands in the dermis and normal duct structure could be seen (black circle) (hematoxylin and eosin, ×200).

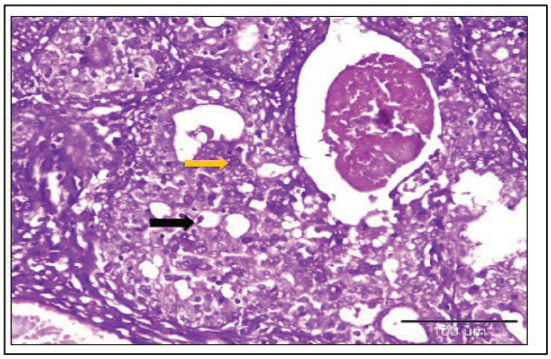
Figure 8



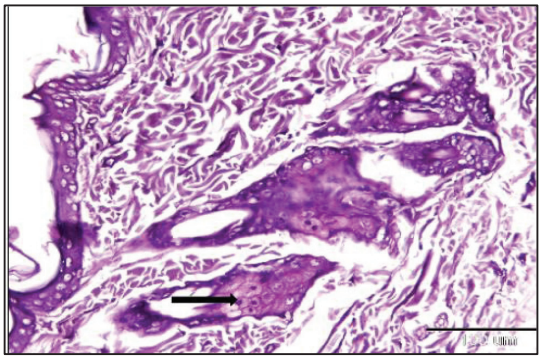
Invasive breast carcinoma. They can grow in tubules with distinct lumina (ductal differentiation), trabecularly, or solidly with nests or cords surrounded by stromal desmoplasia (yellow arrows) (H&E, x400).



Distinct neuroendocrine carcinoma was seen in this group. Malignant cells (arrows) (H&E, x400).



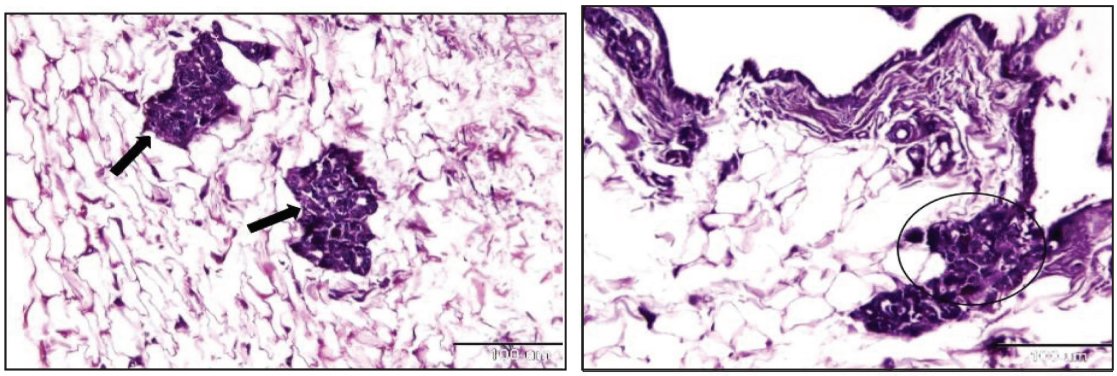
Invasive micropapillary breast carcinoma with morula-like tumor cell clusters without any fibrovascular core, surrounded by small sinus-like spaces could be seen. Mitotic figure (black arrow) and apoptotic figure (yellow arrow) on breast tumor tissues. (H&E, x400).



Sebaceous pattern of invasive breast carcinoma with vacuolated cytoplasm (black arrow) was seen in this group. (H&E, x400).

Photomicrographs of different sections of the mammary tissue of female albino rats with DMBA-induced breast cancer (group 3). The images show different malignancy findings as illustrated below each image.

Figure 9



Pleomorphic invasive lobular carcinoma was also detected (black arrow) (H&E, x200).

In the epidermis, a solid pattern of cell mass was detected (black circle) (H&E, x200).

Photomicrographs of different sections through the mammary tissue of female albino rats with DMBA-induced breast cancer treated with spirulina (group 4). The image shows considerable improvements as illustrated above; however, other malignant changes still appear when compared with DIMBA group.

Unfortunately, mammary glands also activate and deposit DMBA through cytochrome P450 enzymes, which transform DMBA into DMBA-3,4-diol-1,2-epoxide, and other active metabolites. The oxidation process of DMBA disrupts the redox balance, leading to the production of free radicals that can bind to hydrophilic sites on macromolecules within the cell through covalent bonds. Moreover, these end products can form adducts with DNA, initiating tumorigenesis in a manner mimics biochemically, immunologically, and histopathologically what happens in women [28,29]. SOD is the initial line of defence that eliminate oxyradicals (produced by DMBA) by transforming the superoxide anion to hydrogen peroxide; then the cytoplasmic GPx and the GSH detoxify the hydrogen peroxide at the expense of GSH [6]. NO is an inflammatory mediator and a highly reactive free radical that produces NO_2, $NO_2^{\bullet}$, N_2O_3, and $ONOO^-$ that in turn could initiate and promote cancer cells growth especially by the formation of peroxynitrite. The presence of oxyradicals and peroxynitrite causes lipid peroxidation along with the elevation of MDA. Moreover, NO is reduced due to its oxidation in the epithelial and immune cell producing nitrites [30]. Many studies reported the imbalance in the redox state during carcinogenesis and DMBA-induced mammary cancer [6,28,29,31]. Similar results were obtained in the current study showing a marked elevation in the oxidative markers (MDA and NO) alongside with a detectable reduction in the antioxidant bioagents (SOD, GPx, and GSH).

Proinflammatory cytokines such as TNF-α, IL-1β, and IL-6 play a pivotal role in angiogenesis and metastasis; their high concentrations are highly correlated with occurrence, invasiveness, and aggressiveness of mammary gland cancer. The main factor that initiates the proinflammatory and chemokines response is nuclear factor-kappa B which in turn controls the survival and the proliferation of the malignant cells [30]. This was in consistent with the present study findings as TNF-α, IL-1β, and IL-6 are increased significantly in the modelled BC animals group in comparison to those of control group. Moreover, results of modelled BC-group showed a detestable raise in the relative expressed proteins of both p-Akt and mTOR; this result agreed with that of Mathiyazhagan *et al.* [31]; activation of the mTOR pathway in DMBA-induced mammary cancer increases the tumor growth and inhibition of cell apoptosis [16].

Regarding apoptotic markers, caspase 3, a member of cysteine proteases family, has a proteolytic role in programmed cell death in response to intrinsic and/or extrinsic factor; its reduction results in inhibition of the apoptosis process, and subsequently leads to excessive proliferation and growth of the mammary cancer tumor. In addition to, CD4 cells play a crucial role in preventing tumor progression through their infiltration in the tumor and initiation of immune-response, therefore the drop in CD4 and caspase-3 are considered markers of the tumor progression [32] that has been occurred in the BC group that showed a marked decrease in the activity of caspase-3 and concentration of CD4.

Numerous oncologists use CA 15.3 test as a biomarker to fellow up the early stage of mammary cancer

especially in asymptomatic patients. Moreover, it is used in monitoring metastasis and recurrence of the disease after the treatment; however, they reported its increment in BC patients [29,33]; this report was in line with the elevation in CA 15.3 concentration in BC modelled animals' group when compared with its corresponding value of control group.

Dyslipidemia is an imbalance of the lipid profile in blood plasma, and it is correlated with BC. Cholesterol affects the pathophysiology of the mammary cancer with many mechanisms. Elevated cholesterol alters the fluidity of the cell membrane and the signaling pathways. Moreover, it is compulsory precursor of steroid hormones consequently, it is a key player of all types of steroid hormone-dependent tumors. 27-Hydroxycholesterol, an active cholesterol metabolite can act as modulator to liver X receptor and estrogenic receptor. Also, it modulates lipid raft synthesis through Akt pathway. Furthermore, the hedgehog-oncogenes pathway and TOR signaling are activated by oxysterols [34,35]. The results of this study detected dyslipidemia represented by elevation of the cholesterol, triglycerides, LDL-cholesterol, and reduction of HDL-cholesterol this agrees with Laskar *et al.* [35].

Liver and kidney are significant organs in dealing with noxious substances and chemotherapy. DMBA administration induces renal and liver damage [28,30]. This was in line with our study results which showed a significant elevation in both liver and renal tested functions and the decrease in total protein and albumin. GGT is an important enzyme which catalyzes gamma-glutamyl cycle, and the increased activity of this enzyme is correlated with BC patients. Furthermore, this enzyme is included in glutathione synthesis and its accumulation affects the efficiency of the chemotherapy or even develop drug resistance [36].

Due to the high cost of chemotherapy, drug resistance and the hazard effects on patient health, more efforts are made to discover new therapies for BC [8]. Spirulina was tested in this study for its ability to alleviate the various consequences of DMBA administration to the experimental rats.

The treatment of BC modelled animals with spirulina resulted in a marked amelioration in the oxidative stress status as the antioxidant markers (SOD, GPx, and GSH) increased significantly matched with a valuable reduction in the oxidative biomarker (NO and MDA). This improvement was in consistent with recent studies [12,13]. The valuable amounts of total phenolic content, radical scavenging activity, and the reducing power of spirulina, detected in the current study, could be the basic antioxidant power that counteracted the reactive oxygen species and free radicals produced due to the DMBA. Kepekçi *et al.* [11] reported that the phenolic compounds included in algae (spirulina) play an alternative pathway for phytochemical energy consumption and have high antioxidant and reducing power properties. Kumar *et al.* [37] stated that spirulina is rich in polyphenols and other components (tocopherols, carotenoids, and organic acids such as chlorogenic, synaptic, salicylic, and transcinnamic acids) that have the capacity to neutralize free radicals, triplet oxygen, proxygene breaking down, and inhibition of lipid peroxidation, and work synergistically as antioxidants barrier; this suggestion agreed with our *in vitro* findings. Additionally, Akhouri *et al.* [28] confirmed the enzymatic (GPx, CAT, and SOD), and nonenzymatic (vitamins C, E, and GSH) antioxidant activity of spirulina in both aqueous and ethanolic extracts.

AlThobaiti [12] reported that the proinflammatory cytokines (TNF-α, IL-1β, and IL-6) were significantly suppressed after administration of spirulina due to inhibition of COX2 and activation nuclear factor-kappa B; meanwhile, Brito *et al.* [13] noted that systemic inflammation was improved in rats, subjected to strength training, postspirulina ingestion; however, another study reported the anti-inflammatory role of spirulina [38].

PI3K/Akt/mTOR pathway is one of the pathways that modulated by reactive oxygen species. It is one of the targeted mechanisms for treating mammary cancer. Many reports suggested that spirulina supplementation has suppressed PI3K/Akt/mTOR pathway in resistance training murine models or in glioblastoma cell lines [14,15]. The downregulation of this pathway leads to decrease of the proliferation and growth of the malignant cells [16]. Caspase-3 was increased after administration of Fe_3O_4/Ag nanocomposite biosynthesized from spirulina extract to MCF-7 human BC cell line [39]. These reports were in consistent with the results of the BC+spirulina group which showed a down-regulation of p-Akt and mTOR relative expression proteins in comparison to BC group. Moreover, spirulina antitumor properties were reflected on the elevation of caspase-3 activity and CD4 concentration and the decrease in CA 15.3 concentration. These are apoptotic and tumor markers together they reflect the inhibition of the proliferation and prognosis of the treatment by spirulina.

The antitumor effect of spirulina extract enhanced with Braun-type lipoprotein was studied on mouse mammary carcinoma cell line (4T1) implanted in female BALB/c mice. That mixture induced apoptosis, reduced the spontaneous metastasis, and prolonged the survival duration of mice with breast tumors. In addition, it strengthened CD4 and CD8 infiltration in the tumor boosting with that the secondary immune response targeting malignant cells [40]. Ouhtit *et al.* [41] noted that the chemo-preventive ability of the spirulina both *in vivo* and *in vitro*. The Ki-67 and estrogen receptor alpha were inhibited this leads to inhibition of the proliferation through the downregulation of genes controlling cell growth p53 and p21. Furthermore, spirulina induces apoptosis p53-Bcl-2-Bax pathway. Spirulina rich with many effective ingredients that have antitumor properties. Selvakumar *et al.* [42] reviewed the role of polyphenol and flavonoid as epigenetic modifiers in mammary cancer. It was found that they could prevent the tumor growth, proliferation, and metastasis through epigenetic modifications such as up-regulation of histone acetylation and down-regulation of DNA methyltransferase, in addition to activation of tumor suppressor genes. Another review described the polyphenol role at the multistage process of mammary tumor initiation, promotion, and progression. In these stages polyphenols regulates and modulate the antioxidant mechanisms, reduce inflammation, and boost the autophagy process. The autophagy process was induced by polyphenols through different signaling pathways such as such PI3K/Akt, RAS/RAF/ERK [43]. Liao *et al.* [44] observed that phycocyanin induced the apoptosis in pancreatic cancer (PANC-1) cells through dysregulation PI3/Akt/mTOR signaling pathway. Phycocyanin is COX2 inhibitor and subsequently it enhances immune response against tumor [40].

The BC+spirulina group showed an improvement in lipid profile components (reduction of the cholesterol, triglycerides, LDL-cholesterol, and elevation of HDL-cholesterol). In addition, the elevation of serum protein and albumin in comparison to the BC group results this was in consistent with the previous findings that detected the amelioration of the lipid profile components in *Oreochromis niloticus* after spirulina uptake [38]. These results boost the treatment of the mammary cancer because the attenuation of normal levels from cholesterol restore the membrane fluidity and decrease the lipid rafting synthesis required for the proliferation of the malignant cells [34].

The main organs that eliminate the body from noxious and hazard compounds are liver and kidney. They also severely impacted by chemotherapy administration during mammary cancer treatment [28]. However, the spirulina supplementation able to alleviate liver toxicity induced by CCl_4 [11]. Awad *et al.* [38] stated that the spirulina uptake attenuated the liver and renal functions after *O. niloticus* infection with hydrophila. These previous results were in line with the current study findings as the spirulina administration to DMBA-induced mammary cancer modelled rats was successful in ameliorating the normal levels of renal and liver functions.

The BC group in the current study showed a significant reduction in body weight gain in comparison to control group this agrees with Silihe *et al.* [29]. However, the administration of the spirulina in BC+spirulina group elevated the weight gain this was in consistent with Zhou *et al.* [45]. This reflects the nutritional value of the spirulina as it is rich with protein, tannin, carbohydrate, fatty acids, minerals, and vitamins. Therefore, spirulina is considered as a nutraceutical and functional food [37].

Conclusion

Collectively, this study revealed that spirulina exhibited antimammary tumor efficacy, and the supposed mechanism of its role in suppression of DMBA-induced mammary cancer was through the downregulation of p-Akt/mTOR pathway; the included spirulina components such as phenolic compounds, flavonoids, phycocyanin and mineral vitamins boost the antioxidant capacity in the body via their high radical scavenging activity that stabilized or eliminate the free radicals, and boost the immune-response and infiltration of the immune cells. Therapeutic efficiency of spirulina was monitored from the improvement in the CA 15.3, oxidative stress status, lipid profile, and apoptotic biomarkers associated with the notable improvement in the histological architecture of the mammary tissue. On contrary to chemotherapy, spirulina has a good impact on the body, it increased the body weight, alleviated the renal and hepatotoxicity induced by DMBA administration.

Acknowledgments

The authors acknowledge Medical Physiology Department (National Research Centre), Nematology & Biotechnology Research Lab (Faculty of Agriculture), and Chemistry Department (Faculty of Science), Fayoum University for their participation and support throughout this study.

Data availability: The datasets obtained during the current study are available from the corresponding author on reasonable request.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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