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Bisdemethoxycurcumin chemoprevents 7,12-dimethylbenz(a)anthracene-induced mammary toxicity via modulation of oxidative processes

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Bisdemethoxycurcumin (BDMC) is a naturally occurring compound having anti-cancer properties. We investigated the effect of BDMC on DMBA-induced mammary toxicity in female *Wistar* rats. Forty-eight virgin female rats were divided into six groups at random. Group 1 received corn oil, group 2 received DMBA (50 mg/kg), groups 3 and 4 received DMBA and BDMC (25 mg/kg and 50 mg/kg), group 5 received BDMC (50 mg/kg), and group 6 received DMBA and vincristine. A single dosage of DMBA was administered (i.p.) at six weeks, followed by BDMC (orally) and vincristine (i.p.) three times a week for thirteen weeks. The DMBA significantly increased lactate dehydrogenase activity by 1.3 folds. Similarly, DMBA increased nitric oxide, malondialdehyde, and myeloperoxidase activities by 12, 204, and 6.3%, respectively. DMBA-rats decreases glutathione-S-transferase, superoxide dismutase, and glutathione peroxidase activities. Immunohistochemistry analysis revealed that B-cell lymphoma-2, estrogen receptor, and human epidermal receptor-2 were strongly expressed in DMBA-rats, but progesterone receptor and Bcl-2 associated protein were weakly expressed. In DMBA rats, histology revealed mammary glands with moderate proliferating ducts and fibrosis. Co-treatment with BDMC reduces hormone receptors activities, improved antioxidant and apoptotic status. BDMC protected the mammary gland from DMBA toxicity by targeting cellular pathways involved in oxidative stress and apoptosis.

Keywords Dimethylbenz(a)anthracene, Bisdemethoxycurcumin, Apoptosis, Oxidative stress, Inflammation, Hormone receptors, Antioxidants

The mammary gland is a hormone-sensitive organ that grows during pregnancy and changes during a woman's menstrual cycle and puberty[1]. Important biological alterations required for the subsequent stages of structural and functional development are mediated by hormonal signals[2]. With monthly cycles of proliferation and regression, complete differentiation during the first full-term pregnancy, and more severe regression following menopause, this ongoing dynamic development puts the mammary gland at risk for alteration, which can have long-term consequences for the mother and her children[3]. There have been more cases of early breast growth in recent decades due to frequent exposure to environmental chemicals[4]. Despite these alarming developments, chemically induced changes to mammary gland development and function are understudied, and research into the emergence of these negative effects is urgently needed to protect women and future generations.

Breast cancer can affect women in any country in the world, developing at any age after puberty, with rates increasing significantly in later life[2–4]. Numerous chemicals are immunosuppressive and organ-specific carcinogens, and many research studies in rats and human cohorts have connected them to changes in breast development and cancer.

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Exposure to carcinogens like 7,12-dimethylbenz(a)anthracene (DMBA) is one of the primary causes of breast cancer. Many mouse models have been developed over time to better understand the progression of breast cancer[5,6]. Similarly, previous research from our findings has also documented mouse model progression of breast cancer using *N*-methyl-*N*-nitrosourea (NMU) and benzo(a)pyrene (BaP)[6,7]. The most common types are breast carcinomas caused by carcinogens such as DMBA. After exposure to a single dosage of DMBA during the peripubertal stage of mammary gland development (4–10 weeks of age), 30–70% of rats normally develop breast tumors 42–60 days later, which occasionally migrate to the lungs. This multi-step process is considered to be one of the most accurate and pertinent models to study mammary gland toxicity causing mammary gland damage because it closely resembles DMBA-induced carcinogenesis. Furthermore, while some carcinogen exposures have reduced as a result of evolving laws and practices, others, including DMBA, NMU, and BaP, have become more common because many of these substances are found in everyday items like food, dust, consumer goods, and drinking water[8]. Every stage of life exposes women and children to these harmful substances. Some of the causes contributing to the increasing number of breast cancer cases in Nigeria include the ongoing production of hazardous materials, such as air, water, and soil pollution, as well as the effects of fast food and cigarettes[9]. Lack of exercise and staying up too late are additional factors that lead to this[10].

Nearly 60% of toxicity studies use natural herbs, making them a promising area for toxicology research. Turmeric, a yellow spice from India and numerous African countries is derived from the rhizomes of the Zingiberaceae plant *Curcuma longa* Linn. The natural ratios of curcuminoids are roughly 6% bisdemethoxycurcumin (BDMC), 17% demethoxycurcumin, and 76% curcumin[11]. Curcuminoids have been the subject of research for many years due to their multiple biological effects, which include anti-cancer[12], anti-fibrotic[13], antiviral[14], antibacterial[15], and anti-ulcer[16] activities. Due to its low toxicity and cost, this class of compounds has recently become crucial to toxicology studies and is generally recognized as a promising fighting agent. Moreover, numerous studies have examined the pharmacological effects of BDMC worldwide[17,18]. It has been shown to have good antioxidative and cytotoxic properties against gastric adenocarcinoma (AGS), colorectal adenocarcinoma (SW-620), and hepatocellular carcinoma (HepG2) cell lines[17]. Additionally, it inhibits TNF-α-induced inflammation in human endothelial cells by interfering with NF-kB[18]. In this study, we induced mammary gland damage by exposing the rats to a single dose of DMBA at the peripubertal stage of mammary gland development of the rats. Therefore, it is hypothesized that giving BDMC to DMBA-administered rats may prevent the growth of mammary gland tumors and the subsequent carcinogenesis. We explore the anti-oxidative processes of BDMC on DMBA-induced mammary gland toxicity in female *Wistar* rats.

Materials and methods

Chemicals

The BDMC and DMBA were obtained from AK Scientific in California, USA, and stored at 4 °C in the dark, while vincristine was purchased from Kunle Ará Pharmacy (a pharmaceutical firm in Nigeria). Trichloroacetic acid (TCA) and thiobarbituric acid (TBA) were acquired from British Drug House (BDH) Chemical Ltd. in Poole, United Kingdom. AK Scientific's other products include reduced glutathione, 5, 5'-dithios-bis-2-nitrobenzoic acid (DTNB), o-Dianisidine, and epinephrine. The remaining chemicals used were of an analytical grade.

Animals

Forty-eight virgin female *Wistar* rats (33 and 52 g) were procured from the Veterinary Medicine Experimental Animal Facility at Nigeria's University of Ibadan. After being relocated, the animals were housed in plastic cages at Dominion University's Animal House of the Department of Chemical Sciences. They were acclimatised for one week at 25 ± 3 °C, 60 ± 10% humidity, and a 12-h light/dark cycle. They received unlimited water and laboratory feed from Ladokun Feeds Industry in Ibadan, Nigeria. The National Institutes of Health's guidelines for the care and use of laboratory animals were followed during all experimental procedures. The University of Ibadan Animal Ethics Committee approved the experimental design and procedures for handling and treating rats (UI-ACUREC/App/2015/061). The ARRIVE guidelines were followed by the authors.

Study design

A total of 48 virgin female *Wistar* rats (N = 48; 6 weeks old) were randomly divided into six groups of eight animals each. Group 1 (the control group) received corn oil only. Corn oil was used as a gavage vehicle for BDMC and DMBA-exposed rats. Group 2 received 50 mg/kg of DMBA. Groups 3 and 4 were administered DMBA at 50 mg/kg and co-treated with BDMC at 25 mg/kg and 50 mg/kg, respectively. Group 5 received only 50 mg/kg of BDMC. Group 6 was administered DMBA and received treatment with vincristine at a dosage of 0.5 mg/kg for 13 weeks. Groups 3, 4, and 5 received BDMC orally three times per week at doses of 25 and 50 mg/kg, vincristine was given intraperitoneally three times a week for thirteen weeks. Vincristine was used as a standard drug. The dosage and delivery methods for DMBA, BDMC, and vincristine complied with the studies of Adefisan et al.[6,7], Song et al.[12], and Kosemani et al.[35] with slight modifications. The animals were sacrificed by cervical dislocation after being put to sleep with isoflurane.

Preparation of tissues

The animals were fasted all night before being sacrificed via cervical dislocation. The mammary tissues were removed, weighed, and washed with 1.15% cold potassium chloride solution. A segment of each group's mammary gland was kept in a 10% formalin solution for histology analysis. The remaining tissues were homogenized in 4 L of 50 mM phosphate buffer (pH 7.4) using an electronic Teflon homogenizer. The homogenates were spun at 10,000 g for 15 min in an ice-cold (4 °C) ultracentrifuge to isolate the post-mitochondrial fraction (PMF), which was required for biochemical investigations.

Preparation of serum

Blood samples were taken via ocular puncture and placed in centrifuge tubes. The blood was centrifuged for 10 min at 3000 g to obtain the supernatant, which was then used for additional biochemical investigation.

Biochemical assays

Determination of aminotransferases

The activities of aspartate and alanine aminotransferases were assessed using Reitman and Frankel's[19] approach, which is based on the amount of oxaloacetate hydrazone produced by 2, 4-dinitrophenyl hydrazine. A diluted sample (0.1 ml) was mixed with phosphate buffer (100 mmol/L, pH 7.4), L-aspartate (100 mmol/L), and α-oxoglutarate (2 mmol/L) and incubated at 37 °C for 30 min. To stop the reaction, 5.0 mL (0.4 mol/L) of NaOH was added to the mixture after adding 0.5 mL (2 mmol/L) of 2, 4-dinitrophenylhydrazine and leaving it at 25 °C for 20 min. The absorbance was measured at 546 nm in comparison to the reagent blank.

Determination of lactate dehydrogenase (LDH) activity

The lactate dehydrogenase activity was measured using the Weisshar and colleagues[20] method. Pipette 1.0 mL of R1a (phosphate buffer and pyruvate) and R1b (NADH), then add 0.02 mL of the sample to the cuvette. Incubate at 37 °C for two minutes. Absorbance was measured and recorded at 340 nm at one-minute intervals for three minutes.

Determination of protein

Protein concentrations in the samples were determined using the method described by Gornall et al.[21], with bovine serum albumin (BSA) as a standard. The PMF was diluted with distilled water (1:10), and 4 mL of Biuret reagent was added to 1.0 mL of the diluted PMF. After 30 min of incubation at room temperature, the mixture was tested for absorbance at 540 nm against distilled water as a blank, and protein amounts were calculated using the BSA calibration curve.

Assessment of biomarkers of mammary oxidative stress

The mammary glands were extracted, rinsed with an ice-cold 1.15% KCl solution to eliminate bloodstains, dried, and weighed. The post-mitochondrial fraction (PMF) was prepared by homogenizing the gland in four volumes of 50 mM phosphate buffer solution at pH 7.4 for 15 min before it was centrifuge at 10,000 g for 15 min. The entire operation was carried out at 4 °C. The McCord and Fridovich technique were used to quantify the activity of superoxide dismutase (SOD)[22]. The Moron et al. method was used to quantify reduced glutathione (GSH) at 412 nm[23]. Moron et al. described the methods for determining glutathione-s-transferase (GST) activity[23] with CDNB as a substrate. The Rotruck et al. approach[24] was used to evaluate glutathione peroxidase (GPx) activity. Buege and Aust described a method for measuring lipid peroxidation using malondialdehyde (MDA)[25]. Mammary nitrite level was quantified using a sodium nitrite curve and represented as μM of nitrites/mg protein, as described by Palmer et al.[26]. Trush et al. used the technique[27] to quantify myeloperoxidase (MPO) activity in breast tissues.

Immunohistochemistry

Abcam Chemical Inc. (Cambridge, MA) supplied the immunochemical staining kits for the estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor-2 (HER-2), BCL-2 associated X-protein (Bax), and B cell lymphoma-2 (BCL-2)[28]. A secondary antibody and enzyme were employed to incubate the antibody-antigen combination that was developed. When the chromogen and substrate are present, the enzyme acts on the substrate, leaving colored deposits where the antibody binds to its antigen. Following that, a binocular microscope was used to analyze this. Cells that showed specific and distinct colors in the cytoplasm, nucleus, or cell membrane were considered positive based on the antigenic location and comparison to external controls. The pieces were warmed on a heated plate at 100 °C for 15 min to remove the antigen from a citric acid solution (pH 6.0). The color intensities were examined and evaluated using Image J software.

Histopathological examination of mammary tissues

Mammary tissues were preserved in 10% formalin. The tissues were dehydrated with 95% ethanol and extensively cleansed with xylene. The tissues were then cut into blocks and placed in a hot air oven at 56 °C for 30 min to soak in liquid paraffin. Following haematoxylin and eosin staining, a histopathologist examined microscopic sections under a microscope.

Statistical analysis

The results are presented as the mean ± standard deviation (SD) of six to eight animals per group. Statistical analysis of the biochemical data was conducted using one-way ANOVA followed by the post-hoc Duncan's multiple range test, performed with SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was defined as $p < 0.05$.

Results

Table 1 depicts the animals' body weight gain, mammary tissue weight, and organo-somatic weight. DMBA administration reduced the animals' body weight gain by 29% when compared to the control group. However, co-administration with BDMC at 25 mg/kg and 50 mg/kg increased animal body weight to near-normal control levels. In contrast, when compared to control rats, the organo-somatic weight of mammary tissue increases by 67%. In a dose-dependent way, co-treatment with BDMC (at both doses) and vincristine lowers organo-somatic weight by 45, 53, and 45%, respectively.

Groups	WEIGHT (g)			MAMMARY TISSUE	
	Initial Body Weight (g)	Final Body Weight (g)	Body Weight Gained (g)	Organ Weight (g)	Relative Body Weight (g)
CONTROL	48.50 ± 7.78	149.50 ± 9.19	101 ± 1.41	1.54 ± 0.41	1.03 ± 4.46
DMBA	64.00 ± 4.24	135.50 ± 4.95	71.51 ± 0.71*	2.31 ± 0.27	1.72 ± 5.39*
DMBA + BDMC1	59.25 ± 9.91	138.60 ± 3.51	79.35 ± 6.4	1.30 ± 0.17	0.94 ± 4.82
DMBA + BDMC2	58.75 ± 6.24	141.20 ± 8.97	82.45 ± 2.73	1.13 ± 0.28	0.80 ± 3.14
BDMC ONLY	66.33 ± 6.11	152.67 ± 18.04	86.34 ± 11.93	0.74 ± 014	0.48 ± 0.76
DMBA + VIN	68.17 ± 4.31	142.97 ± 19.02	74.8 ± 14.71	0.93 ± 0.36	0.93 ± 0.36

Table 1. The effect of BDMC on weight gain and organosomatic weight of the mammary gland in rats treated with 7, 12-dimethylbenz(a)anthracene. The values are presented as the mean ± SD of five to eight animals per group. DMBA = 7, 12-Dimethylben[a]anthracene (50 mg/kg), BDMC1 = bisdemethoxycurcumin (25 mg/kg); BDMC2-bisdemethoxycurcumin 2 (50 mg/kg), and VIN = Vincristine (0.5 mg/kg). *: significantly different from the control group ($p < 0.05$).

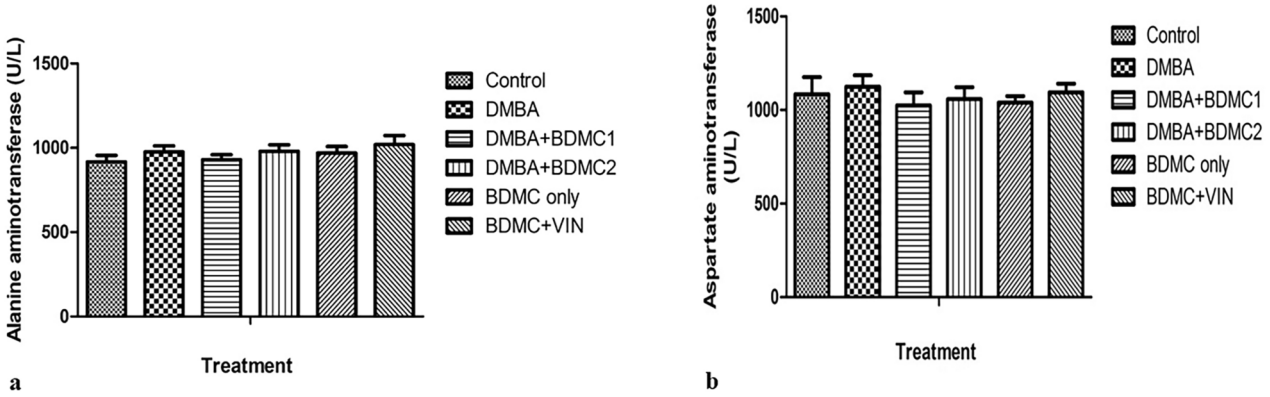

Fig. 1. Effects of BDMC on serum aspartate and alanine aminotransferases activities in DMBA-rats. DMBA = 7,12-dimethylbenz(a)nthracene; BDMC1 = bisdemethoxycurcumin (25 mg/kg); BDMC2 = bisdemethoxycurcumin (50 mg/kg); VIN = Vincristine.

Effects of BDMC on biochemical indices in DMBA-administered rats

Figure 1 depicts rats exposed to DMBA had a slight increase in alanine and aspartate aminotransferase activities by 4 and 6%, respectively, when compared to the control groups (Fig. 1a,b). However, co-administration of BDMC at 25 mg/kg and 50 mg/kg reduced their activities when compared to rats exposed to DMBA alone. Lactate dehydrogenase activity increased significantly by 1.3-fold in rats induced with DMBA when compared to the control (Fig. 2). Similarly, serum malondialdehyde and nitric oxide levels rise by 10 and 12%, respectively, compared to controls (Fig. 3a,c). However, malondialdehyde, nitric oxide, and lactate dehydrogenase activity in DMBA-rats co-treated with BDMC at both doses were reduced by 51, 54%; 18, 10%; and 71, 75%, respectively in a dose dependent manner (Figs. 2 and 3a,c).

Effects of BDMC on antioxidant parameters and inflammatory indices in DMBA-rats

DMBA exposed rats showed drastic increased in the level of mammary malondialdehyde, myeloperoxidase activity by 2.4 and 6.3 folds and a slight increase in mammary nitric oxide levels by 13% when compared to controls (Figs. 3b,d and 4). BDMC drastically reduced the nitric oxide level and myeloperoxidase activity in a dose-dependent manner. In contrast, DMBA administration decreases the levels and activities of reduced glutathione, glutathione-S-transferase, superoxide dismutase and glutathione peroxidase by 15, 39, 31 and 33%, respectively, when compared to controls (Fig. 5a–d). Interestingly, co-treatment with BDMC at both doses increased the levels and activity of reduced glutathione, glutathione-S-transferase, superoxide dismutase and glutathione peroxidase by 30, 33, 76, 48%; 17, 112, 37, 41%, respectively, compared to DMBA-rats.

Immunohistochemistry staining of hormone receptors and apoptosis indices on DMBA-administered rats treated with BDMC.

DMBA-exposed rats show weak expression of BAX activity. On the contrary, DMBA slightly increases BCL-2 activity when compared to control groups (Figs. 6 and 7). The co-administration of BDMC at both dosages lowered and increased the levels of anti-apoptotic and pro-apoptotic proteins. Furthermore, DMBA-treated rats display high expression of the estrogen receptor and human epidermal receptor-2 when compared to controls

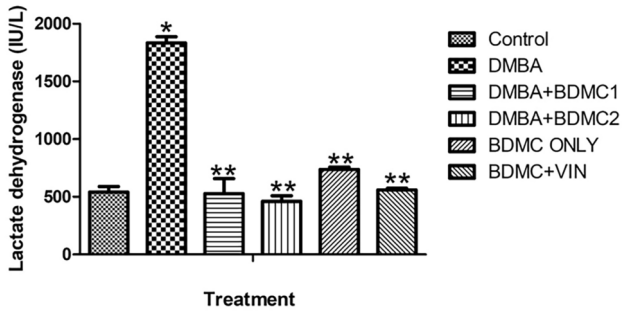

Fig. 2. Effects of BDMC on serum lactate dehydrogenase activity in DMBA- rats. For each group of five to eight animals, values are given as Mean ± Standard Deviation. * denotes a significant difference from the control group (*P* value < 0.05); ** denotes a significant difference from the DMBA group (*P* value < 0.05). DMBA = 7,12-dimethylbenz(a)nthracene; BDMC1 = bisdemethoxycurcumin (25 mg/kg); BDMC2 = bisdemethoxycurcumin (50 mg/kg); VIN = Vincristine.

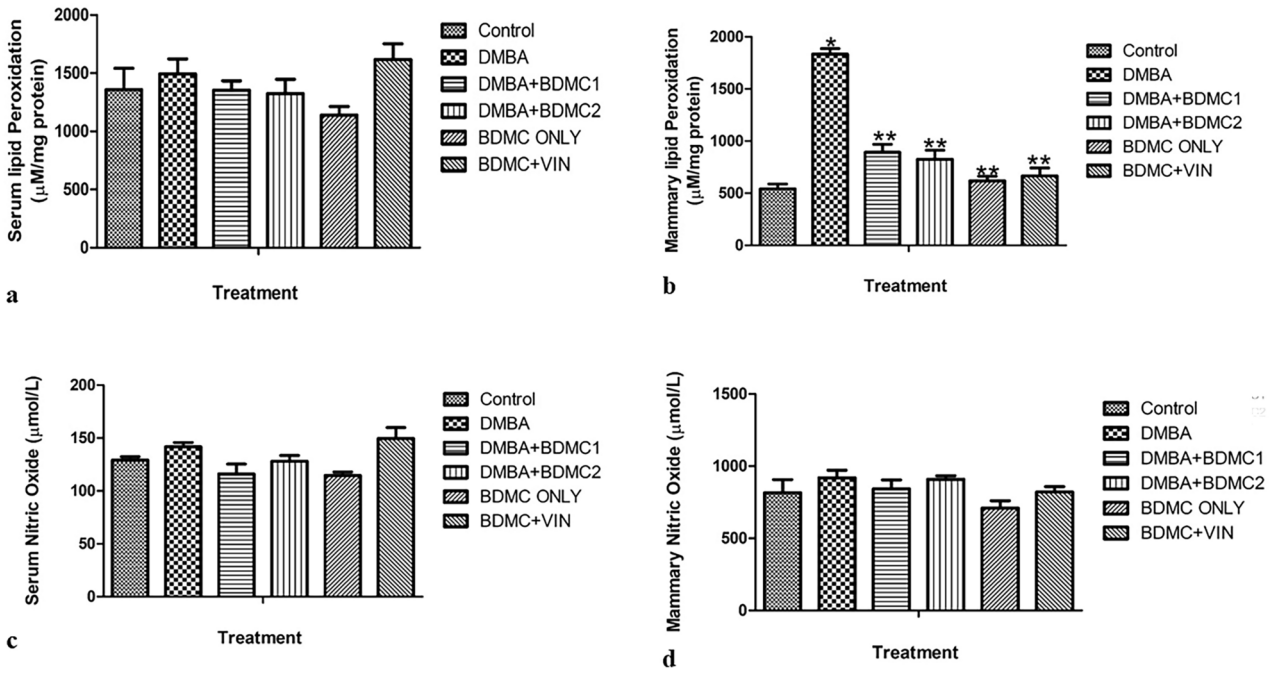

Fig. 3. Effects of BDMC on serum and mammary malondialdehyde and nitric oxide levels in DMBA- rats. For each group of five to eight animals, values are given as Mean ± Standard Deviation. * denotes a significant difference from the control group (*P* value < 0.05); ** denotes a significant difference from the DMBA group (*P* value < 0.05). DMBA = 7,12-dimethylbenz(a)nthracene; BDMC1 = bisdemethoxycurcumin (25 mg/kg); BDMC2 = bisdemethoxycurcumin(50 mg/kg); VIN = Vincristine.

(Figs. 8 and 9). Also, progesterone receptor showed mild expression in DMBA-rats (Fig. 10). However, co-administration with BDMC attenuated the expression of hormone receptors, specifically at high dose.

Representative photomicrographs of BDMC's effects on mammary tissues in rats treated with DMBA

Rats exposed to DMBA only developed tumors at six–seven weeks after DMBA was administered. The histology analysis of rats given DMBA showed proliferating ducts and fibro-collagenous stroma in their mammary gland tissue, along with a high collagen deposit and mild infiltration of inflammatory cells (Fig. 11). The presence of mammary gland tumor was confirmed by macroscopic and histological examinations after 13 weeks of DMBA-administration depicting lymphocytic infiltration (Fig. 11). However, BDMC co-administration, specifically at high dose, depicts non-proliferating ducts and a mild infiltration of inflammatory cells. Similarly, co-treatment with vincristine shows non-proliferating duct and fibro-collagenous stroma with duct lined by inner columnar epithelium hyperplasia and outer myoepithelium in comparison to BDMC-treated groups.

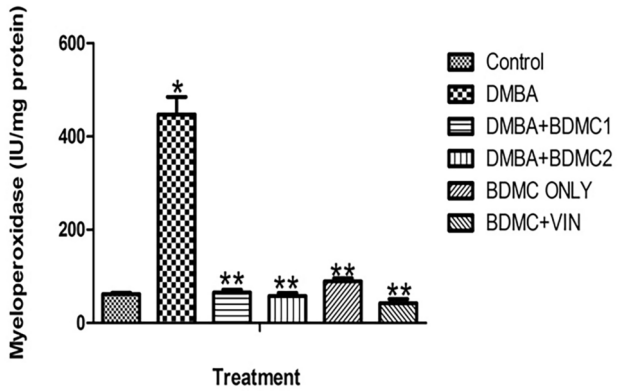

Fig. 4. Effects of BDMC on myeloperoxidase activity in DMBA- rats. For each group of five to eight animals, values are given as Mean ± Standard Deviation. * denotes a significant difference from the control group (P value < 0.05); ** denotes a significant difference from the DMBA group (P value < 0.05). DMBA = 7,12-dimethylbenz(a)nthracene; BDMC1 = bisdemethoxycurcumin (25 mg/kg); BDMC2 = bisdemethoxycurcumin (50 mg/kg); VIN = Vincristine.

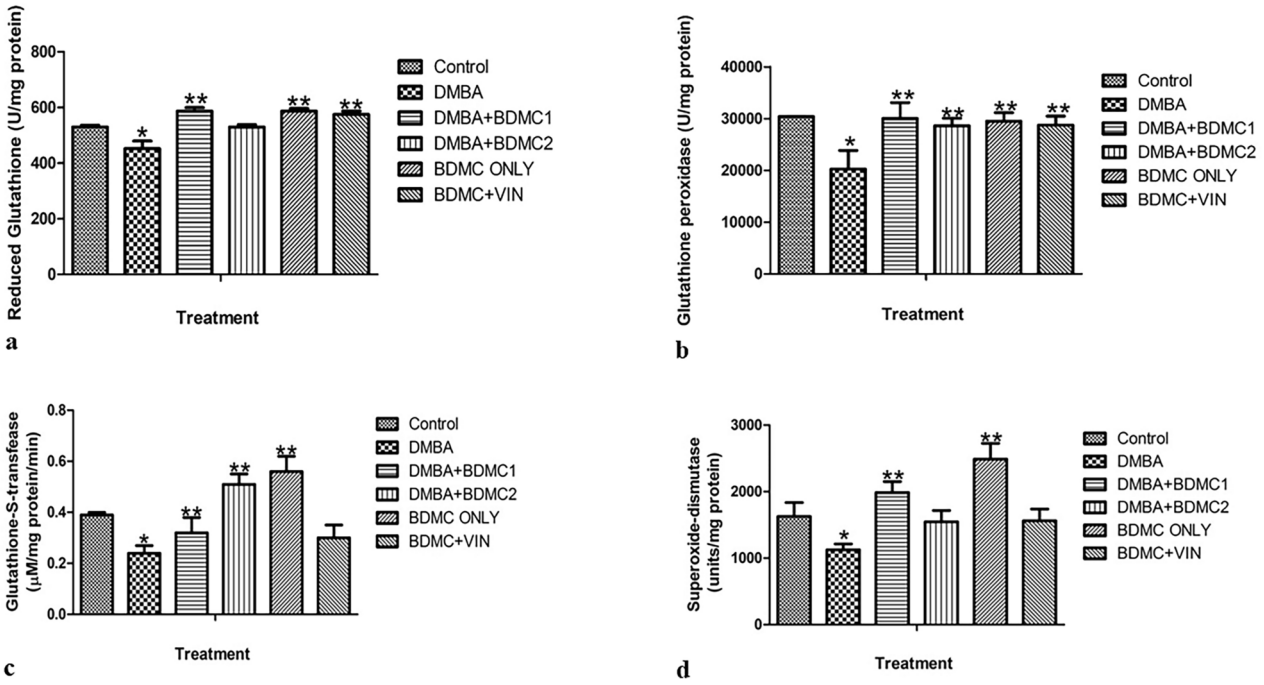

Fig. 5. Effects of BDMC on reduced glutathione, glutathione peroxidase, glutathione-S-transferase and superoxide-dismutase activities in DMBA- rats. For each group of five to eight animals, values are given as Mean ± Standard Deviation. * denotes a significant difference from the control group (P value < 0.05); ** denotes a significant difference from the DMBA group (P value < 0.05). DMBA = 7,12-dimethylbenz(a) nthracene; BDMC1 = bisdemethoxycurcumin (25 mg/kg); BDMC2 = bisdemethoxycurcumin (50 mg/kg); VIN = Vincristine.

Discussion

The current study's investigation shows that administering BDMC and vincristine to rats suppresses the activation of the DMBA multi-step carcinogenesis process, hence preventing the development of tumors in the mammary glands. Our study's results were consistent with those of Singh and his colleagues, who demonstrated that a tiny molecule called Withaferin A, which comes from the medicinal plant *Withania somnifera*, prevents the growth of human breast cancer xenografts and the development of mammary tumors in mouse models without causing any harm[29]. They reported that the fatty acid synthesis pathway is a novel target of Withaferin A in mammary tumors by using *N*-methyl-*N*-nitrosourea to induce mammary tumors in rats in vivo and MCF-7/MDA-MB-231 cells in vitro[29]. In this study, we used DMBA to induce tumors in rats exposed to DMBA

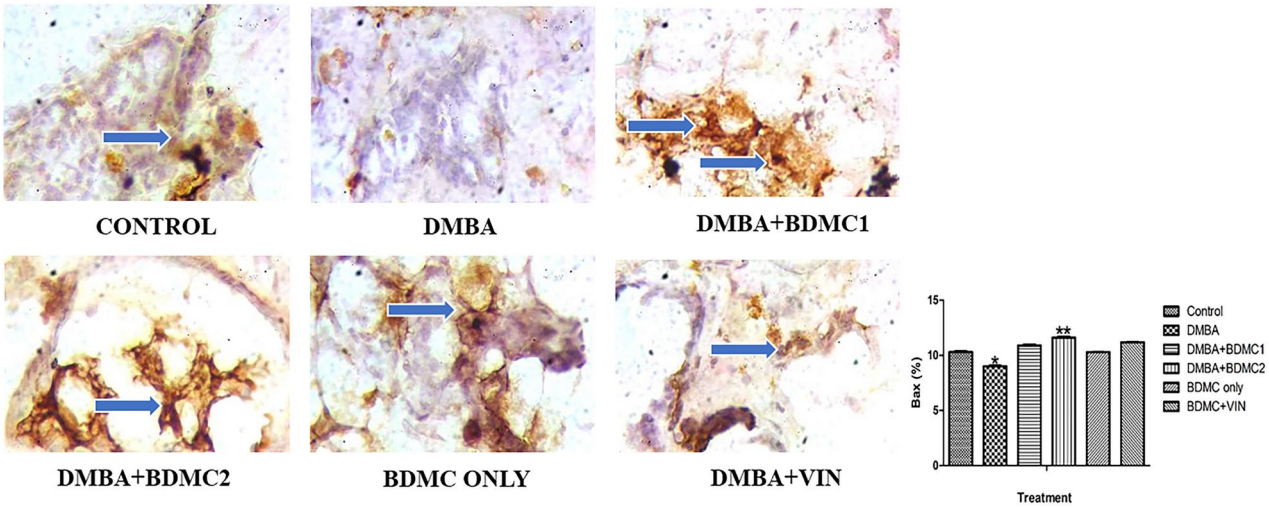

Fig. 6. Effect of BDMC and VIN on BCL-2 associated X-protein in DMBA-rats. The expression of Bax is indicated by the blue arrow.

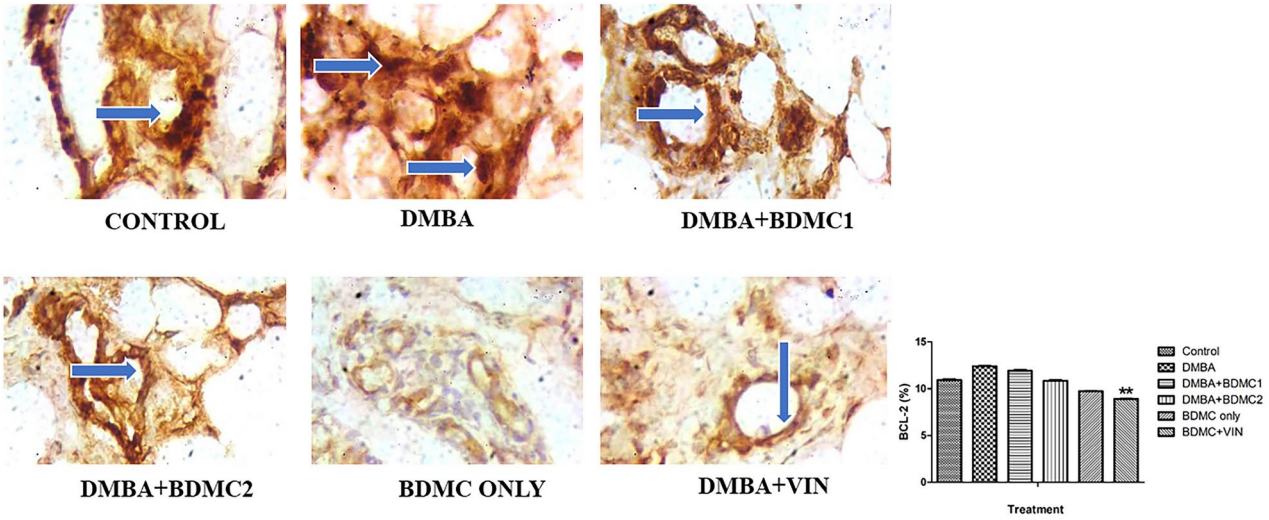

Fig. 7. Effect of BDMC and VIN on B cell lymphoma-2 in DMBA-rats. The expression of BCL-2 is indicated by the blue arrow.

only, while causing damage to the mammary glands in DMBA co-treated groups with BDMC. We discovered that BDMC, at both low and high dosages, safely and effectively suppressed mammary tumor initiation and progression, however, animals who received BDMC alone did not exhibit any particular toxicity or side effects. Furthermore, the apoptotic and antioxidant parameters' results were completely consistent with the histological findings of this study.

The amount of body weight gained by rats exposed to DMBA alone was considerably reduced. This observation aligns with the discoveries of Glory and Thiruvengada[30], who documented a significant decrease in the body weight of rats exposed to *N*-nitrosodiethylamine. However, body weight was not drastically affected in groups treated with BDMC, particularly when the dosage was 50 mg/kg. This suggests that BDMC may help these animals' energy metabolism and increase their appetite. However, mammary gland disorders can result in complicated metabolic issues in test animals, which can lead to tissue degradation and rapid weight loss[31]. Treatment with BDMC decreased organo-somatic and mammary tissue weight in DMBA rats while increasing body weight growth, suggesting that it may be able to restore body weight gain.

The most valuable liver serum enzymes for tracking treatment response and identifying toxicity are aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase[32]. According to a study, the liver metastases of breast tumors showed a substantial rise in aspartate/alanine aminotransferases and alkaline phosphatase[32]. Compared to the controls, the serum aminotransferase concentration dropped over the treatment period[32]. In line with a prior publication on the assessment of nano diosgenin against DMBA-induced renal

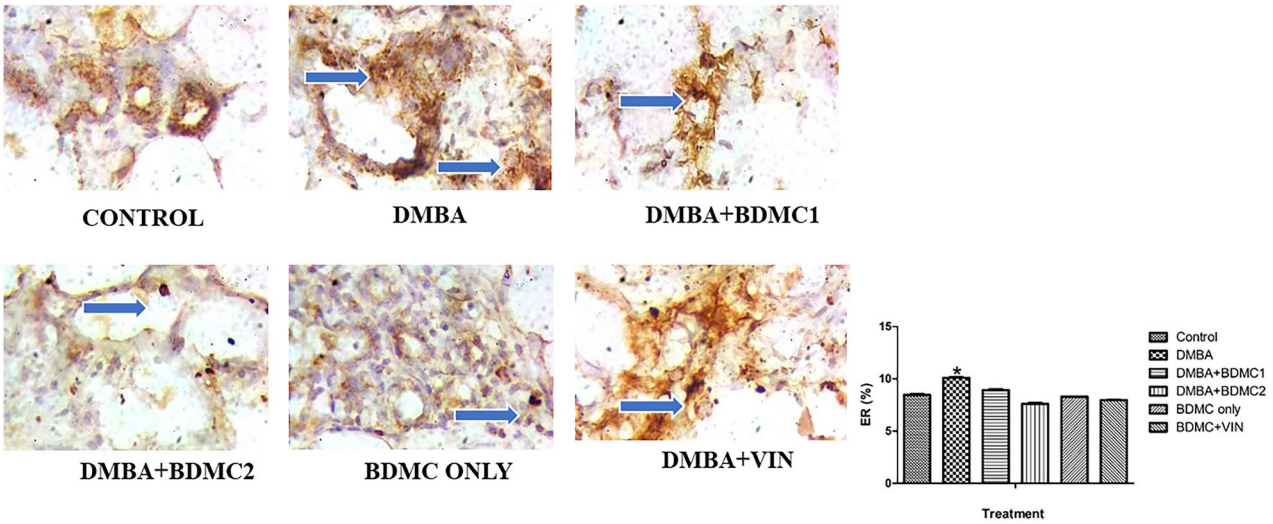

Fig. 8. Effect of BDMC and VIN on human epidermal receptor-2 in DMBA-rats. The expression of HER2 is indicated by the blue arrow.

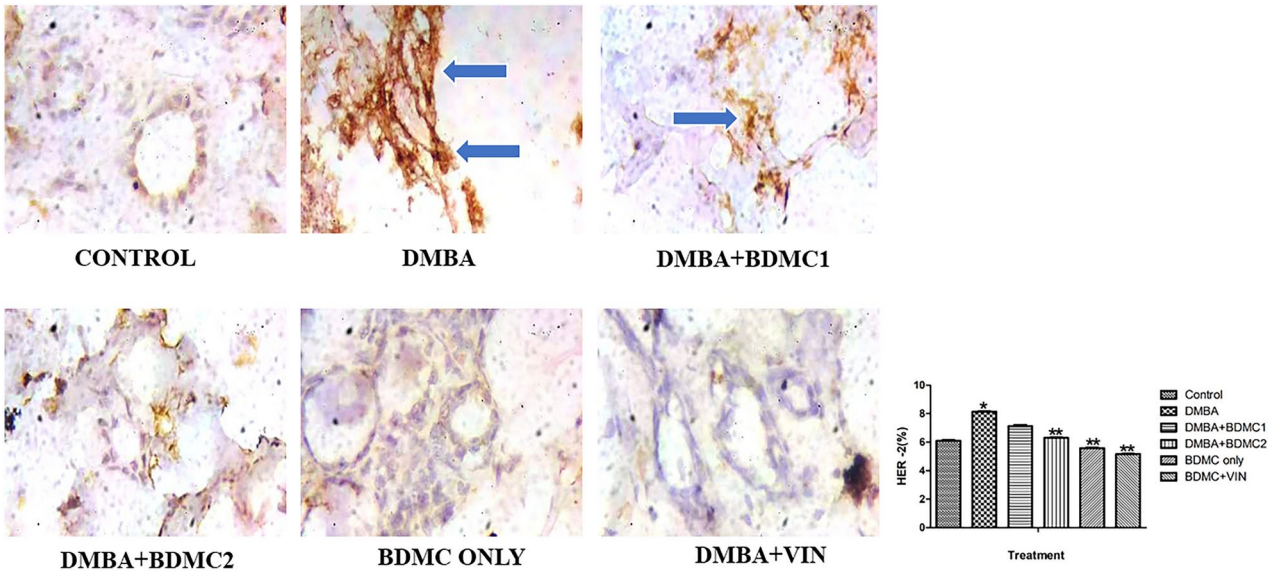

Fig. 9. Effect of BDMC and VIN on estrogen receptor in DMBA-rats. The expression of ER is indicated by the blue arrow.

and hepatic toxicities, this investigation indicates that BDMC and vincristine administration had an impact on serum aspartate/alanine aminotransferases concentrations in DMBA-rats[33]. Since aminotransferases are found in numerous body tissues, it should be mentioned that they are not enzymes that are particular to any specific organ. Nonetheless, identifying mammary gland tumors may benefit from lactate dehydrogenase detection and aminotransferase analysis[34,35]. Patients with breast cancer, ovarian cancer, and endometrial cancer had markedly elevated serum lactate dehydrogenase levels[36]. In contrast to the DMBA-rats, the rats treated with BDMC and vincristine in our investigation exhibited a drastic decrease in serum lactate dehydrogenase, which was indicative of a low tumor volume and a consequent decrease in the release of cytoplasmic lactate dehydrogenase. Thus, BDMC might be a useful chemo-preventive agent for treating mammary gland toxicity. These findings align with a prior case report study[37].

As the first line of defense against reactive oxygen species, endogenous antioxidant enzymes may be useful in determining the likelihood of oxidative damage brought on by carcinogenesis. The primary antioxidant enzyme, superoxide dismutase, combats oxy-radicals by accelerating the conversion of superoxide anion to hydrogen peroxide (H_2O_2). In addition, an enzyme called catalase is responsible for catalysing the removal of H_2O_2 during the superoxide dismutase-catalysed process. Thus, antioxidative enzymes, which offer a prophylactic defence against reactive oxygen species, are stimulated by superoxide dismutase and catalase

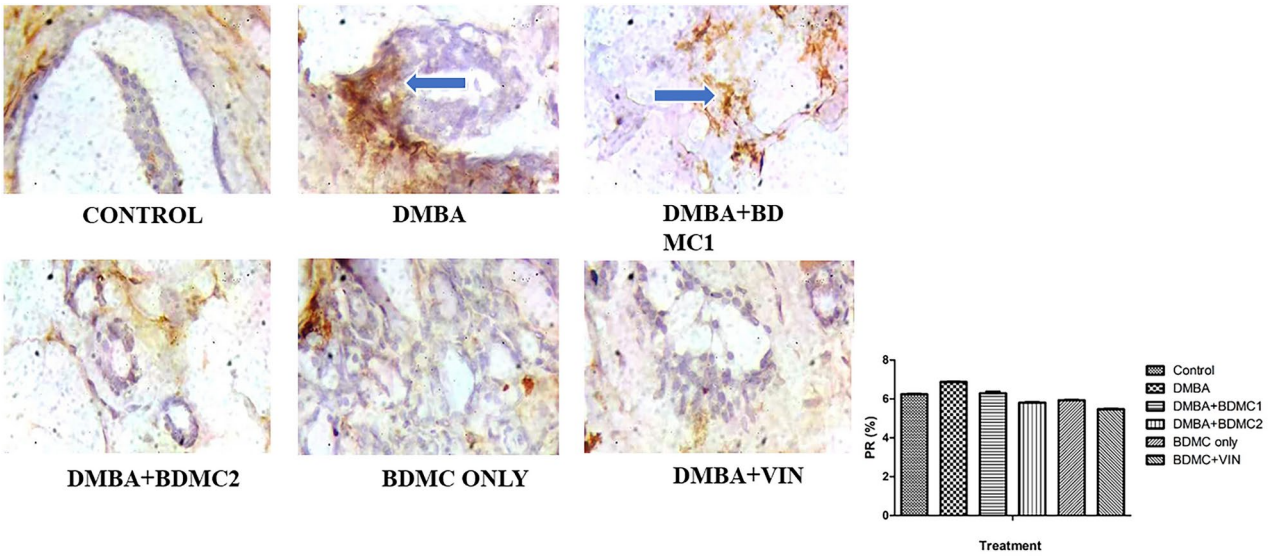

Fig. 10. Effect of BDMC and VIN on progesterone receptor in DMBA-rats. The expression of PR is indicated by the blue arrow.

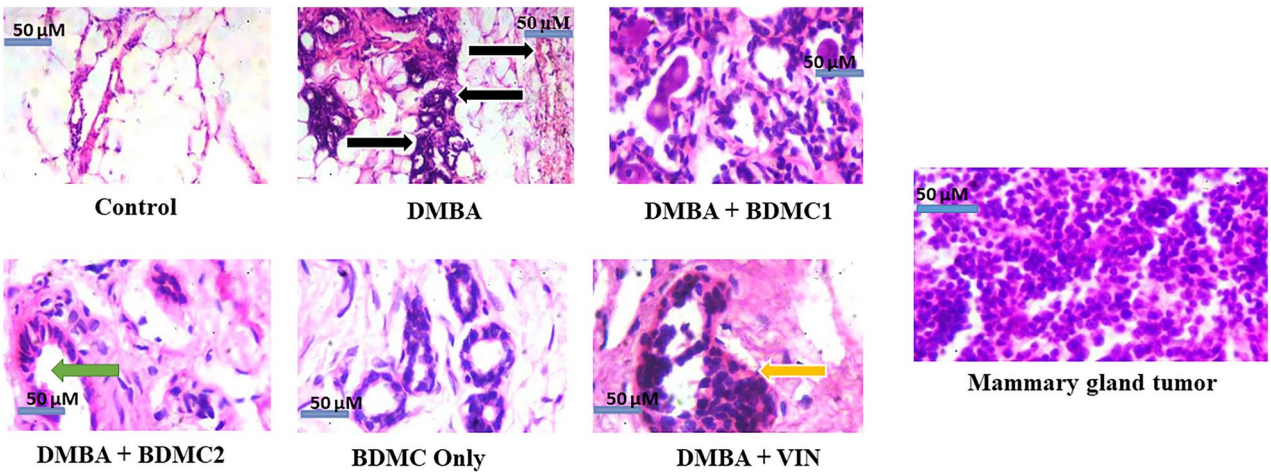

Fig. 11. Effects of BDMC and VIN on mammary gland histology in DMBA-rats. Black arrow depicting fibrocollagenous stroma and proliferating ducts. Green arrow indicating mildly inflammatory cells and non-proliferating ducts. Yellow arrow displays a fibrocollagenous stroma and a non-proliferating duct bordered by hyperplasia of the inner columnar epithelium. White arrow depicting lymphocytic infiltration.

performances in turn[38]. Furthermore, glutathione is a crucial component of glutathione peroxidase, an enzyme that protects against oxidative damage. At the expense of H_2O_2, glutathione peroxidase catalyses the conversion of glutathione to glutathione-disulfide[38,39]. According to the current study, DMBA-rats had lower superoxide dismutase concentrations, which could be related to the decline in antioxidant status brought on by mammary carcinogenesis. The catalase enzyme assay was shown to be decreased in people with benign breast diseases and breast cancer[38]. According to our findings, the DMBA-rats similarly had a lower level of catalase, which may be explained by the use of antioxidant enzymes in the DMBA administration process to eliminate H_2O_2. Our results corroborate the findings of Ibrahim and his colleagues, who demonstrated that α-Mangostin elicits anticancer effects on rat mammary gland tumors induced by LA7 cells[39]. By using anti-proliferative and antioxidant mechanisms, they reported the anti-cancer activities of both low and high doses of α-mangostin on mammary tumors generated by LA7 cells[39].

Malondialdehyde, an index of lipid peroxidation, is highly cytotoxic and acts as a tumor promoter[21]. An increase in free radicals produces an excess of malondialdehyde levels[40]. Malondialdehyde can indicate oxidative stress and affect antioxidant status in cancer patients[40]. The findings from our study revealed that experimental rats exposed to DMBA alone exhibited high levels of malondialdehyde, indicating excessive generation of free radicals causing oxidative stress. This discovery corroborates with previous reports by Kosemani et al.[41], who found elevated amounts

of malondialdehyde and oxidative stress after exposing experimental rats to 7,12-dimethylbenzanthracene. However, our results demonstrated a dose-dependent substantial drop in malondialdehyde levels after co-treatment with BDMC. This further confirms BDMC's anti-oxidative properties.

Furthermore, persistent inflammation has been associated with certain human cancers[42]. A persistent inflammatory environment is thought to be a risk factor for breast cancer and other types of cancer[43]. Myeloperoxidase, a heme-containing peroxidase, is an important component of human white blood cells and the innate immune system. Polymorphonuclear neutrophils largely release it into extracellular fluids to defend against invading pathogens or in reaction to oxidative stress and various inflammatory responses[44,45]. Myeloperoxidase has been related to tumor development by promoting a hyper mutagenic environment, which is caused by the activity of myeloperoxidase-derived oxidants that can oxidize and alter DNA. As a result, myeloperoxidase is recognized as a marker of inflammation and oxidative stress and has frequently been found to be raised in cancer patients[46]. Recent research, however, has linked nitric oxide, a key signaling molecule and inflammatory response produced from L-arginine by nitric oxide synthase, to cancer[44–47]. When DMBA-treated rats were compared to controls, we found higher levels of nitric oxide and myeloperoxidase activity in mammary tissues. Co-administration with BDMC considerably reduced nitric oxide levels and myeloperoxidase activity, indicating its anti-inflammatory properties.

Cancer cells have essential traits that include evasion of apoptosis and dysregulated cell growth. Apoptosis, on the other hand, is critical for removing unwanted, damaged, or infected cells from the body, and several studies have linked it to a variety of biological processes such as cell development, differentiation and proliferation, immunity, and the maintenance of normal cellular homeostasis[48–50]. Several studies have found that down-regulating Bax, a pro-apoptotic member of the Bcl-2 family that promotes apoptosis, prevents apoptosis, whereas up-regulating Bcl-2, an anti-apoptotic member of the Bcl-2 family, also prevents apoptotic cell death[51]. The findings from our study revealed a down-regulation of the pro-apoptotic marker (Bax) and an overexpression of the anti-apoptotic marker (Bcl-2) in the DMBA-rats, indicating apoptosis evasion. However, when rats were treated with BDMC at low and high doses, Bax was up-regulated compared to the DMBA exposed rats, suggesting that BDMC promotes apoptosis. Conversely, Bcl-2 was down-regulated in rats co-treated with BDMC at both doses, respectively. These findings are consistent with the studies of Huang et al.[18] and Xiang et al.[52], who reported the apoptotic potentials of BDMC, stating BDMC treatment resulted in down-regulation of Bcl-2 and Bax expression. Thus, our data further corroborates these findings.

Several research papers have proposed hormonal explanations for the etiology of breast cancer, with a focus on the regulation of sex hormones[53–55]. Evidence from epidemiological, in vivo, and in vitro research has demonstrated that estrogen and progesterone signaling through their receptors play critical roles in the initiation, development, and clinical prognosis of not only breast cancer but also other cancer types[55–57]. As a result, these proteins have emerged as significant and attractive anticancer targets, as well as emerging drivers of anticancer resistance to first-line chemotherapy and ionizing radiation. Our current findings revealed that out of the three protein receptors investigated, estrogen and human epidermal receptor-2 proteins were both elevated, while progesterone proteins were mildly expressed in the mammary tissues of DMBA-rats. However, co-treatment with BDMC considerably reduces the expression of these proteins. Thus, BDMC's simultaneous modulatory effects on estrogen receptor and human epidermal receptor-2 expression in DMBA-induced mammary lesions point to the chemoprotective and anti-tumorigenic potential of BDMC.

Histology of rats exposed to DMBA revealed proliferating ducts and fibrocollagenous stroma with a high collagen deposit and moderate inflammatory cell infiltration. Similarly, the macroscopic and histological examinations following 13 weeks of DMBA administration confirmed the presence of a mammary gland tumor, showing lymphocytic infiltration, which frequently indicates an inflammatory response due to an immune system reaction within a tumor mass. The DMBA-rats co-treated with low and high doses of BDMC demonstrated a significant improvement in the overall tissue structure of the mammary glands, as well as a significant regression in the fibrocollagenous stroma. The rats also showed a lower record of collagen deposits figures compared to the DMBA-rats. These results further support our biochemical findings. This study concludes that BDMC lessens the effects of DMBA-induced toxicity to the mammary glands. As anticipated, we found that in rats given DMBA, BDMC increased antioxidant levels, decreased inflammation, promoted apoptosis, elicited anti-toxicity effects, and restored the cytoarchitecture of the mammary gland. Overall, this study provides insightful information about bisdemethoxycurcumin's potential as a possible defence against lesions of the mammary gland.

Data availability

All data are available on request to the corresponding author (Adedoyin O. Adeoye, PhD).

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Author contributions
A.A. and O.A. Conceived, designed, and supervised the experiments; A.A. and A.F. wrote the main manuscript; A.F., A.A., T.J. and T.A. carried out the investigation; A.F. and T.A. carried out the data analysis. A.A., J.U., T.A., O. A., S.L. reviewed and edit the manuscript. All authors reviewed the manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

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