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Impact of *Clostridium sporogenes* endospores and boswellic acid on breast cancer induced by N-Nitroso-N-methyl urea (MNU) in female rats

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ABSTRACT

Recently, a greater focus has been on investigating bacterial endospores as possible anticancer agents. To improve treatment procedures for cancer, this study examines the effects of gamma radiation-attenuated Clostridium sporogenes endospores as moderators of immune control and Boswellic acid on breast cancer carcinoma growth. Methods: breast carcinoma was induced in adult female albino rats using NMethyl-N Nitrosourea MNU. (50 mg/kg)) i.p. on the days 33, 40, 47, 54, and 61 of age alternately through the left and right abdominal wall) and subsequently treated with endospores (intravenous injection of endospores once at a dose of (60 million spores /Rat) and boswellic acid injected orally at a dose of 250mg/kg/day by oral gavage needle, and samples were taken from rats after one month of endospore injection). Multiple parameters, including oxidative stress (MDA), antioxidant enzyme (GSH), interferon-gamma (IFN-γ), nuclear factor-κB (NFκB), tumor necrosis factor-alpha (TNF-α), vascular endothelial growth factor (VEGF), Caspase-3 and P53, Urea and Creatinine, NO and H_2O_2, JAK and STAT, IL-2 and IL-10 and liver function enzymes were assessed; In addition to histopathological examination of mammary tissue. Results: a remarkable suppression of tumor proliferation and inducing apoptosis through Cas-3 and P53 stimulation was noticed. There was a notable improvement in inflammatory markers (TNF-α, VEGF, IF-γ, and NF-κB) and immunological parameters such as JAK, STAT-3, IL-2, and IL-10. In the serum of the endospore-treated rats, there was a decrease in lipid peroxidation and an improvement in antioxidant status compared to the untreated rats. Elevated liver function enzymes provided additional support for these observed improvements. In conclusion, this work provides strong evidence for the ability of endospores and boswellic acid, either separately or in combination, to target breast tumor cells and modify immune responses, indicating their potential uses as adjuvants to enhance cancer treatment regimens.

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Introduction

Cancers, or malignant tumors, pose a serious risk to people. They are the second greatest cause of death in wealthy countries, behind cardiovascular disease, and the third most common cause in underdeveloped countries, accounting for one in eight fatalities globally (Hulvat et al. 2020). Cancers and their aftereffects dramatically lower patient and family quality of life and raise healthcare and medical costs (Osoba 2021). Nowadays, the most popular and frequently successful treatment method involves surgically removing the tumor and combining it with chemotherapy and radiation (Kareliotis et al. 2020). Numerous novel strategies for the treatment of cancer have been developed as a result of the high death rate and related adverse effects that often follow chemotherapy and/or radiation therapy, including the use of live or attenuated microorganisms (Yang et al. 2021). This led to the theory that bacterial pathogen infection activates macrophages and lymphocytes, resulting in the production of cytotoxic chemicals with anticancer effects (Rommasi 2022). Nevertheless, introducing live bacteria into the body to treat cancer can have serious adverse effects. These reactions can result in infections that worsen and eventually kill the patient, which are thought to be caused by the release of harmful bacterial compounds. As a result, their usage should be limited (Al-Hilu & Al-Shujairi 2020). Therefore, the focus of the research is on separating soluble components produced by bacteria, such as proteins, enzymes, secondary metabolites, and derived peptides and toxins, which may have a targeted effect on cancer cells and so serve as possible anticancer medicines (Ramírez-Rendon et al. 2022).

Bacterial peptides and their metabolites, each with unique features, have been used as anticancer agents (Mohan et al. 2022). The key benefits of potentially therapeutic bacterial peptides are their short size, easily manipulated characteristics, and swift, usually straightforward synthesis (Wang et al. 2022). Bacterial peptides have the capacity to cross cell membranes and can also be very selective and affinite in their inhibition of the growth of many cancer cell types (Tornesello et al. 2020). Additionally, these peptides have demonstrated negligible drug-drug interaction and do not accumulate in particular organs (liver or kidney, for example), which reduces their potentially harmful side effects (Berillo et al. 2021).

Boswellic acid is isolated from the gum resin of Boswellia serrata and Boswellia carteri (Ragab et al. 2024). Boswellic acids [BAs] are pentacyclic triterpenoids belonging to ursane group, which are the major constituents of the gum derived from the plant *Boswellia serrata* Roxb. Ex Colebr. [Family Burseraceae, Syn. B. glabra] commonly known by the names Salai guggal, white guggal, Indian olibanum (Qurishi et al. 2010). Gum resin derived from Boswellia species contains a variety of pentacyclic triterpenes, including boswellic acids, which include b-boswellic acid, 3-acteyl b-boswellic acid, 11-keto-b-boswell lic acid, and 3-acetyl-11-keto-b-boswellic acid (Solanki et al. 2024). Because of their anti-inflammatory and antioxidant properties, BAs have been utilized to treat a variety of inflammatory and infectious conditions, including cancer, endotoxin-induced hepatitis, ulcerative colitis, and Crohn's disease (Thabet et al. 2022). It has been observed that boswelic acid mitigates the metastasis of brain tumors and breast cancer. It is known to induce apoptosis, and tests using an ethanolic formulation against leukemia and brain tumor cells have demonstrated that it can also operate as a strong anti-proliferative agent in addition to inducing apoptosis (Sharma & Jana 2020).

Breast cancer (BC) is the most commonly diagnosed and the second leading cause of cancer-related deaths among women worldwide (Afifi et al. 2020). One in eight women will be diagnosed in her lifetime (Zhu et al. 2023). One of the significant challenges for its treatment is its heterogeneous nature, which determines the therapeutic options (Zeng et al. 2021). Breast cancer is a highly heterogeneous disease. Consequently, breast cancer has fairly complex classifications (Testa et al. 2020). Breast cancer is often first classified based on histopathologic type (do Nascimento et al. 2023). Although invasive ductal carcinoma accounts for the majority of occurrences of breast cancer, other less common subtypes continue to get interest due to their aggressive nature and tendency to arise in diverse patient subpopulations (inflammatory breast cancer, for example, frequently affects younger patients) (Shirsat et al. 2020). The tumor's stage is typically the next most important factor to consider. The primary breast tumor (stage 1) often spreads to surrounding tissues and lymph nodes (stages 2-3) or to distant organs (distant metastasis, or stage 4) as the disease advances (Kavan et al. 2022). Lung, bone, liver, and brain are the most frequent sites of breast cancer metastasis (Kim 2021). Staging is crucial because once the tumor metastasizes; the mortality rate dramatically increases (Barsouk et al. 2020).

In addition, breast cancer is also classified based on the grade and the molecular subtypes, including luminal A, luminal B, HER2 type, and triple-negative type (Al-Thoubaity 2020). For instance, tumors of high grade and without expressing hormone receptors (eg, TNBC) are significantly more aggressive and tend to metastasize

(da Silva et al. 2020). However, many typical treatment methods lose most of their usefulness after cancer spreads because they are either not appropriate for systemic usage or are not very effective against high-grade, metastasized cancer (Wynn & Tang 2022). Therefore, new medicines that are more effective in treating breast cancer are still desperately needed. The purpose of this study is to assess the anticancer potential of Clostridium sporogenes in combination with boswellic acid on BC breast carcinoma in rats that has been caused by N-nitroso-N-methylurea (MNU).

Materials and Methods

Chemicals

Reinforced clostridial medium (RCM) (catalog no. CM0149B), Reinforced clostridial agar (RCA) (catalog no. CM0151B), and peptone (catalog no. LP0037B) were purchased from OXOID LIMITED (England). Trypticase soya broth (TSB) (catalog no.VM836259) and sodium chloride (catalog no. K49451104) were purchased from EMD. Millipore Corporation (Germany). Ammonium sulfate (catalog no.BP212) was obtained from Thermo Fisher Scientific Inc. (USA). N-Methyl-N-nitrosourea MNU (cas no. 684-93-5), purchased from Sigma Chem. Co., (St. Louis, U.S.A.) and all other chemicals were of analytical grade and were obtained from standard commercial suppliers.

Boswellic acids (BA) was obtained from Nature's Way Products (Springville, Utah, USA) in form of tablets (Each tab let contains 307mg Boswellia serrata extract (65% boswellic acids).The tablets were then grounded and suspended in 0.9% pyrogen free isotonic saline (0.9% NaCl) delivered from Otsuka Pharmaceuticals, Japan. While the BPA (purity 99%, CAT # 80–05-7, Sigma-Aldrich, St. Louis, MD, USA) was dissolved in 0.01% (v/v) corn oil as a vehicle for fat-soluble compounds (CAT # 8001–30-7, Sigma-Aldrich, St. Louis, MD, USA).

Irradiation of clostridium for endospore production

Clostridium was irradiated with an attenuating dose of 2 kGy gamma radiations for endospores production at the National Center for Radiation Research and Technology, Cairo, Egypt. This was done using an Indian Cobalt-60 gamma chamber 4000 A irradiator at a dose rate of 2.5 Krad/h at the time of experimentation (Cote et al., 2018).

Clostridium sporogenes Endospore preparation

The American Type Culture Collection (ATCC) provided Clostridium sporogenes ATCC 19404 (catalog no. 0317), which was anaerobically cultivated in RCM for three days at 30-35ºC. After that, 500 mL of sporulation medium (consisting of 1% peptone, 3% trypticase, and 1% (NH4)2SO4)) was made and put into screw-cap tubes, each of which held 20 ml of media. Next, each tube of sporulation media was infected with 1 milliliter of growing culture.

All procedures were carried out in an aseptic environment with laminar airflow in a biosafety cabinet, following the guidelines of Perkins (1965) and Yang et al (2009) with minor adjustments. Then, in order to promote endospore production, inoculated tubes were incubated for three days under strictly anaerobic conditions (at 30-35ºC for two days and 40-45ºC for the final day).

Bacterial cells were heated and shocked (80°C for 15 minutes) in a water bath before being shocked in an ice-cold container according to Yang et al (2009) with brief modification.

Irradiation and Purification

The bacterial culture was irradiated using a gamma irradiation unit of cobalt (Co 60) for a dose of 2 kGy at the National Center for Radiation Research and Technology, Cairo, Egypt. This was done using an Indian Cobalt-60 gamma chamber 4000 A irradiator at a dose rate of 2.5 Krad / h at the time of experimentation (Cote et al., 2018). Endospores were purified by centrifugation (12,850 xg for 10 min at 4℃) and multiply washed by deionized water. To remove any leftover vegetative cells and enforce endospore release, endospore pellets were resuspended in PBS (1x) supplemented with lysozyme (500µg/ml) and incubated for 2 hours at 37℃ followed by ultrasonication for 5 min. Finally, endospores were extensively washed with deionized water according to various investigators (Brown et al. 1957; Powers 1968; Nicholson & Setlow 1990; Yang et al 2009).

Endospore Culturability and Calculation

To test the endospore culturability, firstly saline solution of 0.9 % sodium chloride for serial dilution purposes and RCA 52.5 g/L for culturing each dilution plate were prepared. The purified spores were suspended in 10 ml of saline making stock solution followed by serial dilution till 10-8 dilution. Then, 2 ml from each dilution were separated into 2 Petri dishes then RCA media were poured into plates Followed by incubation in an anaerobic condition GasPakTM EZ Anaerobe pouch system (Becton Dickinson, Sparks, MD) at (30-35ºC) for 3 days. Colonies were counted and the average number was designated as colony forming units (CFU) per volume of the original sample and the number of spores in stock solution equaled almost 6.1×10^{10} spore/ml. The culturability was

determined as the percentage of spores capable of forming colonies.

Animals

The female adult Swiss albino rats (120-150g) used in this study were obtained from the breeding unit of the National Centre for Radiation Research and Technology. Rats were subjected to acclimatization and maintained on a standard commercial pellet diet and water ad libitum for one week.

Ethics approval statement

This experiment was carried out according to recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH no. 85:23, revised 1996) and in compliance with ethical regulations of the National Centre for Radiation Research and Technology (NCRRT) and was approved by the Central Scientific Publishing Committee of the Egyptian Atomic Energy Authority (Ref No: 188/10/2019). All efforts were made to minimize suffering of animals. Also, the study is reported in accordance with ARRIVE guidelines (https://arriveguidelines.org).

Induction of breast cancer

MNU (50 mg/kg) will be injected i.p. on days 33, 40, 47, 54, and 61 of age alternately through the left and right abdominal wall. The MNU always dissolved immediately before use in 0.9 % NaCl adjusted to pH 4 with acetic acid. The solubility of MNU in water at room temperature was 1.4 % (w/v). The experiment will be terminated on the 117th day of the animal's age.

Endospore injection

Endospores were intravenously IV administered once at a dose level of (60 million spores /Rat) according to Möse and Möse (1964) with brief modification.

Experimental design

Rats were categorized into 5 equal groups of 10 rats each as follows: Group 1 (Control): Normal healthy control animals. Group 2 (Tumor bearing rats BC): animals received Methyl-N-nitrosourea (MNU (50 mg/kg)) injected i.p. on days 33, 40, 47, 54, and 61 of age alternately through the left and right abdominal wall. Group 3 (Tumor bearing rats treated with Boswellic acid): rats received MNU (250 mg/kg) as in group 2 and treated with BA at dose of 250mg/kg/day, by oral gavage needle (Barakat et al. 2018). Group 4 (Tumor bearing rats treated with Endospore): animals received (MNU) as in group 2 then treated with Endospore (IV administered once at a dose level of (60 million spores /Rat). Group 5 (Tumor bearing rats treated with Endospore and Boswellic acid): received (MNU) then treated with Endospore (IV administered once and treated with BA.

Sampling and preparation of blood and tissue

Rats were anesthetized with urethane at the end of the experiment (after one month of intravenous injection of endospores). Blood samples were gathered by cardiac perforation, permitted to coagulate at ambient temperature, and then centrifuged at 4000 rpm for 15 min using a centrifuge (Hettich Universal 32A, Germany). The collected sera was further separated and kept at −80°C until use. Mammary tissues were excised. A portion was then washed in ice-cold saline solution and homogenates were prepared for biochemical estimation, while the remaining part was used for histopathological investigation.

Biochemical Assay

The oxidative status was evaluated in serum by measuring the malondialdehyde (MDA) (as an indicator of lipid peroxidation) (ab287860), and reduced glutathione (GSH) (as an indicator of antioxidant level) (CAT NO MBS265966). Hydrogen Peroxide/Peroxidase (MBS9355746) and Rat Total Nitric Oxide (MBS723386). Urease Activity Assay Kit (Colorimetric) (ab204697) and Creatinine Assay Kit (Colorimetric) (ab204537). Albumin (BCG) Assay Kit (Colorimetric) (ab235628).

Activities of Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined in serum according to the method of Reitman and Frankel (1957) to check liver functional status. All photometric methods were done using (Thermo Electron UV-Visible Spectrophotometers USA). The level of IL-10 (CAT #MBS702776) was estimated by ELISA kits My BioSource according to the manufacturer's instructions, for the enzyme-linked immunosorbent assays via ELISA microplate reader (DV990 BV 416; Gio. DE VITA and CO., Rome, Italy). Rat-specific ELISA kits (R&D Systems) were used to measure the levels of inflammatory mediators, tumor necrosis factor-α (TNF-α), and NF-KB in enzyme-linked immunosorbent assays using an Elisa microplate reader (DV990 BV 416; Gio.DE VITA and CO., Rome, Italy) following the manufacturer's instructions.

The level of Rat VEGF (ab100786) was estimated by ELISA kits abcam according to the manufacturer's instructions, for the enzyme-linked immunosorbent assays via ELISA microplate reader. Interferon Gamma (IFN-γ) level in liver tissue homogenate of each group was assayed using Rat specific (IFN- γ) ELISA kit

purchased from Cloud-Clone Corp. (CCC, USA), Catalog no. SEA049Ra. Caspase 3 levels were also detected in the tumor tissue using the ELISA kit from LSBio (Rat CASP3 / Caspase 3 (Sandwich ELISA) ELISA Kit - LS-F4138) as per the manufacturer's instructions.

Histopathological examination

Rats from various groups had autopsies, and the breast tissues were removed. The breast samples were preserved in 10% formalin saline for a whole day. After that, tissue were cleaned with tap water and serial dilutions of alcohol (methyl, ethyl, and 100% ethyl) were applied to dehydrate them. Following that, the specimens were cleaned in xylene and immersed in paraffin for 24 hours at 56oC in a hot air oven. The tissue blocks' paraffin wax was created, and a slide microtome was used to segment the tissue blocks at a thickness of 4 mm. In accordance with Banchroft et al (1996), the resultant tissue sections were then collected on glass slides, de-paraffinized, and stained with hematoxylin and eosin (H&E) stain for routine examination using a light electric microscope.

Statistical analysis

Statistical analysis was accomplished by one-way analysis of variance (ANOVA) then followed by Tukey–Kramer multiple comparison testsGraph Pad prism 8 was used for statistical analysis (Graph Pad Software Inc, San Diego, California, USA). Data were expressed as mean values ± standard error of the mean (SEM) and differences between values are considered significant at $P < 0.05$.

Results

Endospore culturability and colony count

To ensure that endospores still can regerminate, the culturability of the purified endospores was done. Then serial dilution was performed to select the applicable concentration used in the treatment dosage. Dilution (10^{-7}) was the applicable dilution to determine the concentration of endospores dosage (Table 1).

Effect of gamma radiation attenuated endospore along with Boswellic Acid, on oxidative stress (MDA) and antioxidant activity (GSH)

Oxidative stress in the serum was determined in terms of MDA (end product of lipid peroxidation) and non-enzymatic (GSH) antioxidants. The figure 1 shows that the serumlevels of MDA and GSH increased and decreased significantly ($P < 0.05$) in rat of the BC group, respectively compared to normal control rats. The concentration of MDA in the serum decreased significantly ($P<0.05$) in BC+BA, BC+E and BC+BA+E groups while the activity of GSH increased significantly ($P< 0.05$) in BC+BA, BC+E and BC+BA+E groups; compared to their equivalent values in control rat (Fig 1). The severity of changes for the two previously mentioned parameters is at the most in BC+E+BA group.

Table 1 Serial dilution of purified endospores stock solution..

	CFU/ plate no.1	CFU/ plate no.2	Average (CFU/ plate)	CFU/ml
Stock solution	NA	NA	NA	$6.11*10^{10}$
Working solution	NA	NA	NA	$6.11*10^{9}$
10^{-1}	TNTC	TNTC	NA	$6.11*10^{8}$
10^{-2}	TNTC	TNTC	NA	$6.11*10^{7}$
10^{-3}	TNTC	TNTC	NA	$6.11*10^{6}$
10^{-4}	TNTC	TNTC	NA	$6.11*10^{5}$
10^{-5}	TNTC	TNTC	NA	$6.11*10^{4}$
10^{-6}	TNTC	TNTC	NA	$6.11*10^{3}$
10^{-7}	558	664	611	$6.11*10^{2}$

TNTC: Total Numerous Total Count. CFU: Colony Forming Unit. NA: Not Applicable

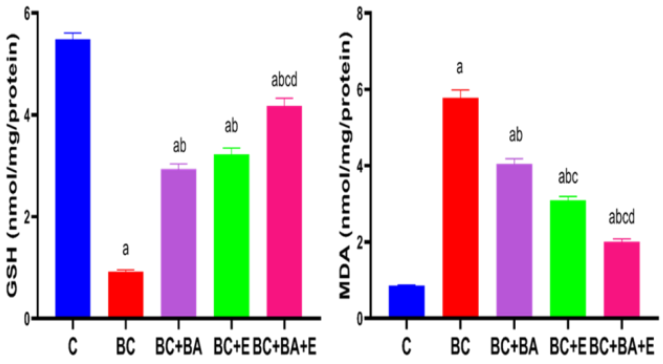

Fig 1. Effect of endospores along with Boswellic Acid on MDA level and GSH activity in different animal groups. C: normal control, BC: MNU injected Rat (untreated BCC), BC+BA: rats injected with MNU then treated by Boswellic Acid, BC+E: Rat injected with MNU and treated by endospores. BC+BA+E: rats injected with MNU then treated by combination of Boswellic Acid and Endospores. Columns denoted with "a" significant from C, "b" significant from BC, "c" significant from BC+BA, "d" signifcant from BC+E, Each value represents the mean ± SE (n = 6) at ($p<0.05$).

Effect of gamma radiation attenuated endospore along with Boswellic Acid on NO and H_2O_2

Herein, we investigated the impact of gamma radiation attenuated endospore along with Boswellic Acid on NO and H_2O_2. In figure 2, a significant decrease in NO levels in the untreated (BC) group when compared with the normal rats group (C). Treatment with Endospores showed a significant increase in NO levels in serum of the BC+BA, BC+E and BC+BA+E groups; when compared with MNU-injected rats (BC). In the other hand, the concentration of H_2O_2 decreased significantly ($P < 0.05$) in BC group, compared with its corresponding value in control group (Fig.2). Meanwhile, the concentration of H_2O_2 increased significantly ($P < 0.05$) in serumof the BC+BA, BC+E and BC+BA+E groups when compared with MNU-injected rats (BC) ($P < 0.05$). In the BC+BA+E group, the modifications for these two parameters are the most severe.

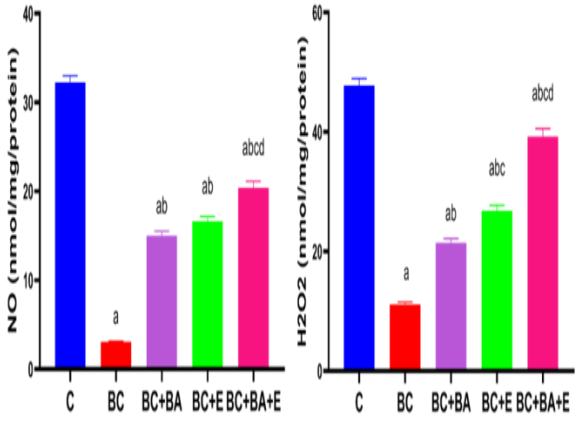

Fig 2. Effect of endospores along with Boswellic Acid on NO and H_2O_2 in different animal groups. C: control, BC: MNUinjected Rat (untreated BCC), BC+BA: rats injected with MNU then treated by Boswellic Acid, BC+E: Rat injected with MNUand treated by endospores. BC+BA+E: rats injected with MNU then treated by combination of Boswellic Acid and Endospores. Columns denoted with "a" significant from C, "b" significant from BC, "c" significant from BC+BA, "d" signifcant from BC+E, Each value represents the mean ± SE (n = 6) at ($p<0.05$).

Effect of gamma radiation attenuated endospores along with Boswellic Acid on Urea and Creatinine

The urea and creatinine levels were surveyed in this study. As depicted in figure 3, rats of BC group showed a pronounced increment ($p < 0.05$) in serum urea and creatinine levels when compared to the control set. Meanwhile, injection of Boswellic acid or Endospores; each alone or combined significantly abolished levels of urea and creatinine in BC+BA, BC+E and BC+BA+E groups ($p < 0.05$) when compared with the BC group (Fig. 3).

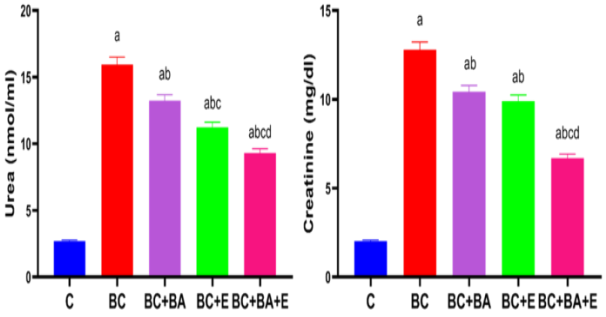

Fig 3. Effect of endospores along with Boswellic Acid on serum urea and creatinine in different groups. C: control, BC: MNUinjected Rat (untreated BCC), BC+BA: rats injected with MNU then treated by Boswellic Acid, BC+E: Rat injected with MNUand treated by endospores. BC+BA+E: rats injected with MNU then treated by combination of Boswellic Acid and Endospores. Columns denoted with "a" significant from C, "b" significant from BC, "c" significant from BC+BA, "d" signifcant from BC+E, Each value represents the mean ± SE (n = 6) at ($p<0.05$).

Effect of gamma radiation attenuated endospores along with Boswellic Acid on ALT and AST

To ascertain the severity of liver injury provoked by BC induction, liver function tests (AST and ALT) were performed. The activity of ALT and ALT enzymes in the serum of different groups was recorded and showed a significant increase in the untreated group (MNU group-BC) when compared with normal control rats.
Treatment with Endospores and Boswellic acid, each alone or combined; showed a significant decrease in ALT and AST activities when compared to the untreated BC group. Fig.4 represents ALT & AST activity in the serum of different groups (BC+BA, BC+E and BC+BA+E groups) ($p < 0.05$) respectively. The severity of changes for AST and ALT, is at the most in BC+BA+E group.

Effect of gamma radiation attenuated endospores along with Boswellic Acid on Immunological Parameters (NFkB, TNFα, VEGF and IFNγ)

Our data indicated that NFkB, TNFα, VEGF and IFNγ levels in breast tissue showed a significant increase in the untreated group (BC group) when compared with control normal rats (Fig.5). In the other hand, treatment with Endospores and Boswellic acid, each alone or combined in BC+BA, BC+E and BC+BA+E groups ($p < 0.05$) respectively; showed a significant decrease in

NFkB, TNFα, VEGF and IFNγ levels; when compared to the untreated BC group (P<0.05-Fig.5). The severity of changes for all the previously mentioned parameters is at the most in BC+BA+E group. However, the changes in all of these parameters are significantly ameliorated in BC+BA, BC+E and BC+BA+E groups when compared with their corresponding values in BC group, as shown in fig. 5.

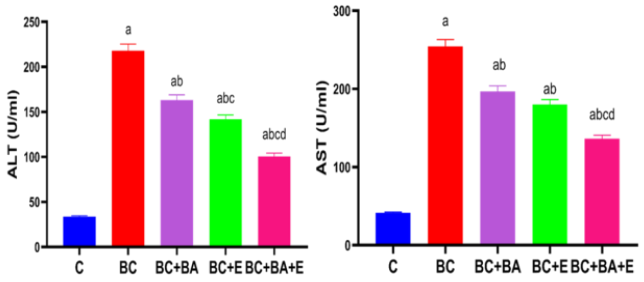

Fig 4. Effect of endospores or Boswellic Acid, each alone or combined; on ALT and AST activities in different rat groups. C: control, BC: MNUinjected Rat (untreated BCC), BC+BA: rats injected with MNU then treated by Boswellic Acid, BC+E: Rat injected with MNUand treated by endospores. BC+BA+E: rats injected with MNU then treated by combination of Boswellic Acid and Endospores. Columns denoted with "a" significant from C, "b" significant from BC, "c" significant from BC+BA, "d" signifcant from BC+E, Each value represents the mean ± SE (n = 6) at (p<0.05).

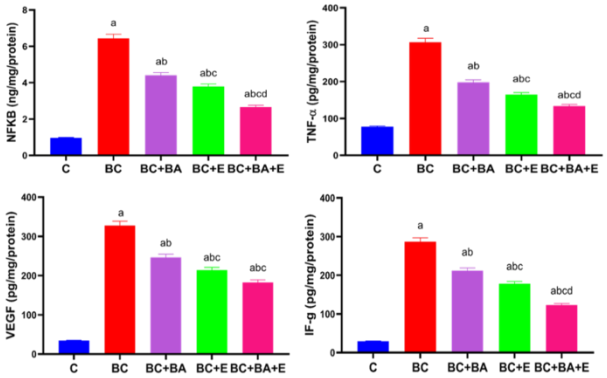

Fig 5. Effect of γ-irradiation attenuated Endospore or Boswellic Acid, each alone or combined in NFkB, TNFα, VEGF and IFNγ levels in Breast carcinoma in rats. C: control, BC: MNU injected Rat (untreated BCC), BC+BA: rats injected with *MNU* then treated by Boswellic Acid, BC+E: Rat injected with MNU and treated by endospores. BC+BA+E: rats injected with *MNU* then treated by combination of Boswellic Acid and Endospores. Columns denoted with "a" significant from C, "b" significant from BC, "c" significant from BC+BA, "d" signifcant from BC+E, Each value represents the mean ± SE (n = 6) at (p<0.05).

Influence of gamma radiation attenuated endospores along with Boswellic Acid on Caspase-3 and P53

Apoptosis is regulated by the protein Caspase-3. Cas-3 activity in the serum of each group was assayed as shown in Fig. 6, the expression of caspase-3 was found to significantly decrease in BC group when compared with control normal rats.

In the other hand, treatment with Endospores and Boswellic acid, each alone or combined in BC+BA, BC+E and BC+BA+E groups (p < 0.05) respectively; showed a significant increase in Caspase-3 activity (Fig.6). Moreover, the concentration of P53 decreased significantly (P< 0.05) in BC group compared to normal control (Fig. 6). However, in BC+BA, BC+E and BC+BA+E groups (p < 0.05), the concentration of P53 increased significantly compared to their corresponding values in BC group (P< 0.05) (Fig. 6). The severity of changes for all the previously mentioned parameters is at the most in BC+BA+E group. However, the changes in all of these parameters are significantly ameliorated in BC+BA, BC+E and BC+BA+E when compared with their corresponding values in BC group.

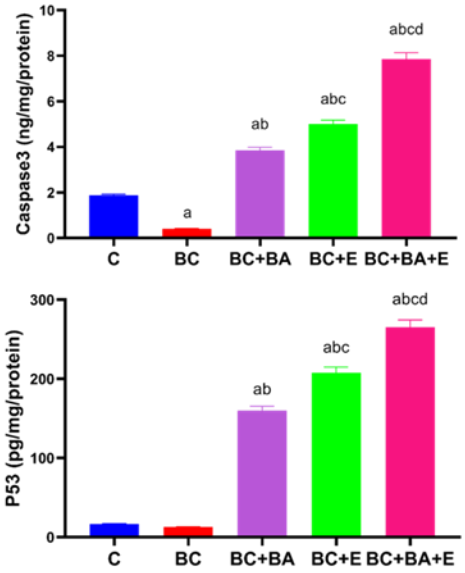

Fig 6. Immune stimulating effect of γ-irradiation attenuated Endospore or Boswellic Acid, each alone or combined in cas-3 level in Breast carcinoma in rats. C: control, BC: MNU injected Rat (untreated BCC), BC+BA: rats injected with MNU then treated by Boswellic Acid, BC+E: Rat injected with MNU and treated by endospores. BC+BA+E: rats injected with MNU then treated by combination of Boswellic Acid and Endospores. Columns denoted with "a" significant from C, "b" significant from BC, "c" significant from BC+BA, "d" signifcant from BC+E, Each value represents the mean ± SE (n = 5) at (p<0.05).

Effect of gamma radiation attenuated endospores or Boswellic Acid, each alone or combined on JAK and STAT-3

The Figure 7 shows that the mammary levels of JAK and STAT-3 decreased significantly ($P < 0.05$) in rat of the BC group compared to the normal group (C). While results showed a significant increase in JAK and STAT-3 levels in the BC+BA, BC+E and BC+BA+E groups ($p < 0.05$), when compare the values of these parameters to their equivalent values in BC (Fig. 7). The severity of changes for JAK and STAT-3 is at the most in BC+BA+E group. However, the changes in all of these parameters are significantly ameliorated in BC+BA, BC+E and BC+BA+E groups when compared with their corresponding values in BC groups.

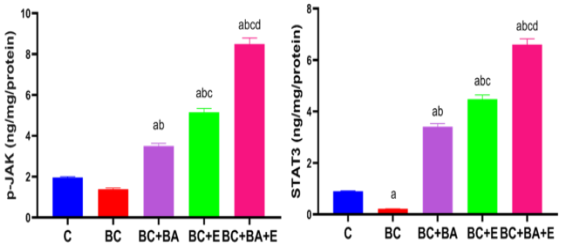

Fig 7. immune stimulating effect of γ-irradiation attenuated Endospore or Boswellic Acid, each alone or combined in JAK and STAT-3 in Breast carcinoma in rats. C: control, BC: MNU injected Rat (untreated BCC), BC+BA: rats injected with MNU then treated by Boswellic Acid, BC+E: Rat injected with MNU and treated by endospores. BC+BA+E: rats injected with MNU then treated by combination of Boswellic Acid and Endospores. Columns denoted with "a" significant from C, "b" significant from BC, "c" significant from BC+BA, "d" signifcant from BC+E, Each value represents the mean ± SE (n = 6) at ($p<0.05$).

Effect of gamma radiation attenuated endospores or Boswellic Acid, each alone or combined on IL-2 and IL-6 levels

Figure 8 shows that the serum levels of IL-2 and IL-6 decreased significantly ($P < 0.05$) in rats of the BC group. In the other hand, results showed a significant increase in IL-2 and IL-6 levels in the BC+BA, BC+E and BC+BA+E groups ($p < 0.05$), when compare the values of these parameters to their equivalent values in BC (Fig 8). The severity of changes for all the previously mentioned parameters is at the most in BC+BA+E group. However, the changes in all of these parameters are significantly ameliorated in BC+BA, BC+E and BC+BA+E when compared with their corresponding values in BC group, especialy for IL-10 level.

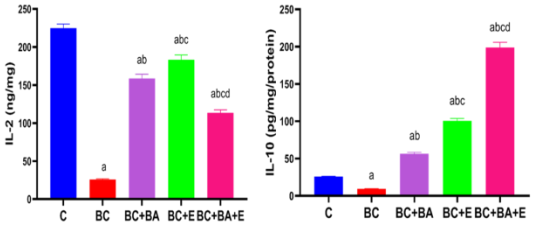

Fig 8. Immune stimulating effect of γ-irradiation attenuated Endospore or Boswellic Acid, each alone or combined in IL-2 and IL-10 in Breast carcinoma in rats. C: control, BC: MNU injected Rat (untreated BCC), BC+BA: rats injected with MNU then treated by Boswellic Acid, BC+E: Rat injected with MNU and treated by endospores. BC+BA+E: rats injected with MNU then treated by combination of Boswellic Acid and Endospores. Columns denoted with "a" significant from C, "b" significant from BC, "c" significant from BC+BA, "d" signifcant from BC+E, Each value represents the mean ± SE (n = 6) at ($p<0.05$).

Histopathological examination

The microscopic examination of breast tissue of normal control rats showed normal histology structure which consisted of tubuloalveolar glands that was formed of branched system of ducts and secretory alveoli arranged in multiple lobules (Fig. 9). Meanwhile, examinations of mammary gland sections from BC group revealed complete destruction of the mammary gland architecture and replaced by dense populations of malignant tumor cells. The mammary tissue showed malignant myoepithelioma arranged in a characteristic storiform pattern. The cells appeared as plump spindle cells with ovoid nuclei and eosinophilic cytoplasm. The tumor cells showed characteristic signs of malignancy, including hyperchromatic, pleomorphism, and frequent atypical mitosis with numerous mitotic figures. Anaplastic cells were frequently detected (Fig. 10).

BC+BA group showed some dysplastic changes that illustrated by hyperchromatic nuclei of epithelial lining with mild to moderate anisocytosis and anisokaryosis. Marked reduction of number of neoplastic cells was observed in the examined tissue sections accompanied by abundant fibroplasia in mammary gland of BC+E group (Fig.11). Examination of BC+BA+E group showed moderate improvement that characterized by reduction in the neoplastic mass with storiform pattern of neoplastic cells with individual scattered necrosis of neoplastic cells. Fewer number of anaplastic cells were observed in the mammary neoplasm compared to PC group. Mild hyperplasia of

ductal epithelium was observed in some examined sections (Fig. 12).

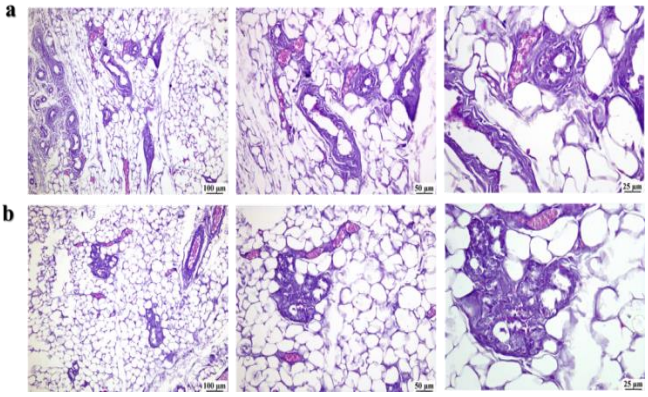

Fig 9. Photomicrograph of normal breast tissue section showing photomicrograph of mammary gland from control group showing normal mammary lobules embedded in adipose tissue (H&E) (a), photomicrograph of mammary gland from control group showing normal mammary acini and duct (H&E) (b).

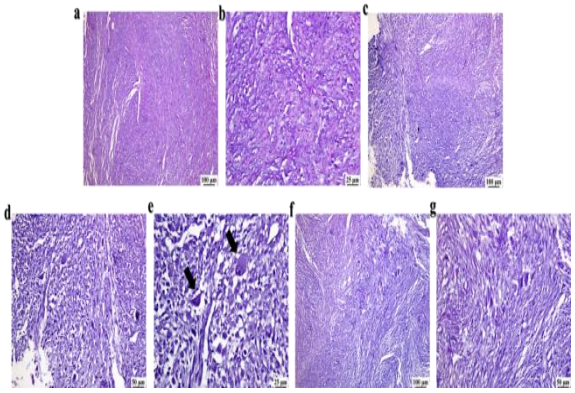

Fig 10. photomicrograph of mammary gland from BC group showing Malignant myoepithelial cells arranged in a characteristic storiform pattern (H&E) (a), plump spindle malignant myoepithelial cells displaying criteria for malignancy (H&E) (b), plump spindle malignant myoepithelial cells (H&E) at higher magnification (c), plump spindle malignant myoepithelial cells displaying criteria for malignancy (karyomegaly and pleomorphism) (H&E) (d), plump spindle malignant myoepithelial cells displaying criteria for malignancy (karyomegaly and pleomorphism) in the presence of anaplastic cells (arrows) (H&E) (e), malignant myoepithelial cells arranged in a characteristic storiform pattern (H&E) (f), a typical mitosis with numerous mitotic figures (arrow) (H&E × 50) (g).

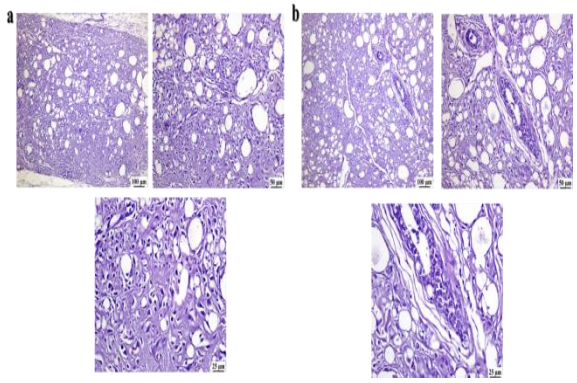

Fig 11. Photomicrograph of mammary gland from BC+BA group (a) showing abundant fibroplasia replacing neoplastic cells (H&E) and from BC+E group (b) showing abundant fibroplasia replacing neoplastic cells with mild hyperplasia of ductal epithelium (arrow) (H&E) (b).

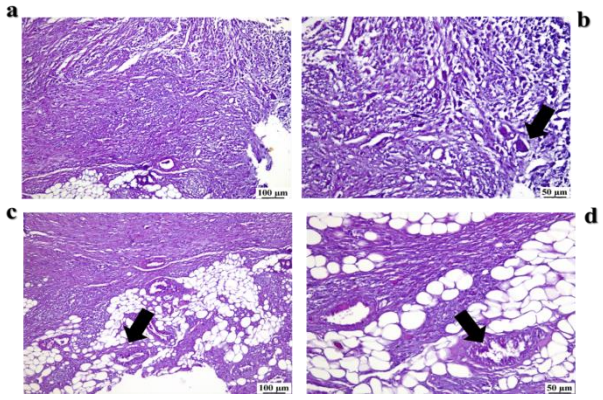

Fig 12. photomicrograph of mammary gland from BC+BA+E group showing interlacing bundles of neoplastic cells (H&E) (a), fewer number of giant cells (arrow) (H&E). (b), moderate fibroplasia with mild proliferation of ductal epithelium (arrow) (H&E). (c), moderate fibroplasia with mild proliferation of ductal epithelium (arrow) (H&E) (d).

Discussion

Adopting bacteria for cancer treatments has been associated with promising outcomes in diverse cases (Rommasi 2022). Anaerobic bacteria's spores can be used to create, develop, and manufacture anticancer drugs. They might also be employed as transporters to deliver drugs and genes to tumor tissues (Lin et al. 2022). These bacterial spores are able to proliferate and carry out their anticancer activity in hypoxic-necrotic tissues (Khadem et al. 2020).

The obtained data showed that the BC group had a marked increase in the lipid peroxidation markers linked to a decrease in antioxidant enzyme activity, such as GSH.

In agreement with Malkoç et al (2018), rats administered MNU alone showed decreased enzymatic antioxidant activity, decreased GSH levels, and elevated MDA forms. Engwa et al (2022) stated that antioxidant enzymes like GSH, which also function as antioxidant regulators, are in charge of eliminating ROS and other free radicals from tissues and cells. According to Meghalatha et al (2022), there are noticeably higher MDA concentrations in cancerous tissue than in healthy tissue. Additionally, research by Cheng et al (2022) indicates that malignant breast tissue may have a higher turnover of membrane lipids.

A higher concentration of thiobarbituric acid receptor-active material (gmol malondialdehyde/g tissue) has been seen in breast cancer tissue in previous reports (Didžiapetrienė et al. 2020). Tumor tissues are known for having higher protein and fat contents relative to their weight (Molendijk et al. 2020), than the corresponding healthy tissues; which appear in BC group compared to NC group. This indicates that the body produces higher serum quantities of lipid peroxidation products when a malignant tissue proliferates, and theoretically this might be because of a high turnover of membrane lipids (Punnonen et al. 1994). Because polyunsaturated fatty acids are more efficiently absorbed into cellular lipid than saturated fatty acids, this may also be a factor in the high concentrations of polyunsaturated fatty acids found in malignant breast tissue (Rizzo et al. 2021).

As shown by our findings, rats were shielded from MNU-induced oxidative damage by endospores and boswellic acid, particularly when injected in the same group. This resulted in much lower levels of lipid peroxidation and higher levels of GSH, which is consistent with Wahia et al (2022). Boswellic acid administration is investigated for its potential to reduce oxidative stress by raising GSH, which in turn neutralizes free radicals and prevents radical chain reactions, hence decreasing lipid peroxidation (i.e. MDA reduces) (Khafaga et al. 2021). According to our research, the combination of endospores and boswellic acid increased GSH and decreased MDA the most, indicating the anticancer effect of each treatment when used in conjunction with the other to treat breast carcinoma.

Free radicals are sometimes referred to as "reactive oxygen species" (ROS), but they also comprise reactive nitrogen (RNS), iron, copper, and sulfur species (Mandal et al. 2022). It should be noted that certain ROS, such hydrogen peroxide (H_2O_2), are not free radicals. H_2O_2 and superoxide anion (O2•-) are among the most well-characterized reactive oxygen species (ROS) generated by vascular cells, while nitric oxide (NO) is the primary physiologically active reactive nitrogen intermediate (Costa et al. 2021). Furthermore, the data showed that the group with breast cancer had much lower levels of NO and H_2O_2 than the normal control group. Because low NO levels (picomolar to nanomolar) stimulate cell division and anti-apoptotic reactions, they directly affect cellular function (Khan et al. 2020). Both beneficial and detrimental effects can be seen in the stress response brought on by cancer when it comes to ROS like peroxide (H_2O_2) and RNS such nitric oxide (NO) (Zarkovic 2020). Furthermore, at low H2O2 concentrations, a variety of cancer cells are stimulated to divide, whereas at greater concentrations, cell growth is inhibited and even cell death occurs (Kohan et al. 2020). Our findings demonstrated that endospores and boswellic acid significantly increased NO and H_2O_2. It has been demonstrated that elevated NO (micromolar) levels can cause senescence, apoptosis, and cell cycle arrest indirectly by causing oxidative and nitrosative stressors (Khan et al. 2020). Since S-nitrosylation of GSH generates inactive S-nitrosoglutathione, which leads to the accumulation of ROS in highly glycolytic and hypoxic cells and promotes apoptotic cell death, the presence of NO can impede GSH's ability to eliminate ROS (Mijatović et al. 2020). Furthermore, increased levels of NO and peroxynitrite, which are formed when NO and ROS interact; have the direct ability to destroy tumor cells (Cao et al. 2020). A high concentration of H_2O_2 in the mitochondrial compartment may prevent the cell cycle from progressing, but high concentrations of H_2O_2 at the cell surface typically cause the cell cycle to be activated (Heo et al. 2020). Being a free radical in and of itself, nitric oxide (NO•) can cause lipid peroxidation and damage to cells when it reacts with oxygen (O_2) to form the very reactive species peroxynitrite (ONOO-) (Bratovcic 2020).

Rats in the BC group had significantly higher serum urea and creatinine levels ($p < 0.05$) than the control group, as Figure 3 illustrates. Since renal damage is the only source of raised creatinine, the level of creatinine in serum is thought to be a more sensitive kidney function test than BUN (Prabhu et al. 2022). The reason for the increase in both urea and creatinine can be ascribed to either renal tissue damage or an inherent physiological problem that hampered the kidneys' ability to operate (Werdi & Al-Hadidy 2023). The body's increased concentration of free radicals and the formation of oxidative stress could be the cause of the elevated urea percentage (Nwogueze et al. 2023). These cause proteins and amino acids to oxidize, which raises the percentage of urea produced as a consequence of this process and the blood's creatinine levels (Werdi & Al-Hadidy 2023). As a result of damage to the renal glomeruli brought on by oxidative stress, which permitted the release of a certain amount of creatinine into the blood, there is a lack of

filtration of creatinine presence in the blood by the renal glomeruli (Kumahor 2024).

When compared to the BC group, the injection of either boswellic acid or endospores, alone or in combination, dramatically reduced the levels of urea and creatinine in the BC+BA, BC+E, and BC+BA+E groups ($p < 0.05$) (Fig. 3). Since high blood pressure is mostly caused by the elimination of cholesterol and triglycerides, which have a detrimental effect on renal functions, the concentration of creatinine and urea in serum has decreased (Podkowińska & Formanowicz 2020). Based on the findings, it is believed that the endospores were able to germinate and used a probiotic-like mechanism to reduce the levels of urea and creatinine in the serum. Wang et al (2023) findings, which showed a considerable improvement in renal functioning as shown by a decrease in serum creatinine and urea, were consistent with our findings. When compared to one another, the treatment involving both endospores and boswellic acid had the greatest impact on renal function.

When compared to normal control rats, the untreated group (MNU group-BC) had a significantly higher level of ALT and AST enzyme activity in their serum. The liver's altered metabolism, which is followed by the generation of toxins and damage, may be the cause of the increase in these enzyme levels (Hastings et al. 2020). The increase in ALT and AST points to potential tumor invasion-related impairment of liver and renal function (Albalawi et al. 2022). Similar to other investigations, the levels of ALT and AST were shown to be significantly elevated in the current study (Chauhan et al. 2020).

When compared to the untreated BC group, treatment with endospores and boswellic acid, either separately or in combination, significantly reduced ALT and AST activity. Because bacterial endospores can enhance liver activities, critical processes, and metabolic markers, they can lower the concentration of liver enzymes (Zhang et al. 2023). It functions to move the injured tissues back toward the normal region (Holzer et al. 2020). Additionally, it lessens the enzymes' leaking from cells into plasma, which is thought to be proof that the damage has been repaired and the issues associated with high fat and circulatory system damage have been addressed (Mahdavi-Roshan et al. 2022). Similarly, Kherouf (2021) discovered that the active ingredient of boswellia serrata, boswellic acid, has a hepatoprotective effect by preventing the formation of 5-lipoxygenase, an enzyme that causes inflammation in liver cells. Serum ALT and AST values were lowest in those treated with both endospores and boswellic acid.

Comparing the BC group of rats to the control normal group, our data revealed a considerable elevation of NFkB, TNFα, VEGF, and IFNγ levels in breast tissue after MNU-induced breast cancer growth (Fig.5). Nuclear factor kappa B is a significant predictor of breast cancer cell apoptosis (NF-κB). In cancer cells, it has potent anti-apoptotic effects. Nuclear factor kappa beta (NF-κβ) is one of the key proinflammatory cascade inducers that, when activated, promotes the growth and progression of BC tumors by promoting tumor cell proliferation, angiogenesis, invasion, metastasis, clonogenicity and stemness, and apoptotic evasion—all well-known malignancies (Diep et al. 2022).

TNF is a two-edged sword that can both activate transcription factor nuclear factor and promote the growth of tumor cells. NF-b in a minimal dosage (Veljkovic et al. 2024). Numerous tumor cells, including breast cancer cells, produce VEGF (Yang & Cao 2022), and some evidence suggests a correlation between the prognosis of breast cancer patients and the VEGF concentration of tumor cells (Al Kawas et al. 2022). According to certain research, VEGF promotes the growth and multiplication of breast cancer cells (Madu et al. 2020). By enhancing the activation of specific immunosuppressive systems that facilitate tumor development and metastasis, IFN-γ may actually diminish immune responses (Peña-Romero & Orenes-Piñero 2022).

On the other hand, NFkB, TNFα, VEGF, and IFNγ levels were significantly lower in the BC+BA, BC+E, and BC+BA+E groups ($p < 0.05$) after treatment with endospores and boswellic acid, either alone or in combination, than in the untreated BC group ($P<0.05$-Fig. 5). Numerous studies have shown that inhibiting NF-κB activity by various methods makes cancer cells more susceptible to the apoptotic effects of various effectors, including tumour necrosis factor-α (TNF-α), chemotherapy, and radiation therapy (Gielecińska et al. 2023). Zhou et al (2021) demonstrated that BA inhibits the transcription of genes controlled by NF-κB and genes that are involved in the angiogenic process, such as VGEF.

Experimental in vitro and in vivo studies have shown that downregulating NF-kB activity by natural or synthetic compounds suppresses the growth of cancer cells, inhibits the development of tumors induced by carcinogens, and induces apoptosis by changing the expression of genes essential for the regulation of carcinogenesis and the survival of cancer cells (Haque et al. 2021). Our findings demonstrated that rats given boswellic acid injections had improved tumor microenvironments, as seen by the reduction of inflammation and inflammatory markers. This was consistent with Efferth & Oesch (2022).The tumor microenvironment improved the most when endospores and boswellic acid were combined. Furthermore, it was discovered that throughout the anticancer therapy, levels of VEFG (vascular endothelial growth factor), TNF-a, IL-6, IL-8, and IFN-g also increased (Tolomeo & Cascio 2022).

A form of planned cell death called apoptosis is brought on by specific circumstances. It is essential for aberrant cell development and proliferation, and immune cells in the human body can remove apoptotic cells (Obeng 2020). Many molecules take part in activating apoptosis, in particular the caspase signaling cascade (Boice & Bouchier-Hayes 2020). Aspartic acid residues play a crucial role in apoptosis, and caspases are cysteine proteases that particularly target these residues (Asadi et al. 2022). Since abnormal caspase expression and/or activation have been linked to a number of cancer types, it makes sense that caspase depletion might encourage the growth of tumors (Lou et al. 2021). It was discovered that the BC group's expression of caspase-3 was much lower than that of the control group of normal rats. It has been shown that loss of caspase-3 expression and activity occurs in primary human breast malignancies, and it has been proposed that this loss may facilitate tumor formation by preventing and/or lowering transformed cell senescence and death (Saleh et al. 2020).

Furthermore, in comparison to the normal control group, the P53 levels in the BC group dropped dramatically ($P<0.05$) (Fig.6). On the other hand, there was a significant increase in Caspase-3 activity following treatment with Endospores and Boswellic acid, either separately or in combination in the BC+BA, BC+E, and BC+BA+E groups ($p <0.05$) (Fig.6). Caspases-3 has a strong correlation with a poor prognosis and the production of proteins linked to apoptosis in breast cancer (Ke et al. 2021). By raising caspase 3 activity in cancer cell lines, bacterial supernatant was also successful in causing apoptosis (Dolati et al. 2021). Nevertheless, P53 concentrations in the BC+BA, BC+E, and BC+BA+E groups ($p <0.05$) were considerably higher than those in the BC group ($P< 0.05$) (Fig. 6). Additionally, the data point to an increase in P53 gene transcription in these cells when boswellic acid is present. P53 expression changed over time, and this could be a way for cells to die off in reaction to boswellic acid (Ragab et al. 2024).

One member of the Janus kinase family is Janus kinase-1 (JAK1). In breast cancer cells that are activated by ERBB2 receptor tyrosine kinase signaling, JAK1 promotes the persistent oncogenic activation of STAT3, is crucial for the advancement of metastatic cancer, and is required for IL-6-class inflammatory cytokine signaling (Gu et al. 2020). The Signal Transducers and Activators of Transcription (STAT) are transcription. It was discovered that JAK1-deficient cell lines were more carcinogenic than wild-type cells (Shrestha et al. 2024). According to the evidence, JAK1 functions as a tumor suppressor or oncogene depending on the circumstances or cell type (Xie et al. 2021). Factors that are involved in normal physiological functions such as cell proliferation, apoptosis and differentiation (Mohassab et al. 2020). Many biological processes within cells, such as organogenesis, fetal development, programmed cell death, differentiation, growth, inflammation, and the immune system, are regulated by STAT proteins (Butturini et al. 2020).

According to our findings, the mammary levels of STAT-3 and JAK were considerably ($P <0.05$) lower in the rats in the BC group than in the normal group (C). Preliminary research demonstrating low or undetectable STAT in the breast cancer lines supports this (Yue et al. 2022). According to a new study, JAK/STAT inhibition increases the production of protumorigenic inflammatory factors in the tumor microenvironment of breast cancer patients, which encourages treatment resistance (Habanjar et al. 2023). Proliferation, survival, inflammation, invasion, development of new blood vessels, and metastasis are all influenced by dysregulated JAK/STAT signaling and are linked to the start, spread, and progression of cancer (Rah et al. 2022).

According to recent research, deregulation of the JAK/STAT pathway has been linked to the promotion of oncogenic phenotypes such as tumorigenesis, proliferation, anti-apoptosis, invasion, angiogenesis, metastasis, and immune evasion. The pathway is crucial for the growth of both normal and cancer stem cells (Zarezadeh et al. 2024). In breast cancer, this pathway was altered by the following mechanisms: (1) down-regulation of phosphotyrosine specific phosphatases (Xin et al. 2020); (2) down-regulation of negative regulators of STAT (Yin et al. 2021); an increase in the amount of IL-6 (Brooks & Putoczki 2020); and (4) activation of other upstream oncogenic pathways, such as c-Src, ERBB1, or PI3K/ mTOR (Sathish et al. 2024).

The JAK/STAT signaling may act either directly or indirectly by triggering nuclear factor-κB (NF-κB) activation (Bose et al. 2023). By inhibiting JAK/STAT signaling in cancer cells, one can prevent the expression of target genes that regulate vital cell processes and make it more difficult for cancer cells to evade growth control mechanisms including invasion and death (Garg et al. 2021). When comparing the levels of JAK and STAT-3 to their corresponding values in BC, the results revealed a significant rise in these parameters in the BC+BA, BC+E, and BC+BA+E groups ($p <0.05$) (Fig.7). Previous research suggests that STAT can also drive tumor suppression through an epigenetic process that appears to be separate from the standard JAK/STAT signaling pathway (Pereira et al. 2021). The antitumorgenic pathways are maintained when radiation exposure and baswilic acid are combined. Thus, adding baswilic acid to the mix could be a suitable therapeutic option for treating cancer (Sharma & Jana 2020).

Low molecular mass glycoprotein interleukin-2 (IL-2) is required for the activation and development of multiple diverse lineages of normal hemopoietic cells (Saha 2020). Interleukin 10 (IL-10), of all, plays an important coordination role in the occurrence of BC (Al-Ameri et al. 2020). One of the anti-inflammatory cytokines, IL-10, works by opposing the co-stimulatory molecules that are expressed on APCs to prevent inflammatory reaction (Habanjar et al. 2023). The data presented in Figure 8 indicates a significant ($P < 0.05$) drop in the serum levels of IL-2 and IL-10 in the rats belonging to the BC group. Tumor growth is facilitated by the positive correlation between the production of IL-1, an inflammatory cytokine, and depleted IL-10 in a mouse model (Nagata & Nishiyama 2021). These findings were consistent with those of Gonda and colleagues, who showed that there were considerable variations in serum IL-2 levels between patients and controls and that there was no correlation between IL-2 levels and the stages of BC disease (Gonda et al. 2021).

However, when comparing the values of these parameters to their equivalent values in BC, the results revealed a significant rise in IL-2 and IL-10 levels in the BC+BA, BC+E, and BC+BA+E groups ($p < 0.05$) (Fig 8). There are currently three identified biological functions of IL-10 that support the pleiotropic impact (Rallis et al. 2022). Firstly, IL-10 can stimulate the activation and multiplication of CD8+ T lymphocytes, which can directly or indirectly cause the cancer cells to become cytotoxic (Huang et al. 2023). Secondly, IL-10 suppresses antigen presentation by APCs, which impedes T cell-stimulated tumor-killing immunity (Paul 2023). Lastly, IL-10 can inhibit tumor-promoting inflammation (Chang et al. 2021). Activated by IL-2, neutrophils, macrophages, and NK cells provide non-specific defense against tumors. T cells regulate the proliferation of natural killer cells (NK cells) in two ways: first, IL-2 stimulates the proliferation of activated NK cells, and second, IFN stimulates the production and proliferation of IL-2 receptors in NK cell progenitors (Harjianti et al. 2023).

IL-10 inhibits angiogenesis to produce its anti-tumor action. The decrease in vascular endothelial growth factor, TNF-α, IL-1β, IL-6, and MMP-9 was the cause of this anti-angiogenic action (Chang et al. 2021). B cell development into plasma cells, which generate antibodies specific to tumor cells and facilitate antibody-dependent cell cytotoxicity, can be stimulated by IL-10 (Tan et al. 2022). Nuclear factor-κB translocation into the nucleus is suppressed by IL-10, which also blocks inflammatory signaling. IL-10 promotes T cell memory, increases IFN-γ production, and facilitates the infiltration of CD8+ T cells in tissue to carry out its anti-tumor function (Guo et al. 2021).

Following boswellic acid therapy, IL10 was significantly upregulated. This was consistent with Khajehdehi et al (2022), who reported that acetyl-11-keto-β-boswellic acid (AKBA) boosts the anti-inflammatory factors like IL-10 and reduces the pro-inflammatory agents like TNFα and interleukin 6. In line with Ni (2017), endospore treatment also shown a notable elevation of IL-10, which functions as an anti-inflammatory factor for tumors. The greatest notable upregulation was shown when BA and endospores were combined. According to Jamshidi-Adegani et al (2022) boswellic acids had an anti-cancer impact on MCF-7 breast cancer cells in one study. In conclusion, Gamma radiation exhibits the ability to elicit immunity and provide strong immune suppression that particularly targets breast tumor cells by weakening the endospores produced by Clostridium sporogenes. More research is required to understand the mechanisms underlying Endospore's anti-apoptotic and anti-angiogenic properties.

Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Data availability

All data obtained from this study are included in the current manuscript.

Ethical statement

This experiment was carried out according to recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH no. 85:23),

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