Acidic Exopolysaccharide Produced from Marine *Bacillus amyloliquefaciens* 3MS 2017 for the Protection and Treatment of Breast Cancer

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ABSTRACT

PURPOSE: This study was planned to investigate the anti-breast-cancer property of acidic exopolysaccharide produced from marine *Bacillus amyloliquefaciens* 3MS 2017 (BAEPS) in an animal model, which previously showed in-vitro anti-breast-cancer activity, by studying its potential participation in various targeted mechanisms.

METHODS: Mammary carcinoma in female Sprague-Dawley rats, both in prophylactic and in curative designs, was chemically induced using 7,12-dimethylebenz-(a)-anthracene (DMBA). *B. amyloliquefaciens* 3MS 2017 anti-breast-cancer property was evaluated by studying its effects on cancer-growth-rate-limiting enzymes (aromatase and Na^+/K^+ ATPase), sexual hormones (estrogen and progesterone), antioxidant and inflammatory biomarkers (cyclooxygenase-1; COX-1 and cyclooxygenase-2; COX-2). The incidence of breast cancer by DMBA was dependent on the level of carcinoembryonic antigen (CEA) and aromatase.

RESULTS: 7,12-Dimethylebenz-(a)-anthracene female rats were characterized by a significant increase in cancer-related biomarkers with an increase of oxidative stress biomarkers, in comparison with the negative control. Potent BAEPS anticancer activity on DMBA rats was exhibited either as a prophylactic or as a curative agent, which appeared via restoring the aromatase and Na^+/K^+ ATPase subunits levels and CEA close to the normal level. Besides, BAEPS modulated a sexual hormone, in comparison with the cancer control group ($P \leqslant .05$). *B. amyloliquefaciens* 3MS 2017 selectively inhibited COX-2 in parallel with promising antioxidant properties. The curative characters of BAEPS were more promising than the prophylactic.

CONCLUSION: The anti-breast-cancer characters accompanied with a good safety margin may be attributed to its inhibitory effect on cancer-growth-rate-limiting enzymes, estrogen production, COX-2 level and lipid peroxidation, concurrent with enhancing COX-1 level, progesterone production, and antioxidant status.

KEYWORDS: Acidic exopolysaccharide, *Bacillus amyloliquefaciens* 3MS 2017, breast cancer, cancer-growth-rate-limiting enzymes, anti-inflammatory, antioxidant

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Introduction

The breasts are the mammary glands that secrete milk for breastfeeding.[1] Breast cancer occurs when normal breast cells transfer into malignant cells. A lump in the breast is the most common symptom of breast cancer.[2] Breast cancer is considered the main type of invasive-cancer prevalent among females[3] and acts about 22.9% of invasive cancers in women[4] and 16% of all female cancers.[5] The lowest incidence of breast cancer occurs in less-developed countries, and the greatest incidence recorded in the more-developed countries. However, the survival rate of breast cancer in more-developed countries is higher than that in less-developed countries (73% and 57%, respectively) regarding a health care.[6]

7,12-Dimethylbenz[a]anthracene (DMBA) polyaromatic hydrocarbon compound is a carcinogen material with estrogenic characteristics. 7,12-Dimethylbenz[a]anthracene is a procarcinogen that is metabolized by the cytochrome P450 and its carcinogenic metabolites. 7,12-Dimethylbenz[a]anthracene serves as a tumor initiator, and widely used as a laboratories cancer model to study cancer. 7,12-Dimethylbenz[a]anthracene is the main carcinogenic material used for the induction of mammary gland carcinogenesis in animals.[7]

Carcinoembryonic antigen (CEA) is a glycoprotein that is normally produced in gastrointestinal tissue during fetal development, but the production stops before birth. Consequently, CEA is usually present at very low levels in the blood of healthy adults (about 20 ng/mL). Carcinoembryonic antigen is important and is the most commonly expressed biological marker in breast cancer patients, and its level decreased after treatment. Increasing CEA is related to the extent of the disease, degree of differentiation of the tumor, and site of metastasis.[8]

There is a positive relation between tissue prostaglandin concentrations and human breast tumors. Prostaglandins are produced by cyclooxygenase (COX)-2 enzyme and occur with

high concentrations in various human breast cancer cell lines. It was confirmed that COX-2 is highly expressed in breast cancer cell lines and tumors.[8] Cyclooxygenase-2 over-expression leads to a low breast cancer prognosis and survival rate as well as its progression to invasive breast cancer.[9] Therefore, COX-2 inhibitors are considered promising targets for breast cancer therapy.[10]

Aromatase is considered a rate-limiting enzyme of estrogen biosynthesis via the aromatization of androgens to estrogens. It is expressed with a high amount in breast cancer cells leading to estrogen overproduction.[8,11] Therefore, aromatase inhibitors (AIs) can contribute to breast cancer therapy. The AIs are drugs that were at first used as antiepileptic and aminoglutethimide drugs. Richard Santen[12] was the first user of aminoglutethimide for breast cancer treatment in the 1970s. In addition, he illustrated that the aminoglutethimide inhibited aromatase activity, leading to decrease in estrogens production.

Another controlling factor is the sodium/potassium pump (Na^+/K^+-ATPase), which plays an important role in the maintenance of ionic homeostasis, pH, and volume of the cell.[13] Sodium/potassium pump is the key step in preserving a high extracellular Na^+ and a high intracellular K^+ by pumping Na^+ ions outside the cell concurrently with importing K^+ ions inside the cell.[14] The previous process plays an important role in the cell growth and activities. The Na^+/K^+ ATPase have been related to cancer cell motility and migration. Cancer cells express a large amount of Na^+/K^+-ATPase,[8,15] which may serve it as a biological cancer biomarker and a cancer therapeutic target. Inhibition of Na^+/K^+-ATPase by cardiac glycoside ouabain is considered cytotoxic to breast cancer.[16]

In our previous study[17] published in 2017, we produced the present targeted, acidic exopolysaccharide from marine *Bacillus amyloliquefaciens* 3MS 2017 (BAEPS) collected from Egyptian beaches. *B. amyloliquefaciens* 3MS 2017 contains uronic acid (12.3%) and sulfate (22.8%) with constitutions of glucose, galactose, and glucuronic acid in a molar ratio 1.6:1.0:0.9, respectively. *B. amyloliquefaciens* 3MS 2017 has a low molecular mass (3.76×104 g/mol). *B. amyloliquefaciens* 3MS 2017 exhibited strong antioxidant activities including free radical scavenging, reactive oxygen species (ROS; NO, H_2O_2, and O_2–) scavenging, and ferrous chelation capacity. *B. amyloliquefaciens* 3MS 2017 showed selective anti-inflammatory activity against COX-2. *B. amyloliquefaciens* 3MS 2017 proved a strong and selective effectiveness to breast cell cancer MCF7 with 65.20% death percentage and $IC_{50} = 70$ µg/mL and $IC_{90} = 127.40$ µg/mL. *B. amyloliquefaciens* 3MS 2017 suppressed the viability of Ehrlich Ascites Carcinoma tumor model and increased median survival time and life span.

Therefore, this study was carried out to accomplish our previous study through many investigations on anti-breast-cancer characters of BAEPS in chemically induced breast cancer in rats by evaluating its participation in many targeted cancer mechanisms as hormones, cancer cell growth promoter, inflammation, and antioxidant.

Methods

Chemicals

7,12-Dimethylbenz[a]anthracene purchased from Sigma Aldrich, USA. Ethylenediaminetetraacetate, sodium dihydrogen phosphate, and disodium monohydrogen phosphate were purchased from Fin Chem Ltd. Liver and kidney functions, lipid profile, and antioxidant parameters kits were purchased from Biodiagnostic, Egypt. Enzyme-linked immunosorbent assay (ELISA) kits for cyclooxygenases activity (COX-1 and COX-2), aromatase, Na^+/K^+ ATPase, CEA, estrogen, and progesterone were purchased from Sunlong Biotech Co, Ltd, PingShui Street, Gong Shu District, Hangzhou, Zhejiang, China. All chemicals and solvents were analytical grade and were carried out using ELISA reader (NJ 2000; Nihom Inter Med Co). The sensitivity of assay was 12 pgEq/mL, 0.01 pgEq/mL, 150 pgEq/mL, 14 pg/mL, 0.5 ng/mL, 10 ngEq/mL, and 0.1 ngEq/mL for COX-2, COX-1, CEA, estrogen, progesterone, aromatase, and Na/K ATPase, respectively.

Production and isolation of BAEPS from B. amyloliquefaciens 3MS 2017

The strain was isolated from marine and was placed in the culture collection of the Microbial Biotechnology Department, National Research Center, Dokki, Cairo, Egypt. The production of exopolysaccharide (EPS) by *B. amyloliquefaciens* 3MS 2017 was performed by flask fermentation using the previously reported media and assay by El-Newary and colleagues.[17,18]

In-vivo antibreast cancer

Chemically breast cancer induction. The mammary gland tumors in Virgin female Sprague-Dawley rats 50 to 65 days old weighing around 110 to 130 g was performed.[19] One hundred eighty rats were orally force fed a single dose of DMBA in sesame oil (75 mg/kg body weight). Mammary gland tumor induction by DMBA was evaluated using tow biomarkers, which documented as cancer biomarkers, CEA as a cancer biomarker[15] and aromatase[10] as cancer-growth-rate-limiting enzyme with visual observation of tumor (Image 1). After 5 months, the rats were fasted and maintained with tap water overnight. The fasted rats were anesthetized by injection of 87 mg ketamine/kg of body weight and 13 mg of xylazine.[20] Blood samples were obtained from the tail vein of each rat and then centrifuged at 4000 r/min for 10 minutes. Carcinoembryonic antigen and aromatase were determined in serum samples using ELISA kits. Animals that reached 425 ± 7.35 to 475.50 ± 10.45 µgEq/mL of CEA and 4.80 ± 0.38 to 5.35 ± 0.40 µgEq/mL of aromatase were considered cancer animals. Obtained data showed that about 75% of DMBA force-fed rats recorded a significant increase in these biomarkers. It could be mentioned that the control rats ranged between 252.55 ± 10.25 to 280.55 ± 5.65 µgEq/mL for CEA and 0.72 ± 0.09 to 0.88 ± 0.1 µgEq/mL for aromatase.

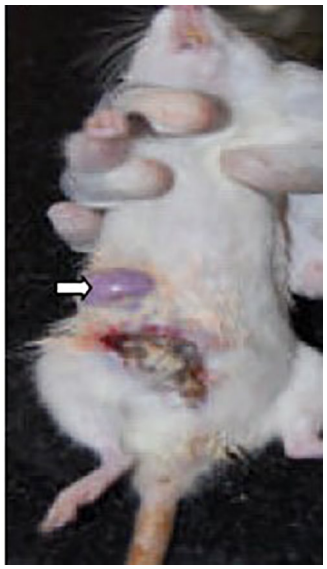


Image 1. DMBA-induced breast cancer image.
DMBA indicates 7,12-dimethylebenz-(a)-anthracene.

Experimental animals

This study was carried out in the National Research Center, Dokki, Egypt, and the experiment was performed for 8 months. In addition, this research protocol was permitted by the National Research Center Medical Ethics Committee, Egypt, with registration no. 6/014.

Female Sprague-Dawley rats were used to evaluate the anti-breast-cancer activity of BAEPS. This rat strain remains alive for 3 years and starts its reproductive function and lasts for about 1 year, at 50 to 60 days of age. One hundred fifty female rats were obtained from the animal house of the National Research Center, 33 St. El-Buhouth, Dokki, Cairo, Egypt. Rats were maintained at 25 ± 2°C, moisture 60% to 65% with a 12-hour-light:12-hour-dark cycle, and food and water were ad labium.

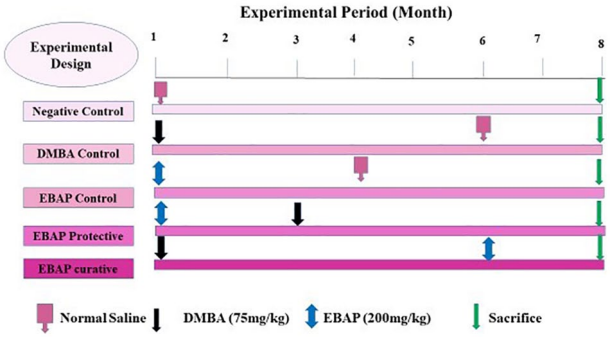
Experimental design

After the adaptation period (2 weeks) under the laboratory facilities, the rats were divided into 4 main groups: negative, positive, prophylactic, and therapeutic.

The negative group was 30 rats force fed with normal saline at all the experimental period.

The positive group was distributed into 2 subgroups (n = 30) as follow:

- The first, cancer control subgroup: rats were orally force fed with 65 mg/kg body weight DMBA as a single dose and were kept under laboratory conditions for 20 weeks, and they received normal saline for 12 weeks.
- The second, BAEPS-control subgroup: rats received normal saline up to the 20th week and then orally received the BAEPS at a dose of 200 mg/kg body weight (as the 10th of the LD_{50} [17]) for 12 weeks.



Scheme 1. Experimental design.

The prophylactic group contained 30 female rats, which orally received BAEPS at a dose of 200 mg/kg body weight (as the 10th of the LD_{50}) for 12 weeks. They received DMBA (65 mg/kg body weight as a single dose orally) and then were kept for 20 weeks until the end of the experiment.

The curative subgroup contained 30 female rats, which first received DMBA (65 mg/kg body weight as a single dose orally) and were kept for 20 weeks. They were then treated with BAEPS at a dose of 200 mg/kg body weight for 12 weeks.

At the end of the experiment (32 weeks), animals were fasted and maintained with tap water overnight. Fasted rats were anesthetized by injection of 87 mg ketamine/kg of body weight and 13 mg of xylazine, beginning 10 to 15 minutes after simultaneous intraperitoneal injection and lasting 15 to 30 minutes. The 2 drugs dissolved in normal saline, and each rat received 0.2 mL/100 g body weight.[20] Animals were sacrificed after anesthesia, and the blood samples were collected for biochemical analysis. Serum was obtained by centrifugation at 4000 r/min for 10 minutes using Sigma Laborzentrifugen (Osterode am Harz, Germany). Organs were collected and were freshly weighted (Citizen analytical balance, CX series 220, USA) for the chronic toxicity evaluation (Scheme 1).

Biochemical Assessment

Toxicity biomarkers

Liver function assessments, total protein and albumin concentrations, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, were spectrophotometrically estimated in serum samples (Jasco, serial No. C317961148, Japan) according to the methods of Henry,[21] Doumas et al,[22] and Reitman and Frankel,[23] respectively. Globulin was calculated as the difference between the total protein and albumin content.[24] The kidney function parameters: urea, uric acid, and creatinine were spectrophotometrically estimated in serum samples as described by Tabacco et al,[25] Gochman and Schmitz,[26] and Faulkner and King,[27] respectively. Lipid profile: total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TGs) were spectrophotometrically determined in serum samples according to methods described by Allain et al,[28] Naito and Kaplan,[29] and Fossati and

Prencipe.[30] Low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), and the risk ratio were calculated according to Friedewald et al,[31] Naito and Kaplan,[29] and Kikuchi et al.[32]

Antioxidant parameters

Glutathione (GSH) concentration and antioxidant enzymes activities, glutathione reductase (GR), glutathione S-transferase (GST), glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) were spectrophotometrically determined in serum samples according to the methods of Griffith,[33] Goldberg and Spooner,[34] Paglia and Valentine,[35] Habig et al,[36] Beers and Sizer,[37] and Fridovich,[38] respectively.

Malondialdehyde (MDA), a lipid peroxidation biomarker, was measured in serum samples spectrophotometrically according to the methods of Ohkawa et al.[39]

Anti-inflammatory and cancer biomarkers

Serum COX-1 and COX-2, cancer rate growth enzyme; aromatase, and α1-Na, K ATPase, tumor biomarker; CEA, and sexual hormones; estrogen and progesterone were determined using ELISA kits of Sunlong Biotech Co, Ltd.

Statistical analysis

All data were mentioned as mean ± SD. Data were analyzed by 1-way analysis of variance (ANOVA; n = 20) using IBM SPSS statistics program (version 23). $P < .05$ was considered as the significant difference.

Results

The effects of the BAEPS administration on the Safety profile of DMBA-induced breast cancer in female rats

The effect of BAEPS administration on chronic toxicity through 12 weeks as the relative weight of vital organs, liver and kidney functions, and lipid profile of DMBA-induced breast cancer in female rats was represented in Tables 1 to 4.

Effect on organs relative weight

The positive control of BAEPS did not show any toxic symptoms; the relative weight of vital organs was close to those values of the negative control.

In contrast, the remarkable increase was recorded in vital organs of cancer control in a response to DMBA administration, compared to the negative control (Table 1). The significantly increased vital organs of cancer rats group were liver (+65.11%), spleen (+143.60%), heart (+18.18%), and total breast weight (+365.20%), compared to the corresponding values of the negative control. On the contrary, kidneys and lungs

Table 1. The prophylactic and curative effect of BAEPS on vital organs of DMBA-induced breast cancer in female rats.

PARAMETERS								
GROUP	BODY WEIGHT	ORGANS WEIGHT (G/100 G)						
		LIVER	KIDNEY	SPLEEN	LUNG	HEART	BRAIN	TOTAL BREAST
Negative control	156.80 ± 1.25[a]	2.78 ± 0.20*	1.01 ± 0.35[c]*	0.39 ± 0.05[e]*	0.80 ± 0.41[f]*	0.44 ± 0.11[a]*	0.99 ± 0.11[b]*	2.27 ± 0.17[c]*
Cancer control	150.5 ± 2.13[a]	4.59 ± 0.86	0.71 ± 0.09	0.95 ± 0.30	0.66 ± 0.12	0.52 ± 0.16	1.06 ± 0.24[a]	10.56 ± 0.56
		+65.11%	−29.70%	+143.59%	−21.21%	+18.18%	+7.07%	+365.20%
BAEPS groups								
Control	158.50 ± 2.33[a]	3.12 ± 0.41[b]*	0.97 ± 0.05[d]*	0.40 ± 0.06[e]*	0.83 ± 0.05[f]*	0.45 ± 0.01[a]*	0.91 ± 0.10*	2.31 ± 0.78[c]*
Prophylactic	111.17 ± 2.15	3.21 ± 0.95[b]*	1.03 ± 0.03[c]*	0.83 ± 0.04*	0.72 ± 0.03[a]*	0.48 ± 0.01*	0.98 ± 0.02[b]*	6.31 ± 0.96[d]*
Curative	157.77 ± 1.68[a]	3.42 ± 1.00*	0.95 ± 0.02[d]*	0.76 ± 0.01*	0.75 ± 0.02[a]*	0.46 ± 0.01[a]*	0.98 ± 0.01[b]*	7.06 ± 1.21[d]*

The presented data are mean of 20 replicates ± SD. Data were analyzed using 1-way ANOVA followed with post hoc analysis for multiple comparisons. The appearance of letters means an insignificant difference between groups that have the same letter as compared to negative controls. The significant difference with cancer animal group is indicated by *.

Table 2. The prophylactic and curative effect of BAEPS administration on liver functions in DMBA-induced breast cancer female rats.

PARAMETERS								
GROUP	AST (U/L	ALT (U/L)	AST/ALT	TOTAL PROTEIN	ALBUMIN	GLOBULIN	ALB/GLO	GSH
Negative group	69.45 ± 1.20[a*]	26.89 ± 2.61[a*]	2.58[a]	8.89 ± 0.14[a*]	4.51 ± 0.31	4.38 ± 0.45	1.04 ± 0.18	7.24 ± 0.97[a*]
Cancer group	161.01 ± 2.11	48.75 ± 2.45	3.30*	3.37 ± 0.03	2.18 ± 0.061	1.20 ± 0.004	1.82 ± 0.11	2.47 ± 0.05
	+131.84%	+81.30%	+27.91%	−62.09%	−51.66%	−72.60%	+74.04%	+65.88%
BAEPS groups								
Control	68.87 ± 2.13 [a*]	28.57 ± 1.43[a*]	2.41	7.76 ± 0.269[b*]	4.411 ± 0.216	3.349 ± 0.430	1.344 ± 0.211	7.23 ± 0.75[a*]
Prophylactic	53.27 ± 2.48[b*]	29.96 ± 1.67[a*]	1.78	8.94 ± 0.376[a*]	3.985 ± 0.281	4.955 ± 0.389	0.811 ± 0.103	6.29 ± 0.83*
Curative	53.27 ± 1.74[b*]	33.71 ± 2.00*	1.58	7.96 ± 0.205[b*]	3.835 ± 0.211	4.159 ± 0.383	0.935 ± 0.145	7.59 ± 0.81*

The presented data are mean of 20 replicates ± SD. Data were analyzed using 1-way ANOVA followed with post hoc analysis for multiple comparisons. * indicates a significant difference between groups and cancer control animals. The appearance of letters means an insignificant difference between groups that have the same letter as compared to negative controls.

significantly minimized (42.25% and 17.50%, respectively) in comparison with values of the negative control.

Co-administration of BAEPS for 12 weeks in the prophylactic group protected vital organs of DMBA-induced female breast cancer rats. The relative weight of the liver, spleen, heart, and breast appeared to be in normal values when compared to cancer control. Meanwhile, the relative weight of kidneys and lungs were higher than that of cancer control, respectively. The relative weight of the brain did not change significantly when compared to the cancer control.

The curative effect of BAEPS appeared in a significant reduction in liver, spleen, heart, and breast weights with a significant increase in kidneys when compared to values of cancer control. The relative weight of liver, spleen, heart, and breasts was decreased from 4.59 ± 0.86, 0.95 ± 0.30, 52 ± 0.12, and 10.56 ± 0.56% for cancer control to 3.42 ± 0.31, 0.76 ± 0.01, 0.46 ± 0.01, 7.06 ± 0.74%, respectively, after BAEPS treatment for 12 weeks. The decreased relative weight of kidneys and lungs of cancer control (0.71 ± 0.09% and 0.66 ± 0.05%, respectively) were enhanced to 0.95 ± 0.02% and 0.75 ± 0.02% after BAEPS treatment. The relative weight of vital organs of prophylactic and curative groups was improved toward normalization than compared to those values of the negative control.

The effects on liver functions

Data in Table 2 showed the hepatotoxic effect of DMBA. Administration of DMBA caused hepatocytes damage, which leads to the release of liver enzymes AST and ALT into the bloodstream, which increased to 161.01 ± 2.11 and 48.75 ± 2.45 U/L, with 131.84% and 81.30% increments, respectively, than those values of the negative control (69.45 ± 1.20 and 26.89 ± 2.61 U/L, respectively). In addition, DMBA disabled liver functions as a significant reduction in total protein and its fractions; albumin and globulin production; 3.37 ± 0.03, 2.18 ± 0.06, and 1.203 ± 0.004 mg/dL, respectively, with 62.10%, 51.66%, and 72.60%, decrease than those values of the negative control; 8.89 ± 0.03, 4.51 ± 0.31, and 4.38 ± 0.45 mg/dl, consequently.

The prophylactic effect of BAEPS exhibited as a significant increase in total protein and its fractions; albumin and globulin, to reach 8.94 ± 0.04, 3.99 ± 0.28, and 4.96 ± 0.39 mg/dl, respectively. *B. amyloliquefaciens* 3MS 2017 prevented hepatocytes destruction and release of AST and ALT to the bloodstream and was evident in keeping AST and ALT levels in the normal range (53.27 ± 2.48 and 29.96 ± 1.67 U/L) compared to values of the cancer control.

Similarly, force-feeding of BAEPS for 12 weeks after DMBA administration showed a curative effect. Liver functions improved presented in a significant increase in total protein production and its fractions albumin and globulin to reach 7.96 ± 0.21, 3.84 ± 0.21, and 4.16 ± 0.38, respectively, in comparison with cancer group. In addition, AST and ALT

Table 3. Prophylactic and curative effect of BAEPS on kidney functions in DMBA-induced breast cancer female albino rats.

PARAMETERS			
GROUP	CREATININE (MG/DL)	URIC ACID (MG/DL)	UREA (MG/DL)
Negative control	3.23 ± 0.71^{b}	2.43 ± 0.57^{a}	9.87 ± 1.25^{c}
Cancer group	4.06 ± 0.77*	3.06 ± 0.53*	12.43 ± 1.67*
	+25.70%	+25.93%	+25.94%
BAEPS groups			
Control	3.30 ± 0.15^{b}	2.51 ± 0.05^{a}	9.30 ± 1.12*
Prophylactic	3.60 ± 0.21*	2.95 ± 0.11*	9.41 ± 1.05^{c}
Curative	3.40 ± 0.16*	2.65 ± 0.31*	9.02 ± 1.02*

The presented data are mean of 20 replicates ± SD. Data were analyzed using1-way ANOVA followed with post hoc analysis for multiple comparisons. * indicates a significant difference between groups and negative controls. The appearance of letters means an insignificant difference between groups that have the same letter as compared to negative controls.

activities were restored to normal levels (53.27 ± 1.74 and 33.71 ± 2.00 U/L, respectively) as compared to AST and ALT activities of the cancer control.

The liver functions of prophylactic and curative groups were close to those of the negative control. Besides, liver function parameters of BAEPS control were also close to those values of the negative control.

Effect on kidney functions

A disturbance in kidney function was observed in female rats force-fed DMBA. It was presented in a significant increment in kidney biomarkers, including creatinine (4.06 ± 0.39 mg/dL; +25.70%), uric acid (3.06 ± 0.30 mg/dL; +25.93%), and urea (12.43 ± 1.67 mg/dL; +25.94%) in comparison with the same parameters of negative control (3.23 ± 030, 2.43 ± 022, and 9.87 ± 1.25 mg/dL, respectively; $P \leqslant .05$).

As present in Table 3, when BAEPS administrated before DMBA, it significantly protected kidney performance of female rats as compared to cancer control rats. Consequently, creatinine, uric acid, and urea concentrations of the prophylactic group significantly decreased, as compared to cancer control ($P \leqslant .05$).

In addition, kidney functions of the curative group were significantly improved toward normalization and were decreased to 3.40 ± 016, 2.65 ± 0.31, and 9.02 ± 1.02 mg/dL for creatinine, uric acid, and urea, respectively, as compared to the corresponding values in the cancer control ($P \leqslant .05$). However, the kidney performance of BAEPS-control group did not change significantly, compared to the negative control.

The effects on lipid profile parameters

7,12-Dimethylbenz[a]anthracene administration was associated with hyperlipidemia (Table 4), compared to the negative control. Total cholesterol, TGs, VLDL-C, and LDL-C were significantly increased to 4.77 ± 0.06, 1.82 ± 0.03, 0.36 ± 0.06, and 3.61 ± 0.06 mmol/L, respectively, instead of 1.75 ± 0.01, 0.98 ± 0.02 and 0.20 ± 0.04, and 0.44 ± 0.07 mmol/L in the negative control. Meanwhile, HDL-C was remarkably decreased from 1.11 ± 0.07 mmol/L in the negative control group to 0.80 ± 0.01 mmol/L in the cancer control ($P \leqslant .05$).

Total cholesterol levels, either in the prophylactic or in the curative groups were suppressed to 1.76 ± 0.02 and 1.53 ± 0.01 than TC of cancer control (4.77 ± 0.06 mmol/L; $P \leqslant .05$). In addition, TC of BAEPS-control was decreased to 1.53 ± 0.05 mmol/L, with 12.57% decreasing percentage than TC of the negative control (1.75 ± 0.01 mmol/L).

Triglycerides and VLDL-C in prophylactic or curative groups presented low levels that recorded 0.87 ± 0.10 and 0.85 ± 0.10 mmol/L for TG, 0.179 ± 0.002 and 0.171 ± 0.002 mmol/L for VLDL-C, respectively, as compared to the corresponding values of cancer control; 1.82 ± 0.024 and 0.364 ± 0.005 mmol/L, respectively, ($P \leqslant .05$). Triglycerides and VLDL-C of BAEPS-control were impressed than negative control; 0.98 ± 0.02 and 0.196 ± 0.004 mmol/L, respectively, ($P \leqslant .05$). Triglycerides and VLDL-C of rats administered DMBA and treated with BAEPS were decreased than that of the negative control ($P \leqslant .05$).

A significant increment percentage of HDL-C was recorded (38.75% and 35% for the prophylactic and curative groups, respectively) over cancer control; 0.80 ± 0.01 mmol/L ($P \leqslant .05$), whereas there is no significant change in HDL-C of BAEPS-control in comparison with HDL-C of negative control animals.

In response to VLDL-C decrease and HDL-C increase, LDL-C was significantly reduced toward the normal level. Low-density lipoprotein cholesterol levels reached 0.473 ± 0.10 and 0.350 ± 0.07 mmol/L for prophylactic and curative groups, respectively, as compared to cancer control, 3.61 ± 0.06 mmol/L ($P \leqslant .05$). Low-density lipoprotein cholesterol level of BAEPS-control was

Table 4. Impact of BAEPS on the lipid profile of DMBA-induced breast cancer in the female rat.

PARAMETERS						
GROUP	TC (MMOL/L)	TG (MMOL/L)	HDL-C (MMOL/L)	VLDL-C (MMOL/L)	LDL-C (MMOL/L)	RR (%)
Negative control	1.75 ± 0.01[a]	0.98 ± 0.02	1.11 ± 0.01	0.20 ± 0.004	0.44 ± 0.07	0.40 ± 0.09
Cancer control	4.77 ± 0.06*	1.82 ± 0.02*	0.80 ± 0.01*	0.36 ± 0.005*	3.61 ± 0.06*	4.51 ± 0.07*
BAEPS groups						
Control	1.53 ± 0.05b*	0.85 ± 0.02a*	1.09 ± 0.04	0.17 ± 0.003	0.27 ± 0.06*	0.251 ± 0.06*
Prophylactic	1.76 ± 0.02a*	0.87 ± 0.01a*	1.11 ± 0.09	0.17 ± 0.002	0.47 ± 0.10	0.433 ± 0.14
Curative	1.54 ± 0.01b*	0.85 ± 0.01a*	1.08 ± 0.002	0.17 ± 0.002	0.35 ± 0.07	0.348 ± 0.08

Abbreviation(s): HDL-C, high-density lipoprotein cholesterol; VLDL-C, very-low-density lipoprotein cholesterol.
The presented data are mean of 20 replicates ± SD. Data were analyzed using 1-way ANOVA followed with post hoc analysis for multiple comparisons. * indicates a significant difference between groups and negative controls.
The appearance of letters means an insignificant difference between groups that have the same letter as compared to negative controls.

significantly declined by about 38.64%, in comparison with that of the negative control; 1.112 ± 0.006 mmol/L ($P \leqslant .05$).

B. amyloliquefaciens 3MS 2017 administration caused HDL-C to increase concurrently with LDL-C decrease; therefore, the risk ratio of rats force-fed *B. amyloliquefaciens* 3MS 2017 was significantly reduced in the prophylactic and curative groups; 0.433 ± 0.14% and 0.348 ± 0.08%, respectively, compared to those of cancer control; 4.51 ± 0.07% ($P \leqslant .05$). *B. amyloliquefaciens* 3MS 2017 control rats produced a low-risk ratio in comparison with that of the negative control ($P \leqslant .05$).

The Effect on lipid peroxidation biomarker (MDA)

Figure 1 demonstrated that DMBA administration was associated with a significant increment in lipid peroxidation represented via 1065.09% increase in MDA concentration of the cancer control than the value in the negative control (24.70 ± 1.50 and 2.12 ± 0.09 mmol/L, for cancer and negative control, respectively) ($P \leqslant .05$). Force-feeding BAEPS as a prophylactic agent maintained MDA level within the normal level and remained at 2.28 ± 0.27 mmol/L, compared to MDA of cancer control (24.70 ± 1.50 mmol/L). *B. amyloliquefaciens* 3MS 2017 administration as a curative agent retains MDA to the normal limit (2.28 ± 0.27 mmol/L), in comparison with cancer control without any significant difference between MDA values of both positive control and negative control.

Effects of BAEPS on antioxidant parameters

Data presented in Table 5 showed the effect of DMBA on enzymatic (GR, GST, GPx, CAT, SOD, and nonenzymatic (reduced glutathione, GSH) antioxidant biomarkers as well as the prophylactic and curative effect of BAEPS. 7,12-Dimethylbenz[a]anthracene-induced breast cancer in female rats suffered from suppression in the antioxidant system. Glutathione reductase, GST, GPx, CAT, and SOD activities were significantly inhibited (0.96 ± 0.09, 1.12 ± 0.16, 0.43 ± 0.03, 4.10 ± 0.15, and 3.69 ± 0.70 µmol/mg protein/min, respectively) with decreasing GSH (0.86 ± 0.09 mg/dL) as compared to the corresponding values of negative control (3.99 ± 0.36, 3.57 ± 0.33, 1.63 ± 0.14, 19.10 ± 1.56, and 12.05 ± 0.56 µmol/mg protein/min and 3.24 ± 0.32 mg/dL, respectively; $P \leqslant .05$).

Antioxidant biomarkers of BAEPS positive control were significantly ameliorated to be 10.73 ± 1.12, 6.28 ± 0.59, 4.38 ± 0.38, 20.26 ± 1.32, and 16.56 ± 1.47 µmol/mg protein/min for GR, GST, GPx, CAT, and SOD, respectively as well as 8.70 ± 0.77 mg/dL for GSH compared to the corresponding value of the negative control.

Antioxidant parameters of prophylactic group rats were significantly improved in comparison with those of the cancer group. Glutathione concentration was significantly elevated to reach 7.27 ± 0.55 mg/dL (+745.35%), while GR, GST, GPx,

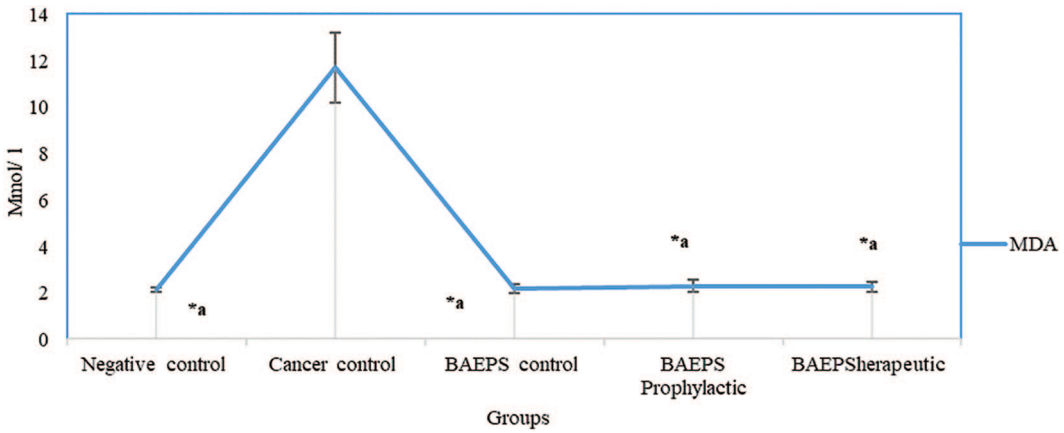


Figure 1. Prophylactic and curative effect of BAEPS on lipid peroxidation of DMBA-induced breast cancer in female rats.
The presented data are mean of 20 replicates ± SD. Data were analyzed using 1-way ANOVA followed with post hoc analysis for multiple comparisons. The appearance of letters means an insignificant difference between groups that have the same letter, while * indicates significant difference as compared to the cancer group.

Table 5. The prophylactic and curative effects of BAEPS on antioxidant status of serum in DMBA-induced breast cancer in female albino rats.

PARAMETERS						
GROUPS	GSH (MG/DL)	GR (μMOL/MG PROTEIN/MIN)	GST (μMOL/MG PROTEIN/MIN)	GPx (μMOL/MG PROTEIN/MIN)	CAT (μMOL/MG PROTEIN/MIN)	SOD (μMOL/MG PROTEIN/MIN)
Negative control	3.24 ± 0.22	3.99 ± 0.29	3.57 ± 0.20	1.63 ± 0.05	19.10 ± 3.12[a]	12.05 ± 0.56
Cancer group	0.86 ± 0.11*	0.96 ± 0.09*	1.12 ± 0.16*	0.43 ± 0.03*	4.10 ± 0.15*	3.69 ± 0.70*
BAEPS groups						
Control	8.70 ± 0.64*	10.73 ± 1.12*	6.28 ± 0.67[a]*	4.38 ± 0.46*	20.26 ± 1.32[b]*	16.56 ± 1.47*
Prophylactic	7.27 ± 0.55*	8.96 ± 0.63*	6.53 ± 0.60[a]*	3.66 ± 0.37*	19.90 ± 1.16[b]*	14.34 ± 0.55[a]*
Curative	6.33 ± 0.57*	7.80 ± 0.65*	5.80 ± 0.48*	3.19 ± 0.37*	19.03 ± 1.07[a]*	14.34 ± 1.33[a]*

Abbreviations: CAT, catalase; GR, glutathione reductase; GSH, glutathione; GST, glutathione S-transferase; SOD, superoxide dismutase.
The presented data are mean of 20 replicates ± SD. Data were analyzed using 1-way ANOVA followed with post hoc analysis for multiple comparisons. The appearance of letters means an insignificant difference between groups that have the same letter, while * indicates significant difference as compared to the cancer group.

CAT, and SOD were significantly increased to 8.96 ± 0.61, 6.53 ± 0.56, 3.66 ± 0.32, 19.90 ± 1.35, and 14.34 ± 0.55 μmol/mg protein/min, respectively in comparison with the cancer group ($P \leqslant .05$).

Curative group rats recorded significant improvement in antioxidant parameters in comparison with the cancer group. Glutathione concentration was significantly raised to 6.33 ± 0.58 mg/dL (+636.05%). In addition, GR, GST, GPx, CAT, and SOD were increased to 7.80 ± 0.65, 5.80 ± 0.48, 3.19 ± 0.73, 19.03 ± 1.07, and 14.34 ± 1.33 μmol/mg protein/min, respectively, compared to the corresponding values of the cancer group ($P \leqslant .05$).

Antioxidant parameters of the prophylactic group or curative group were improved toward normalization and were higher than the corresponding values of the negative control group. The prophylactic administration of BAEPS was more effective than curative effect against oxidative stress status in DMBA-induced breast cancer in female rats.

Effects of BAEPS on COXs production

7,12-Dimethylbenz[a]anthracene, carcinogenic materials, worked as proinflammatory inducer that is shown as a significant rise in COX-2 (326.00 ± 1.38 μgEq/mL with increment +168.31%) synchronized with a significant decrease in COX-1 (102.00 ± 2.24 μgEq/mL with decrease -78.30%) in consideration with the negative control (121.50 ± 0.79 and 470.00 ± 1.35 μgEq/mL, respectively; $P \leqslant .05$; Figure 2).

B. amyloliquefaciens 3MS 2017 exhibited an inhibitory effect on COX-2 gene expression where COX-2 subunit of the prophylactic group remained close to the normal level; 163.33 ± 1.67 μgEq/mL with +49.90% increase than the level of the cancer group. In addition, BAEPS kept COX-2 of the curative group close to normal ranges: 137.00 ± 1.81 μgEq/mL with -57.98% decrease than that of the cancer group. On the contrary, COX-1 was decreased as a response to DMBA-induction while bringing increase to 250.00 ± 2.33 and 290.00 ± 1.94 μgEq/mL in a response to BAEPS administration

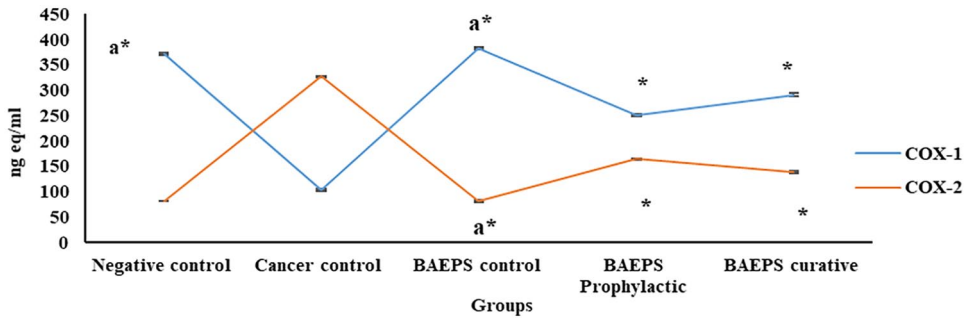


Figure 2. The prophylactic and curative effect of BAEPS on inflammation rate-limiting enzymes of DMBA-induced breast cancer in female rats. The presented data are mean of 20 replicates ± SD. Data were analyzed using 1-way ANOVA followed with post hoc analysis for multiple comparisons. The appearance of letters means an insignificant difference between groups that have the same letter, while * indicates significant difference as compared to the cancer group.

as prophylactic and curative groups, respectively, with 145.10% and 184.31% increment percentage considering COX-1 value of cancer group (102.00 ± 2.14 μgEq/mL; $P \leq .05$).

Determined COX-1 subunit of BAEPS-control group showed a significant elevation and reached 382.00 ± 2.41 μgEq/mL, meanwhile, COX-2 did not significantly change, according to the results of the negative control.

Effects of BAEPS on cancer-growth-rate-limiting enzymes

Aromatase activity. 7,12-Dimethylbenz[a]anthracene-inducing breast cancer in female rats expressed more aromatase level (8.33 ± 0.66 μgEq/mL) with 9.41-fold higher than aromatase of the negative group (0.80 ± 0.04 μgEq/mL; Figure 3A). *B. amyloliquefaciens* 3MS 2017 administration either in a prophylactic group or in the curative group appeared aromatase inhibitory action. Aromatase level of prophylactic and curative groups were declined to 3.81 ± 0.33 and 2.27 ± 0.24 μgEq/mL with 54.26% and 72.75% percentages reduction, respectively. Aromatase of BAEPS-control rats did not change significantly in comparison with the value of the negative control ($P \leq .05$).

Effect of BAEPs on Na^+/K^+ ATPase expression

7,12-Dimethylbenz[a]anthracene administration promoted angiogenesis process as a significant increase in Na^+/K^+ ATPase production to reach 5.70 ± 0.41 μgEq/mL with 11.40-fold higher than that of the negative group; 0.50 ± 0.03 μgEq/mL (Figure 3B). *B. amyloliquefaciens* 3MS 2017 exhibited antiangiogenesis action represented as a significant magnification in Na^+/K^+ ATPase catalytic subunit production. The level of Na^+/K^+ ATPase was reduced to 0.99 ± 0.02 and 0.92 ± 0.01 μgEq/mL for prophylactic and curative groups as compared to the cancer group ($P \leq .05$). *B. amyloliquefaciens* 3MS 2017 adjusted the level of Na^+/K^+ ATPase of breast cancer female rats nearly to normal ranges, in comparison with the negative control. *B. amyloliquefaciens* 3MS 2017 control group appeared normal Na^+/K^+ ATPase catalytic subunit levels.

Effect of BAEPS in cancer biomarkers

Carcinoembryonic antigen. Carcinoembryonic antigen was significantly increased to 524.17 ± 1.02 μgEq/mL in a response to DMBA administration, compared to CEA of the negative control; 279.97 ± 1.04 μgEq/mL (Figure 3C; $P \leq .05$). *B. amyloliquefaciens* 3MS 2017 administration, either before or after DMBA treatment significantly reduced CEA (335.00 ± 2.53 and 281 ± 1.97 μgEq/mL) in the prophylactic and curative groups, respectively, with 36.09% and 46.39% reduction percentage, as compared to CEA in the cancer control (524.17 ± 1.02 μgEq/mL). There was no significant difference observed between CEA level in both BAEPS-control group and negative control (Image 2).

Effect of BAEPs on steroid hormones

Cancer induction with DMBA induced hormonal imbalance in breast cancer rats. Hence, estrogen level increased from 28.35 ± 1.35 ng/mL in negative control rats to 105.14 ± 3.21 ng/mL in DMBA rats, concurrent with the reduction in progesterone level from 8.95 ± 0.88 ng/mL to 2.45 ± 0.18 ng/mL (data in Figure 4; $P \leq .05$). The prophylactic group produced less estrogen (63.84 ± 1.51 ng/mL) and more progesterone (3.36 ± 0.21 ng/mL), as compared to their levels in cancer control. The curative group recorded the same ameliorative effect. The fluctuation in hormonal balance was restored toward normalization in treated groups. Estrogen level recorded for the curative group was reduced to 50.26 ± 1.23 ng/mL with the induction of progesterone, 4.01 ± 0.24 ng/mL, in comparison with cancer control, $P \leq .05$. The ameliorative effect of BAEPS on estrogen and progesterone as curative was superior to the prophylactic effect, as compared to cancer control, $P \leq .05$. The positive control of BAEPS rats showed the same trend, where estrogen level was decreased and the progesterone level was increased in comparison with the negative control.

Discussion

7,12-Dimethylbenz[a]anthracene administration as a single dose produced breast cancer in female rats after 5 months.

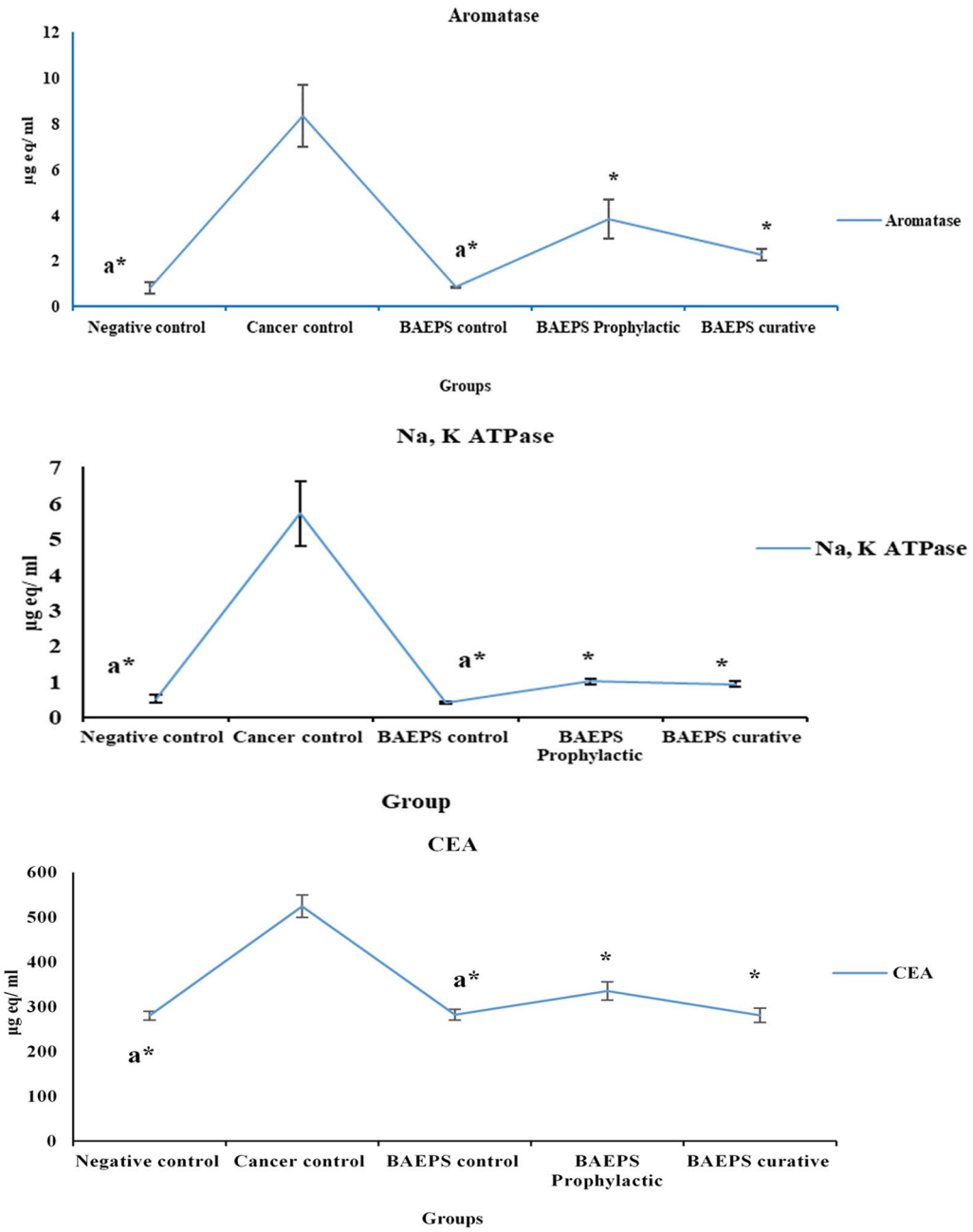


Figure 3. The prophylactic and curative effect of BAEPS on cancer-growth-rate-limiting enzymes (Aromatase (A), Na^+ and K^+ ATPase (B), and CEA (C) of DMBA-induced breast cancer in female rats.
The presented data are mean of 20 replicates ± SD. Data were analyzed using 1-way ANOVA followed with post hoc analysis for multiple comparisons. * indicates a significant difference between groups and negative controls, while the appearance of letters means an insignificant difference between groups that have the same letter as compared to cancer controls.

7,12-Dimethylbenz[a]anthracene-induced breast cancer in rats characterized by a high level of oxidative stress biomarkers, an inflammatory biomarker, cancer-growth-relating enzymes, estrogen, and cancer biomarker concomitant with low progesterone production. In addition, DMBA-female rats appeared to have hepatotoxicity and nephrotoxicity concurrent with hyperlipidemia. Administration of BAEPs either prophylactic or curative exhibited significant improvement in all assessed parameters that were returned toward normalization without toxicity and keeping body maintenance.

Our experiment revealed the capacity of DMBA to increase the proliferation of rat breasts, which increased the relative weight of the breasts. In prophylactic and curative groups, BAEPS protected and suppressed harmful proliferation in the breasts as a significant reduction in breasts weight. These data agreed with the data of Minari and Okeke[40] on *Annona muricata* and Ibrahim and colleagues,[9] on Broccoli. The increase in the relative weight of vital organs in cancer control was due to the toxicity of DMBA causing hypertrophy of cells and accumulation of fats. The susceptibility of the mammary glands to

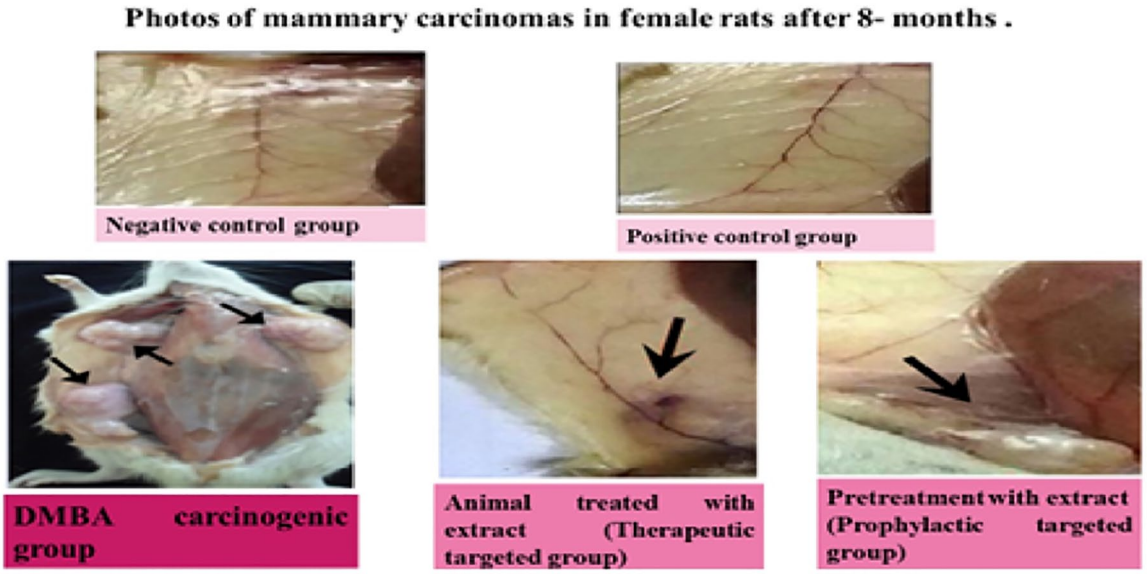


Image 2. The photo of mammary carcinomas in DMBA female rats.

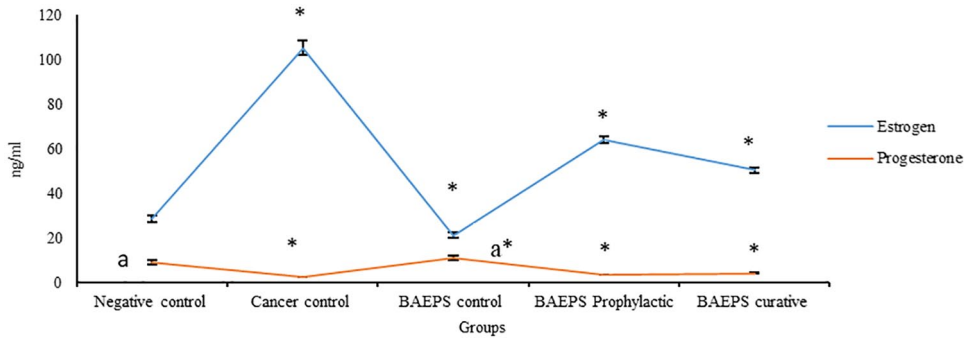


Figure 4. The prophylactic and curative effect of BAEPS on steroid hormones of DMBA-induced breast cancer in female rats.
The presented data are mean of 20 replicates ± SD. Data were analyzed using 1-way ANOVA followed with post hoc analysis for multiple comparisons. The appearance of letters means an insignificant difference between groups that have the same letter, while * indicates significant difference as compared to the cancer group.

DMBA carcinogenesis is strongly age dependent. In the breasts, DMBA is converted to epoxides that are the active metabolites with a damage capacity resulting in a higher cellular proliferation index.[41]

In this study, DMBA induced hepatotoxicity and nephrotoxicity. *B. amyloliquefaciens* 3MS 2017 maintained liver functions in the prophylactic group or curative one within normal levels. These findings were in agreement with those published by Manickam et al,[42] Ibrahim et al,[8] and Dakrory et al.[43] 7,12-Dimethylbenz[a]anthracene metabolized by cytochrome P450 enzyme in the liver to form diol peroxides and other toxic reactive species.[44] Kumar et al [45] reported that DMBA-induced hepatotoxicity[46] and decreasing liver antioxidants lead to harmful changes in liver functions. The ameliorative effect of BAEPS on liver function of DMBA-induced breast cancer female rats may be attributed to its antioxidant characters regarding its composition as a polysaccharide. El-Newary et al[17] reported that BAEPS has antioxidant characters as a potent free radical (DPPH and ABTS) scavenger, ROS scavenger, NO scavenger, Fe^{+2} chelator, reducing agent and lipid peroxidation inhibitor, compared to 2 antioxidant; ascorbic acid as a natural antioxidant and butylatedhydroxytoluene (BHT) as a synthetic antioxidant. *B. amyloliquefaciens* 3MS 2017 inhibited lipid peroxidation with very low IC_{50} (1.10 µg/mL), compared with ascorbic acid and BHT (1.62 and 1.79 µg/mL, respectively). In conclusion, antioxidant characters of BAEPS prevented oxidative stress that occurred by DMBA, induced hepatotoxicity in female rats.

Administration of BAEPS improved lipid profile of DMBA-induced breast cancer rats toward normalization. The explained results are in agreement with those of Nandhakumar et al[47] and Ibrahim et al[9] who demonstrated a hyperlipidemic effect of DMBA administration in breast cancer female rats. Meanwhile, BAEPS administration exhibited hypolipidemic effect. These data were in accordance with those published by Nandhakumar et al[47] on Shemamruthaa (a Siddha formulation which constitutes *Hibiscus rosa sinensis, Emblica officinalis*, and Honey in define ratio) and Arroyo-Acevedo et al[48] on *Piper aduncum* capsule. The disturbance in lipid profile of DMBA-female rats may

reveal to the secretion of proinflammatory cytokines. This disturbance may be attributed to the acute-phase response induced by cytokines transmission by inflammatory cells surrounded by tumor or generated by the tumor cells.[49] In our previous study, BAEPS significantly inhibited the activity of COX-2 in an in-vitro assessment,[17] also in this study, it suppressed the production of COX-2 subunits in-vivo.

It is observed that the positive correlation between DMBA administration and oxidative stress. 7,12-Dimethylbenz [a]anthracene induced the production of ROS and free radicals that promote lipid peroxidation and deficiency of antioxidant enzymes activities (GR, GST, GPx, CAT, and SOD) as well as a decline in GSH concentration.[43] Malondialdehyde, the final product of lipid peroxidation in the cell, leads to tissues destruction and disturbance in the cellular antioxidant defense system. Therefore, the cell cannot prevent the production of excess ROS and free radicals. These results are in line with Arroyo-Acevedo et al,[48] Manickam et al,[42] and Ibrahim et al.[9] Meanwhile, the administration of BAEPS for 12 weeks restored these deteriorations toward normalization as significant elevation in GSH concentration and related enzymes (GR, GST, and GPx), CAT, and SOD activities. On the contrary, BAEPS treatment reduced oxidative biomarkers concentrations; MDA. These results may be attributable to the antioxidant abilities.[17] *B. amyloliquefaciens* 3MS 2017 has the reducing power that helps to reduce oxidized GSSG into reduced GSH, the active form. Glutathione has an important role in maintaining the integrity of the cell by protecting its structure and function. Glutathione can capture free radicals and detoxify, which leads to the balance of the cellular redox homeostasis.[50] In addition, decreasing MDA as lipid peroxidation product leads to increase GSH levels. In parallel, increasing GSH concentration decreased ROS production.[51] Due to ROS scavenging ability of BAEPS as H_2O_2, O_2–, NO radicals, and inhibition of lipid oxidation, BAEPS avoided oxidative stress injury of DMBA-induced breast cancer female rats. The increase that recorded in GR, GST, and GPx activities was due to the increase in GSH, where they are strongly GSH dependent.[52] In addition, the decrease happened in CAT and SOD after DMBA administration is due to an increase in MDA and ROS radicals, whereas the improvement in them after BAEPS treatment may be due to the decrease in MDA and ROS radicals, as it was reported previously for BAEPS.[17]

Cyclooxygenase-1 and COX-2 are cancer-growth-rate-limiting enzymes in the formation of prostaglandins from arachidonic acid. Cyclooxygenase-2 is an inducible be many factors and linked to tumorgenesis through promotion angiogenesis, inhibition apoptosis, promoting proliferation, and suppression immune system.[53] Cyclooxygenase-2 is a biological fuel to hyperproliferation, in cancer cells.[54] *B. amyloliquefaciens* 3MS 2017 reduced the expression of COX-2 (with reduction 49.90% and 57.96%, respectively) and promoted the expression of COX-1 (with increase 145.09% and 184.32%, respectively) in DMBA-induced breast cancer in female rats as a prophylactic agent or as a curative agent, respectively, compared to the cancer control. 7,12-Dimethylbenz[a]anthracene-induced breast cancer in female rats elevated COX-2 and reduced COX-1, while BAEPS administration showed opposite effects. These results harmonized with those published by Abdel-Rahman et al[55] on *Eruca sativa* seeds, and Alessandra-Perini et al[53] on *Euterpa oleracea*. The anti-inflammatory ability of BAEPS appeared in DMBA-induced breast cancer in female rats may be attributable to its antioxidant and anti-inflammatory aspects stated by El-Newary et al.[17] *B. amyloliquefaciens* 3MS 2017 also was proved in our previous study as COX-2 inhibitor and it was close to the standard drug celecoxib, where IC_{50} of BAEPS and celecoxib were 340.75 ± 7.70 and 312.48 ± 9.69 μg/mL, respectively. Therefore, it could be concluded that BAEPs impresses COX-2 by 2 parallel manners, COX-2 production suppressor, and COX-2 inhibitor.

7,12-Dimethylbenz[a]anthracene-induced breast cancer appeared induction of aromatase concentration, while breast cancer female that administrated BAEPS produced a significant decrease in aromatase level. These results agreed with the results of Chen et al, [56] who found that fraction rich with polysaccharides extracted, from white button mushrooms (*Agaricus bisporus*) had a potent aromatase inhibition ability in in-vitro and in-vivo studies.[56] In addition, polysaccharides were found to be an inhibitor of cytochrome P450 1A1-mediated ethoxyresorufin O-deethylase activity and caused a dose-dependent inhibition of aromatase activity in microsomes isolated from human placenta in a study prompted by Kyung-Soo et al.[57] Aromatase is a key enzyme in the biosynthesis of estrogens and plays an important role in the process of breast carcinogenesis of hormone-dependent breast cancers. There is a strong relation between aromatase (CYP19) gene expression and the expression of COX genes. The higher expression of COX-2 leads to higher production of prostaglandin E2, which in turn, increases aromatase expression via elevation of the intracellular cAMP levels. The nonsteroidal anti-inflammatory drugs have an effect on aromatase activity and expression in human breast cancer cells. Cyclooxygenase-2 inhibitors decrease aromatase mRNA expression and enzymatic activity in human breast cancer cells. Cyclooxygenase-2-selective agents are more effective in suppressing aromatase activity, with significant low IC_{50} concentrations than those required for nonselective COX inhibitors.[58] Regarding a previous explanation, BAEPS can be considered as a promising, hormonal-dependent breast cancer therapeutic agent depending on its selective anti-inflammatory property and aromatase inhibitory characters.

7,12-Dimethylbenz[a]anthracene-induced breast cancer in a female caused a significant increase in Na^+/K^+ ATPase level meanwhile, breast cancer females that administrated BAEPS appeared low concentration of Na^+/K^+ ATPase. The explained data are in accordance with that of Ibrahim et al.[9] They found that a significant elevation in Na^+/K^+-ATPase level of DMBA-breast cancer rats, which is decreased by Broccoli alcoholic extract treatment. Yan et al,[59] revealed that ROS

(generated from ouabain treatment) is involved in the Na^+/K^+ ATPase signaling transduction in a feed-forward mechanism as ROS are required to initiate ouabain-stimulated Na^+/K^+ ATPase/c-Src signaling. Pretreatment with the antioxidant N-acetyl-L-cysteine (NAC) prevented ouabain-stimulated Na^+/K^+ ATPase /c-Src signaling, protein carbonylation, redistribution of Na^+/K^+ ATPase, and sodium/proton exchanger isoform 3 (NHE3), and inhibition of active transepithelial Na^+ transport.[22] In this study, there is a significant reduction in Na^+/K^+ ATPase catalytic subunit level in prophylactic and curative groups.

The Na^+/K^+ pump plays a key role in the regulation of normal cellular homeostasis, cell differentiation, and cell proliferation. The proliferation of normal and cancer cells is indispensably coupled with switch-up of the Na^+/K^+ pump power of the cell. Prevention of Na^+/K^+ pump power switches-up by any means block cell proliferation.[60] *B. amyloliquefaciens* 3MS 2017 significantly reduced Na^+/K^+ pump by about 82.63% and 83.86% when it is used as a prophylactic or curative agent, respectively, in comparison with the value of the cancer control.

In this search, CEA that is elevated by DMBA administration, significantly decreased by BAEPS administration in breast cancer female rats. Many authors revealed the same results including Ibrahim et al,[9] on Broccoli and Alipanah et al,[61] on *Viola odorata*. In healthy adults, CEA produced in blood with a very low concentration not exceeding 20 ng/mL. Cancer patients produce CEA with large quantities; therefore, it is considered as cancer biomarkers.[62] Carcinoembryonic antigen decrease in this study may be attributed to the anticancer ability of BAEPS, which killed about 65.20% of MCF-7 cells in in-vitro and antitumor ability in in-vivo model on Ehrlich ascites carcinoma (EAC) bearing mice.[17]

7,12-Dimethylbenz[a]anthracene-induced breast cancer was characterized by high estrogen levels and low progesterone levels. On the contrary, BAEPS modulated both estrogen and progesterone levels. These data are in harmony with those published by Ibrahim et al.[9] Estrogen is the primary female sex hormone. It is responsible for the development and regulation of the female reproductive system and secondary sex characteristics.[63] The aromatase enzyme converts the androgens hormone to estrogens in many human tissues. *B. amyloliquefaciens* 3MS 2017, either prophylactic or curative drug, recorded a significant reduction in aromatase activity by about 54.26% and 72.75%, respectively, with a significant decline in estrogen production by about 39.28% and 52.20%, respectively, compared to the level in the cancer control group. It is evident from the results that BAEPS could be considered as an AI, which is a key enzyme in the biosynthesis of estrogens, and plays an important role in the process of breast carcinogenesis of hormone-dependent breast cancers.

Estrogens are demonstrated to encourage the cellular proliferation associated with certain cancers; [63] meanwhile, progesterone inhibits the proliferation of normal and cancerous breast epithelial cells.[64] Low progesterone is associated with up-regulated growth factor signaling and aggressive tumors.[64] Progesterone reduces the proliferative activity of the estrogen.[60] In addition, progesterone suppresses the cells spreading and induced cell death in malignant mesothelioma cancer cells.[65] Administration of BAEPS as a prophylactic or a curative agent increased progesterone by about 37.14% and 63.67%, respectively, compared to the value of the cancer control.

Finally, anti-breast-cancer features appeared with the administration of BAEPS, either as prophylactic or curative agents might be correlated to its inhibitory effects on cancer-growth-limiting enzymes expression (aromatase and Na^+/K^+ ATPase), selective anti-inflammatory impact, antioxidant characteristics, and selective stimulatory effect on progesterone production.

Conclusion

The recent study demonstrated the anti-breast-cancer characters of acidic EPS produced from marine BAEPS, which showed antibreast cancer (MCF-7) in in-vitro previous study. *B. amyloliquefaciens* 3MS 2017 could prohibit and treat breast cancer in female rats presented via several mechanisms including inhibition of COX-2, aromatase, Na^+/K^+ ATPase, and estrogen production, which were reported as cancer stimulators and proliferator inducers. In addition, BAEPS influencing anticancer defense mechanisms as stimulation of antioxidant system components as well as COX-1 and progesterone production. This exhibited anticancer feature was accompanied by an accepted safety marginal through safety profile parameters for an 8-month follow-up. These results support the incorporation of this EPS in further advanced clinical trials to be considered as new pharmaceutical therapeutic raw material in treating breast cancer.

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Authors Contributions

M.G.M. and M.S.A. prepared the tested polysaccharide. A.Y.I., S.A.E., and E.R.Y. performed the animal experiments. All authors have participated in data analysis and manuscript preparation. All authors have accepted the final manuscript.

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