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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2025.219.228

Research Article
Histological Changes in Breast Tissue via the Expression of Her2, Cox-2 and Caspase 3 after the Administration of *Zanthoxylum acanthopodium* DC and *Moringa oleifera* Leaves

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Abstract

Background and Objective: Breast tissue may experience structural alterations due to variables including infection, illness, inflammation, or exposure to deleterious compounds, with 7,12-Dimethylbenz[a]anthracene (DMBA) being a prevalent carcinogen utilized in cancer research. *Zanthoxylum acanthopodium* DC and *Moringa oleifera* leaves possess anti-inflammatory and anticancer effects. This study aimed to determine the expression of Her2, Cox-2 and Caspase 3 as markers of breast carcinoma after administration of *Zanthoxylum acanthopodium* DC and *Moringa oleifera* leaves. **Materials and Methods:** This study used 36 rats with 6 groups, namely C0: Normal mice, C^-: Only DMBA injection, C^+: DMBA-injected rats+Doxorubicin, P1: DMBA-injected rats+*Z. acanthopodium* fruit, P2: DMBA-injected rats+100 mg/kg b.wt., of *Moringa oleifera* leaves and DMBA-injected rats+50 mg/kg b.wt., of *Moringa oleifera* leaves and 50 mg/kg b.wt., of *Z. acanthopodium* fruit orally for 30 days then dissected and breast tissue was taken for immunohistochemistry procedures. The non-parametric data were analyzed using the Kruskal-Wallis and Mann-Whitney tests with a significance level of $p<0.05$. **Results:** The DMBA injection into the mammary tissue increases Her2 expression, infiltrates the surrounding tissue, including the stroma and forms a solid tumor mass with ambiguous boundaries. *Zanthoxylum acanthopodium* and *Moringa oleifera* alone reduced damaged tissue, however, their combined action boosted breast tissue Her2 expression but did not lower Cox-2 or Caspase 3 scores. The expression of Cox-2 in group C^- differed significantly from P1 ($p<0.05$, $p = 0.001$). **Conclusion:** *Zanthoxylum acanthopodium* and *Moringa oleifera* enhanced Caspase 3, which promotes apoptosis in breast cancer cells. Nevertheless, each plant individually diminished Her2 and Cox-2 expression more effectively than the combination.

Key words: Breast cancer, Caspase 3, Cox-2, Her2, *Moringa oleifera*, *Zanthoxylum acanthopodium*

Citation: Simanullang, R.H., M.M. Hutahaean, J.M. Siahaan, Calen and Kasrawati *et al.*, 2025. Histological changes in breast tissue via the expression of Her2, Cox-2 and Caspase 3 after the administration of *Zanthoxylum acanthopodium* DC and *Moringa oleifera* leaves. Pak. J. Biol. Sci., 28: 219-228.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Breast tissue may experience structural changes due to infection, disease, inflammation or the introduction of harmful substances[1]. Researchers frequently use the carcinogenic chemical 7,12-Dimethylbenz[a]anthracene (DMBA) to induce breast cancer. The DMBA exposure can cause significant histological changes in breast tissue, mostly through genetic mutations and oxidative stress mechanisms[1]. Histologically, tissue exposed to DMBA demonstrates increased epithelial cell proliferation, dysplasia and the development of preneoplastic lesions[2]. These alterations are typically associated with the disruption of the normal lobuloalveolar architecture and an elevation in cells exhibiting hyperplastic nuclei, indicating early malignant transformation[3]. Moreover, DMBA enhances the infiltration of inflammatory cells in the breast tissue stroma, thereby establishing a pro-tumor microenvironment[1]. This condition can exacerbate tissue injury by producing pro-inflammatory cytokines and proteolytic enzymes that compromise the integrity of the extracellular matrix. In advanced stages, the architecture of the mammary glands becomes aberrant, accompanied by the development of a distinctive tumor mass[2]. The alterations indicate that DMBA exposure may initiate breast carcinogenesis via intricate biological processes, encompassing oncogene activation and tumor suppressor gene suppression[3]. Human Epidermal Growth Factor Receptor 2 (HER2) is a membrane-bound receptor protein that plays an essential role in controlling cell proliferation and differentiation. About 20-30% of breast cancer cases, known as HER2-positive breast cancer, may have an overexpression or genetic amplification of HER2[4]. This syndrome amplifies proliferative signals, hence expediting aggressive tumor growth and facilitating more rapid dispersion compared to other breast cancer variants[5]. Establishing HER2 status is essential for developing treatment strategies, as HER2-positive breast cancer generally exhibits a favorable response to targeted therapy[6]. An enzyme known as COX-2 or cyclooxygenase-2, is an important player in inflammation and is frequently found to be overexpressed in breast tissue, particularly in instances of breast cancer[7]. Prostaglandin synthesis in response to COX-2 activation in this tissue can affect cell proliferation, angiogenesis and immunological response suppression, allowing tumors to proliferate[8]. The COX-2 expression is frequently induced by cytokines, atypical cellular proliferation or oxidative stress. Furthermore, elevated COX-2 levels correlate with resistance to apoptosis, obstructing the eradication of cancer cells[9]. The Caspase-3 functions in normal tissues to preserve homeostasis by removing damaged or superfluous cells. The Caspase-3 expression is frequently diminished or impaired in breast cancer tissues, leading to the cancer cells' resistance to apoptosis, hence facilitating tumor development and spread[10]. Investigating Caspase-3 expression in breast tissues may provide essential insights for developing pharmaceuticals that target the apoptosis pathway, perhaps serving as a tool to inhibit cancer progression[11]. The leaves of *Moringa oleifera* are commonly employed by Indonesians as herbal medicine because of their various phytochemical components, such as quercetin, vitamin C and beta-carotene[12]. Antioxidants like these help shield breast tissue from the free radical oxidative damage that can lead to diseases like breast cancer[12]. Because of its anti-inflammatory characteristics, moringa leaf extract may alleviate breast tissue irritation. Moringa leaf extract has been demonstrated in many *in vitro* experiments to reduce cancer cell proliferation, with a focus on breast cancer cells. This is achieved by mechanisms such as reducing cancer cell growth and inducing apoptosis[13]. Furthermore, *Moringa* leaves may aid in the regulation of estrogen hormone levels. Hormonal imbalances can affect breast tissue integrity, thereby increasing the likelihood of benign tumors or malignancies[13]. The fruit of *Zanthoxylum acanthopodium* (andaliman) functions as a culinary spice for the Batak community in Indonesia[14]. This fruit, abundant in bioactive constituents like alkaloids, flavonoids and terpenoids, has garnered interest in cancer research for its capacity to affect molecular pathways associated with the proliferation and dissemination of cancer cells[15]. Numerous studies indicate that andaliman extract possesses antioxidant and anti-inflammatory characteristics that can impede cancer cell proliferation by inducing apoptosis, inhibiting angiogenesis and altering cellular signaling pathways[16]. This study examine the effects of a combination of *Moringa oleifera* leaves and *Zanthoxylum acanthopodium* DC fruit on the expression of Her2, Cox-2 and Caspase 3 in connection with histological changes in breast cancer, which are expected to be substantial.

MATERIALS AND METHODS

Study period: The research was carried out from July, 2023 to August, 2024. The research was performed at the Biology Department of the Faculty of Mathematics and Natural Sciences at the University of Sumatera Utara in Medan, Indonesia.

Ethics statement: All animal care and experimental techniques comply rigorously with the rules set forth by the FMIPA USU Medan Medical Research Ethics Committee (No. 087/KEPH-FMIPA/2023). All conceivable procedures were executed to alleviate the distress endured by the rats.

Chemical and reagents: The reagents utilized included Doxorubicin from Sigma-Aldrich, USA (2023), Invitrogen COX2 Polyclonal Antibody (Catalog # PA1-37505), Invitrogen ErbB2 (HER-2) Polyclonal Antibody (Catalog # PA5-16305) and Invitrogen Caspase 3 Monoclonal Antibody 74T2 (Catalog # 43-7800) from Thermo Fisher Scientific-USA (2023).

Preparation of *Moringa oleifera* leaves: The initial stage in generating a 70% ethanol extract of *Moringa oleifera* is the collection of high-quality, fresh leaves. Subsequently, the leaves are washed to eliminate any impurities and then desiccated in a shady environment to maintain their phytochemical integrity. A fine powder is produced from the desiccated leaves. Maceration is conducted for 24-48 hrs with intermittent stirring to optimize contact between the material and the solvent following immersion in a 70% ethanol solution at a specified ratio. Upon completion of the maceration procedure, the pulp may be filtered to yield the *Moringa* leaf extract. The filtrate may subsequently be evaporated with a rotary evaporator to eliminate any remaining solvent.

Preparation of *Zanthoxylum acanthopodium* DC fruits: To preserve the active constituents, the fresh fruit was meticulously picked, washed and subsequently dried by an indirect drying method. The dried fruit was ground into a coarse powder. The andaliman powder underwent a maceration process by being soaked in 70% ethanol for 48 hrs with occasional stirring. After maceration, the mixture was filtered using a filter cloth or filter paper to separate the extract solution from the pulp. A concentrated extract of andaliman fruit, comprising putative medicinal constituents, was acquired by evaporating the filtrate at a low temperature with a rotary evaporator (Labconco Company, 2022, Missouri, United States of America).

Animal handling: This study utilized 36 female white rats (*Rattus norvegicus*) weighing between 150 and 200 g as a model for breast cancer. The rats were housed under controlled laboratory settings at a temperature of 22-24°C, with a light cycle of 12 hrs of light and 12 hrs of darkness. The rats were given standard pellets and water ad libitum to meet their daily dietary needs. Before treatment, the rats underwent a 2 week acclimatization phase to adjust to the laboratory environment. Animal handling techniques were implemented per Animal Research Ethics Guidelines to reduce stress and pain during the study. Oncological model DMBA was dissolved in corn oil for rats. Female Wistar rats, approximately 8-10 weeks of age, received a single dose of DMBA at 50 mg/kg b.wt., via either intramammary or intraductal injection. This method aimed to directly give DMBA, a known carcinogen, to the mammary tissue of rats to produce breast cancer. After the administration of DMBA, the animals had a treatment-free incubation period of roughly 3 months to promote the development of breast tumors. During this period, regular monitoring was performed to evaluate tumor development, physiological changes and possible harmful effects that arose.

Research design: C0: Normal rats, C^-: Only injection of DMBA, C^+: Rats injected DMBA+Doxorubicin, P1: Rats injected DMBA+*Z. acanthopodium* fruits, P2: Rats injected DMBA+ 100 mg/kg b.wt., of *Moringa oleifera* leaves. Rats injected DMBA+50 mg/kg b.wt., of *Moringa oleifera* leaves and 50 mg/kg b.wt., of *Z. acanthopodium* fruits. The oral administration of *Z. acanthopodium* and *Moringa oleifera* was performed over one month. On the 31st day, anesthesia was delivered to the rat using 4% isoflurane at a flow rate of 1.5 L/min of oxygen within a Perspex chamber. Anesthesia was maintained with 2% isoflurane administered at a flow rate of 0.5 L/min of oxygen. Isoflurane is chosen for its swift induction of anesthesia and its ability to provide a safe recovery while reducing adverse effects. Thereafter, the breast tissue is harvested and preserved in formalin to facilitate the immunohistochemistry procedures.

Immunohistochemistry: Breast tissue is processed with formalin and fixed in paraffin. The tissue was sliced into minute sections measuring 3-5 µm in thickness, employing a microtome. Thereafter, deparaffinize the slides using xylene, followed by dehydration with sequential applications of alcohol (80, 95 and 100%) for several minutes. The tissue was then rehydrated using phosphate-buffered saline (PBS) and prepared for immunohistochemical procedures. The next phase entailed non-specific blocking to prevent unwanted antibody binding[17]. The blocking was achieved via serum or non-specific protein solutions. Subsequently, specific primary antibodies targeting Her2, COX2 or Caspase 3 were administered to the tissue preparation and incubated at room temperature or 4°C for 1-2 hrs. The primary antibody utilized must align with the specific antigen intended for detection, such as the anti-Her2 antibody for Her2 expression, the anti-COX2 antibody for COX2 expression and the

anti-Caspase 3 antibody for Caspase 3 activity. The tissue is rinsed to eliminate any unbound primary antibody following incubation. The next step is to apply a secondary antibody that is conjugated to an enzyme, like Alkaline Phosphatase (ALP) or horseradish peroxidase (HRP). After that, the target antigen is detected by staining with DAB (3,3'-diaminobenzidine) or another appropriate substrate, which results in a brown hue[18]. The stained slide is cleaned, a coverslip is placed over it and the slide is then inspected under a light microscope (The Olympus CX-23, Olympus Corporation, Tokyo, Japan, 2015)[19].

Statistical analysis: The utilized data are non-parametric, employing the Kruskal-Wallis test and the subsequent Mann-Whitney test (SPSS, Inc., Chicago, Illinois, USA) alongside XLSTAT version 2011.4.02 (Addinsoft SARL, France), with a significance threshold of $p<0.05$.

RESULTS

Histological changes of breast tissue through Her2 expression after administration of *Moringa oleifera* leaves and *Zanthoxylum acanthopodium* DC fruits: The findings indicated that the comparison group (P1) exhibited the lowest Her2 Score compared to the other treatment groups, with groups P2 and P3 following subsequently (Fig. 1). The DMBA injection in rat mammary tissue may reduce the elevation of Her2 expression (C^-). The amalgamation of these two herbs sustained the elevated Her2 expression in the tissue (P3). According to the results of the cancer histology analysis using the Mann-Whitney test ($p<0.00$, $p = 0.001$, Fig. 2), there was a significant difference in the Her2 expression score among the groups ($p<0.01$). In the control group, fibrous tissue, composed of collagen and other components, gives structural support to the breasts, facilitating the formation of well-developed milk ducts and lobules, which exhibit negative Her2 expression (Fig. 1a). Histological analysis revealed the epithelial injury and proliferation of atypical epithelial cells frequently accompanied by a breakdown of normal architectural integrity, hyperchromatic nuclei, cellular pleomorphism and heightened mitotic activity (Fig. 1b). The infiltration of adjacent tissue, including the stroma and the development of a solid tumor mass with indistinct margins are essential markers (Fig. 1c). The fruit of *Z. acanthopodium* enhanced epithelial maturation, while fibrosis persisted showing superior results compared to *Moringa oleifera* (Fig. 1d). The *M. oleifera* enhanced epithelial maturation proliferation while reducing fibrosis associated with the inflammatory response (Fig. 1e). The administration of *Z. acanthopodium* and *M. oleifera* separately led to a reduction in damaged tissue (Fig. 1f); however, the synergistic action of the two herbs did not diminish the Her2 score but rather elevated the Her2 expression score in breast tissue.

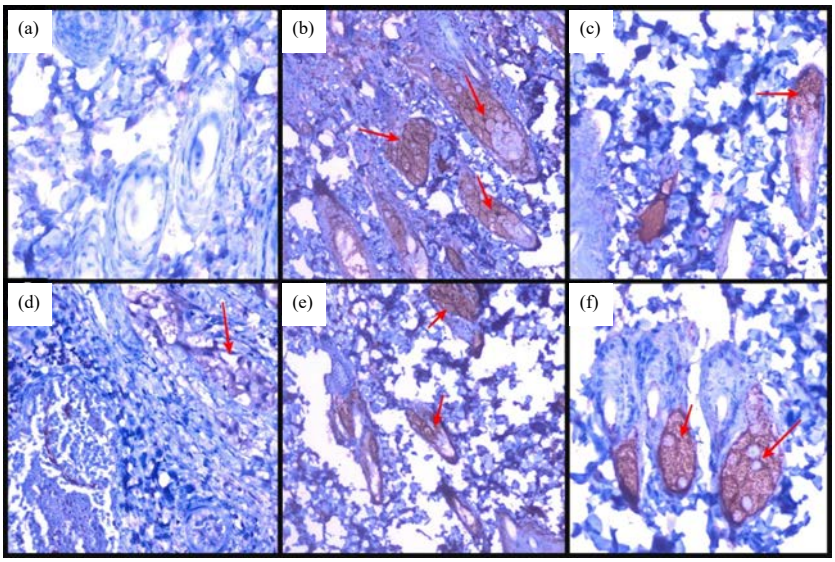

Fig. 1(a-f): Her2 expression in breast tissue after administration of *Moringa oleifera* leaves and *Zanthoxylum acanthopodium* fruit DC, (a) C0: Normal rats, (b) C^-: Only injection of DMBA, (c) C^+: Rats injected DMBA+Doxorubicin, (d) P1: Rats injected DMBA+*Z. acanthopodium* fruits, (e) P2: Rats injected DMBA+100 mg/kg b.wt., of *Moringa oleifera* leaves and (f) P3: Rats injected DMBA+50 mg/kg b.wt., of *Moringa oleifera* leaves and 50 mg/kg b.wt., of *Z. acanthopodium* fruits
Red arrows denote positive Her2 expression in breast tissue

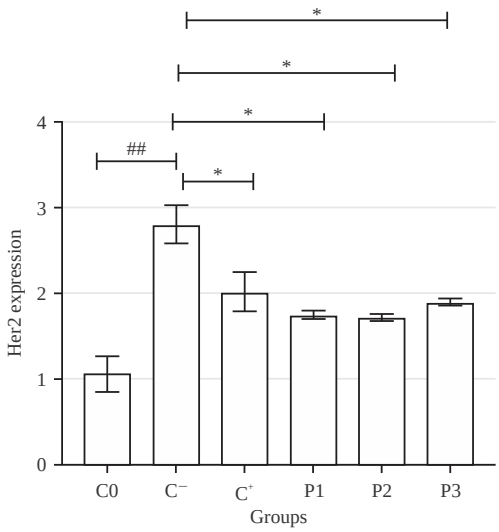

Fig. 2: Positive Her2 score in breast tissue after administration of *Moringa oleifera* leaves and *Zanthoxylum acanthopodium* DC fruit

C0: Normal rats, C⁻: Only injection of DMBA, C⁺: Rats injected DMBA+Doxorubicin, P1: Rats injected DMBA+*Z. acanthopodium* fruits, P2: Rats injected DMBA+100 mg/kg b.wt., of *Moringa oleifera* leaves, P3: Rats injected DMBA+50 mg/kg b.wt., of *Moringa oleifera* leaves and 50 mg/kg b.wt., of *Z. acanthopodium* fruits ($^{\#\#}p<0.01$ vs C0 and $^{*}p<0.05$ vs C⁻)

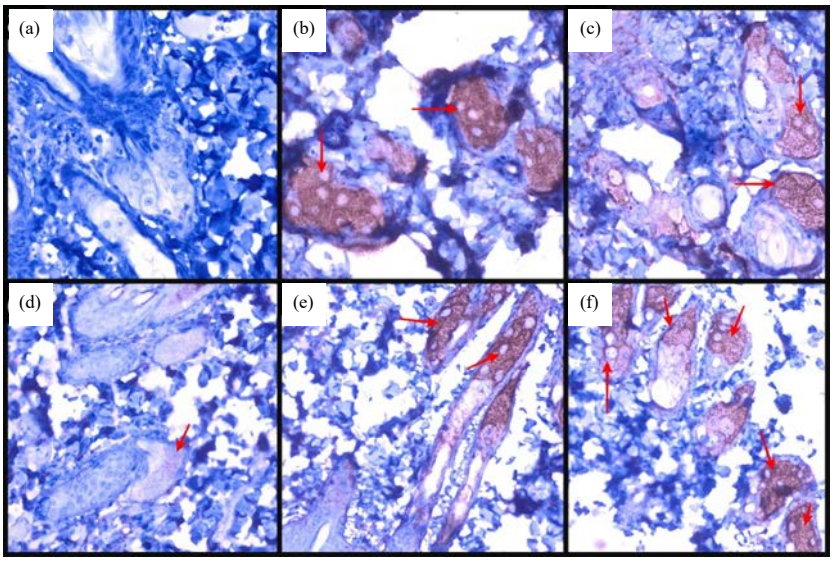

Fig. 3(a-f): Cox-2 expression in breast tissue after administration of *Moringa oleifera* leaves and *Zanthoxylum acanthopodium* fruit DC, (a) C0: Normal rats, (b) C⁻: Only injection of DMBA, (c) C⁺: Rats injected DMBA+Doxorubicin, (d) P1: Rats injected DMBA+*Z. acanthopodium* fruits, (e) P2: Rats injected DMBA+100 mg/kg b.wt., of *Moringa oleifera* leaves and (f) P3: Rats injected DMBA+50 mg/kg b.wt., of *Moringa oleifera* leaves and 50 mg/kg b.wt., of *Z. acanthopodium* fruits

Red arrows denote positive Her2 expression in breast tissue

Histological changes of breast tissue through Cox-2 expression after administration of *Moringa oleifera* leaves and *Zanthoxylum acanthopodium* DC fruits: Breast ductal epithelial cells exhibit a cuboidal or columnar morphology, are systematically organized, possess distinct and uniform nuclei and demonstrate negative COX-2 expression (Fig. 3a). Following DMBA injection, epithelial hyperplasia transpires. Dysplasia may manifest, characterized by the alteration of cellular architecture (Fig. 3b). Epithelial cells have enlarged, hyperchromatic nuclei and non-uniform cytoplasm; nonetheless, there is a reduction in COX-2 (Fig. 3c). The administration of herbs in this category can mitigate fibrosis

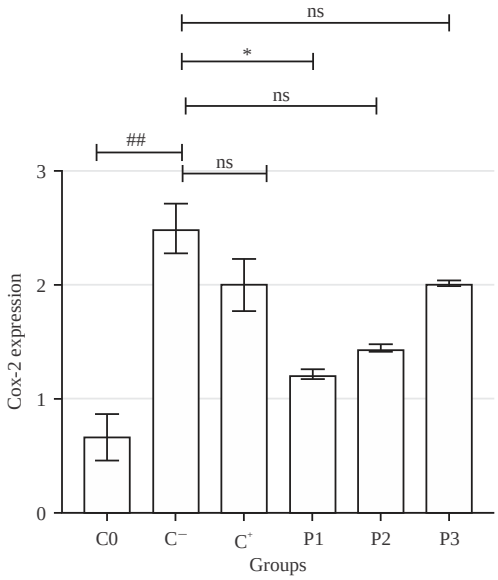

Fig. 4: Positive Cox-2 score in breast tissue after administration of *Moringa oleifera* leaves and *Zanthoxylum acanthopodium* DC fruit

C0: Normal rats, C⁻: Only injection of DMBA, C⁺: Rats injected DMBA+Doxorubicin, P1: Rats injected DMBA+*Z. acanthopodium* fruits, P2: Rats injected DMBA+100 mg/kg b.wt., of *Moringa oleifera* leaves, P3: Rats injected DMBA+50 mg/kg b.wt., of *Moringa oleifera* leaves and 50 mg/kg b.wt., of *Z. acanthopodium* fruits ($^{\#\#}p<0.01$ vs C0, $^{*}p<0.05$ vs C⁻ and $^{ns}p>0.05$ vs C⁻)

resulting from inflammatory reactions while facilitating epithelial cell repair (Fig. 3d). Similar occurrences were observed with the administration of *Moringa oleifera* leaves, accompanied by notable alterations in cell size and morphology (Fig. 3e). Histopathological examination of mice with breast cancer subjected to herbal treatment revealed substantial alterations in tissue morphology relative to the control group. The administration of these herbs might mitigate cellular damage, evidenced by a reduction in the quantity of necrotic cells exhibiting aberrant proliferation, with enhanced tissue organization (Fig. 3f). These modifications suggest that *Z. acanthopodium* and *Moringa oleifera* play a crucial role in mitigating oxidative stress. Breast tissue exhibited elevated expression of Cox-2 following DMBA injection. The results indicated that decreased Cox-2 expression was observed in all groups, with minimal expression recorded in groups P1, P2 and P3. Statistically significant differences in Cox-2 expression were seen among the groups in the histological analysis utilizing the Kruskal-Wallis test. No statistically significant difference in protein expression was observed between groups C⁻ and C⁺, C⁻ and P2 and C⁻ and P3 when analyzed using the Mann-Whitney test. The Cox-2 expression in the C⁻ group exhibited a significant difference with P1 ($p<0.05$, $p = 0.001$) (Fig. 4).

Histological changes of breast tissue through Caspase 3 expression after administration of *Moringa oleifera* leaves and *Zanthoxylum acanthopodium* DC fruits: The control group in this treatment comprised cells exhibiting distinct membrane borders, homogeneous size, regulated mitotic division and low Caspase 3 expression (Fig. 5a). Injured breast tissue exhibited necrotic debris and subsequent infiltration of inflammatory cells, including macrophages (Fig. 5b). The modified tissue architecture exhibited regions of calcification or cyst development as a reaction by the organism to encapsulate necrotic tissue (Fig. 5c). The administration of *Z. acanthopodium* leaves less hyperplasia and dysplasia in the epithelium, as illustrated in (Fig. 5d). The administration of *Moringa oleifera* diminished pleomorphism, specifically notable alterations in cell size and morphology, as well as facilitated epithelial healing (Fig. 5e). The combination of both resulted in elevated Caspase 3 expression and atypia, characterized by enlarged nuclei, hyperchromasia and non-uniform cytoplasm in epithelial cells (Fig. 5f). The administration of *Z. acanthopodium* and *Moringa oleifera* leaves individually yielded superior tissue healing compared to their combined use. The amalgamation of *Z. acanthopodium* fruits and *M. oleifera* leaves had the highest expression of Caspase 3 and showed a significant difference in group C⁻. The fruits of *Z. acanthopodium*, known as Andaliman, markedly suppressed Caspase 3 expression at

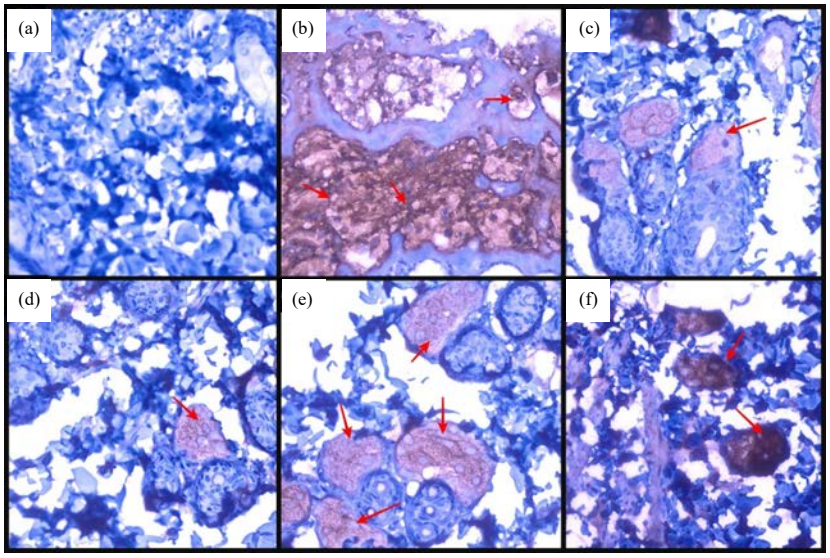

Fig. 5(a-f): Caspase 3 expression in breast tissue after administration of *Moringa oleifera* leaves and *Zanthoxylum acanthopodium* fruit DC, (a) C0: Normal rats, (b) C^-: Only injection of DMBA, (c) C^+: Rats injected DMBA+Doxorubicin, (d) P1: Rats injected DMBA+*Z. acanthopodium* fruits, (e) P2: Rats injected DMBA+100 mg/kg b.wt., of *Moringa oleifera* leaves and (f) P3: Rats injected DMBA+50 mg/kg b.wt., of *Moringa oleifera* leaves and 50 mg/kg b.wt., of *Z. acanthopodium* fruits
Red arrows denote positive Her2 expression in breast tissue

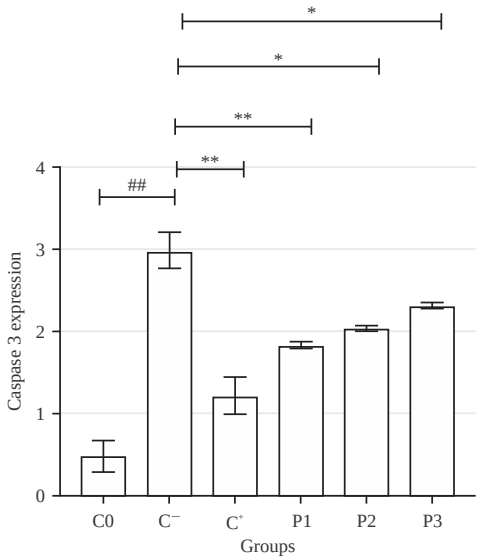

Fig. 6: Positive Caspase 3 score in breast tissue after administration of *Moringa oleifera* leaves and *Zanthoxylum acanthopodium* DC fruit
C0: Normal rats, C^-: Only injection of DMBA, C^+: Rats injected DMBA+Doxorubicin, P1: Rats injected DMBA+*Z. acanthopodium* fruits, P2: Rats injected DMBA+100 mg/kg b.wt., of *Moringa oleifera* leaves, P3: Rats injected DMBA+50 mg/kg b.wt., of *Moringa oleifera* leaves and 50 mg/kg b.wt., of *Z. acanthopodium* fruits ($^{\#\#}p<0.01$ vs C0, $^{*}p<0.05$ vs C^- and $^{**}p<0.05$ vs C^-)

the doses depicted in the Fig. 6. The Kruskal-Wallis test conducted on nonparametric data revealed a considerable disparity in Caspase 3 expression across several groups, as depicted in the Fig. 6. The Mann-Whitney test indicated a statistically significant difference in cytochrome c expression between the specified group and other groups ($p<0.01$). The cohort of rats administered just andaliman (P1) exhibited a statistically significant difference ($p<0.01$) when compared to the groups receiving only *Moringa oleifera* and the combination of both substances (Fig. 6).

DISCUSSION

The fruits of *Zanthoxylum acanthopodium* and *Moringa oleifera* enhance the proliferation of epithelial maturation in breast tissue. The administration of *Zanthoxylum acanthopodium* and *Moringa oleifera* individually results in a decrease in damaged tissue; however, their combined application does not diminish the Her2 score but rather elevates the Her2 expression score in breast tissue. *Zanthoxylum acanthopodium* and *Moringa oleifera* possess potential as natural therapeutic agents in the inhibition of Her2 expression, frequently linked to the aggressive progression of breast cancer. Active chemicals in *Zanthoxylum acanthopodium* and *Moringa oleifera*, including alkaloids and flavonoids, are recognized for their potent antioxidant and anticancer properties[18]. The Her2 is frequently overexpressed in various cancer types, particularly breast cancer and contributes to the proliferation and spread of cancer cells[20]. The inhibitory mechanism is believed to involve the modulation of cellular signaling pathways, whereby bioactive substances can suppress Her2 overexpression, diminish cancer cell growth and trigger apoptosis or programmed cell death[3].

Breast tissue exhibited elevated expression of Cox-2 following DMBA injection[21]. The results indicated that decreased Cox-2 expression was observed in all groups, with minimal expression recorded in groups P1, P2 and P3. The histopathology of rat breast cancer treated with both herbs exhibited significant alterations in tissue morphology compared to the control group. The administration of these herbs may mitigate cellular damage, as indicated by a decrease in the quantity of necrotic cells exhibiting aberrant proliferation, alongside an enhancement in tissue organization. These alterations indicate that *Zanthoxylum acanthopodium* and *Moringa oleifera* play significant roles in mitigating oxidative stress. Compounds derived from *Zanthoxylum acanthopodium* and *Moringa oleifera* can suppress the expression of Cyclooxygenase-2 (Cox-2), an enzyme implicated in inflammatory processes and linked to numerous pathological illnesses, including cancer and degenerative diseases[22]. Oxidative stress resulting from excessive reactive oxygen species (ROS) formation following DMBA injection in mice can activate transcriptional pathways, including Nuclear Factor-Kappa B (NF-κB) and activator protein-1 (AP-1), hence enhancing Cox-2 gene expression[22]. The plant functions by neutralizing reactive oxygen species (ROS) and blocking the activation of these pathways, hence diminishing the synthesis of Prostaglandin E2 (PGE2), the primary product of Cox-2 action[22,23].

The amalgamation of *Z. acanthopodium* fruits and *Moringa oleifera* leaves exhibited the highest expression of Caspase 3 and showed substantial changes in the C^- group. The leaves of *Moringa oleifera* include Quercetin, which possesses antioxidant and anti-inflammatory properties, while kaempferol is recognized for its anticancer, antioxidant and anti-inflammatory benefits[12,13]. Additionally, Apigenin possesses anticancer properties and neuroprotective benefits. Flavonoids in *Zanthoxylum acanthopodium*, commonly referred to as andaliman, exhibit potential as anticancer agents[14]. These flavonoids can induce apoptosis in cancer cells via modulating protein pathways, including p53 and caspase, while also disrupting the cancer cell cycle to halt proliferation[24]. The chemical mitigates inflammation and acts as an antioxidant, inhibiting Caspase-3 production by diminishing oxidative stress that induces apoptosis[24]. Oxidative stress, arising from an imbalance between free radical generation and cellular antioxidant defenses, can initiate the apoptosis process by upregulating pro-apoptotic proteins, including Caspase-3[25]. Antioxidants function by neutralizing free radicals and mitigating oxidative damage to biological constituents, such as DNA, proteins and cell membranes[25]. Consequently, antioxidants can inhibit the activation of Caspase-3, the principal executioner enzyme in the apoptosis cascade, so preserving cell viability and averting more tissue damage[26].

The amalgamation of the two herbs may not be optimal, such as the pairing of *Z. acanthopodium* fruits and *Moringa oleifera*. The individual herbal constituents of the two plants can exhibit significant pharmacological effects. Nonetheless, when combined, it can enhance apoptosis, since the synergistic interaction of bioactive components from each herb activates the apoptotic pathway in target cells[26]. Compounds including flavonoids, polyphenols and terpenoids present in herbs can elevate the generation of reactive oxygen species (ROS), leading to mitochondrial impairment and the activation of pro-apoptotic proteins, including Caspase-3 and Caspase-9[27]. This combination can also suppress the expression of anti-apoptotic proteins like Caspase, therefore expediting planned cell death[28,29]. This synergistic action enhances therapeutic efficacy while minimizing the dosage of each herb, so mitigating the risk of adverse effects and improving the elimination of damaged or abnormal cells[29,30].

CONCLUSION

The fruit of *Zanthoxylum acanthopodium* and *Moringa oleifera* enhance epithelial maturation and mitigate tissue damage in breast tissue by decreasing necrotic cells and increasing tissue organization. Despite both herbs demonstrating individual favorable benefits, their combined application did not reduce Her2 and Cox-2 expression; rather, it augmented both. Nonetheless, both herbs dramatically elevated Caspase 3 expression, showing their involvement in enhancing the apoptotic pathway in breast cancer cells.

SIGNIFICANCE STATEMENT

This study demonstrates that extracts of *Zanthoxylum acanthopodium* and *Moringa oleifera* provide therapeutic potential for breast cancer by influencing critical molecular indicators, including Her2, Cox-2 and Caspase 3. Both extracts show favorable benefits in enhancing breast histology and diminishing neoplastic alterations. This study aimed to investigate the molecular function of *Z. acanthopodium* and *M. oleifera* in modulating these proteins, hence aiding the advancement of cancer treatments. These findings indicate that each herb individually can more effectively control tumor growth and progression at the molecular level than when used in combination. Subsequent research may build upon these findings by investigating the impact of these herbs on additional signaling pathways, potentially opening new possibilities for cancer pharmacotherapy and treatment options for breast cancer and other malignancies.

ACKNOWLEDGMENT

This research is supported by DRTPM funds from the Ministry of Higher Education, Science and Technology through the Fundamental Research-Regular National Competitive Research scheme with contract number 103/E5/PG.02.00. PL/2024, June 11th, 2024.

REFERENCES

1. Machida, Y. and T. Imai, 2021. Different properties of mammary carcinogenesis induced by two chemical carcinogens, DMBA and PhIP, in heterozygous BALB/c *Trp53* knockout mice. Oncol. Lett., Vol. 22. 10.3892/ol.2021.12999.
2. Wiegand, C., U. Schönfelder, M. Abel, P. Ruth, M. Kaatz and U.C. Hipler, 2010. Protease and pro-inflammatory cytokine concentrations are elevated in chronic compared to acute wounds and can be modulated by collagen type I *in vitro*. Arch. Dermatological Res., 302: 419-428.
3. Freudenberg, J.A., Q. Wang, M. Katsumata, J. Drebin, I. Nagatomo and M.I. Greene, 2009. The role of HER2 in early breast cancer metastasis and the origins of resistance to HER2-targeted therapies. Exp. Mol. Pathol., 87: 1-11.
4. Budi, H.S., F.N. Ahmad, H. Achmad, M.J. Ansari and M.V. Mikhailova *et al*., 2022. Human epidermal growth factor receptor 2 (HER2)-specific chimeric antigen receptor (CAR) for tumor immunotherapy; recent progress. Stem Cell Res. Ther., Vol. 13. 10.1186/s13287-022-02719-0.
5. Lin, H., C. Liu, A. Hu, D. Zhang, H. Yang and Y. Mao, 2024. Understanding the immunosuppressive microenvironment of glioma: Mechanistic insights and clinical perspectives. J. Hematol. Oncol., Vol. 17. 10.1186/s13045-024-01544-7.
6. Mercogliano, M.F., S. Bruni, F.L. Mauro and R. Schillaci, 2023. Emerging targeted therapies for HER2-positive breast cancer. Cancers, Vol. 15. 10.3390/cancers15071987.
7. Desai, S.J., B. Prickril and A. Rasooly, 2018. Mechanisms of phytonutrient modulation of cyclooxygenase-2 (COX-2) and inflammation related to cancer. Nutr. Cancer, 70: 350-375.
8. Half, E., X.M. Tang, K. Gwyn, A. Sahin, K. Wathen and F.A. Sinicrope, 2002. Cyclooxygenase-2 expression in human breast cancers and adjacent ductal carcinoma *in situ*. Cancer Res., 62: 1676-1681.
9. Sobolewski, C., C. Cerella, M. Dicato, L. Ghibelli and M. Diederich, 2010. The role of cyclooxygenase-2 in cell proliferation and cell death in human malignancies. Int. J. Cell Biol., Vol. 2010. 10.1155/2010/215158.
10. Huang, Q., F. Li, X. Liu, W. Li and W. Shi *et al*., 2011. Caspase 3-mediated stimulation of tumor cell repopulation during cancer radiotherapy. Nat. Med., 17: 860-866.
11. Yang, X., D.N. Zhong, H. Qin, P.R. Wu and K.L. Wei *et al*., 2018. Caspase-3 over-expression is associated with poor overall survival and clinicopathological parameters in breast cancer: A meta-analysis of 3091 cases. Oncotarget, 9: 8629-8641.
12. Pareek, A., M. Pant, M.M. Gupta, P. Kashania and Y. Ratan *et al*., 2023. *Moringa oleifera*: An updated comprehensive review of its pharmacological activities, ethnomedicinal, phytopharmaceutical formulation, clinical, phytochemical, and toxicological aspects. Int. J. Mol. Sci., Vol. 24. 10.3390/ijms24032098.
13. Vergara-Jimenez, M., M.M. Almatrafi and M.L. Fernandez, 2017. Bioactive components in *Moringa oleifera* leaves protect against chronic disease. Antioxidants, Vol. 6. 10.3390/antiox6040091.
14. Ilyas, S., R.H. Simanullang, S. Hutahaean, R. Rosidah and P.C. Situmorang, 2021. Effect of *Zanthoxylum acanthopodium* methanol extract on CDK4 expression to cervical cancer. Res. J. Pharm. Technol., 14: 5647-5652.
15. Situmorang, P.C., S. Ilyas, S. Hutahaean and Rosidah, 2021. Effect of nano herbal andaliman (*Zanthoxylum acanthopodium*) fruits in NOTCH1 and HES1 expressions to human placental trophoblasts. Pak. J. Biol. Sci., 24: 165-171.

16. Situmorang, P.C., R.A. Syahputra and R.H. Simanullang, 2022. *EGFL7* and *HIF-1α* expression on human trophoblast placental by *Rhodomyrtus tomentosa* and *Zanthoxylum acanthopodium*. Pak. J. Biol. Sci., 25: 123-130.
17. Simanullang, R.H., P.C. Situmorang, M. Herlina, Noradina, B. Silalahi and S.S. Manurung, 2022. Histological changes of cervical tumours following *Zanthoxylum acanthopodium* DC treatment, and its impact on cytokine expression. Saudi J. Biol. Sci., 29: 2706-2718.
18. Situmorang, P.C., S. Ilyas, S. Hutahaean and R. Rosidah, 2021. Histological changes in placental rat apoptosis via FasL and cytochrome *c* by the nano-herbal *Zanthoxylum acanthopodium*. Saudi J. Biol. Sci., 28: 3060-3068.
19. Simanullang, R.H., P.C. Situmorang, J.M. Siahaan, S.S. Widjaja and M. Mutiara, 2022. Effects of *Zanthoxylum acanthopodium* on MMP-9 and GLUT-1 expression and histology changes in rats with cervical carcinoma. Pharmacia, 69: 911-920.
20. Iqbal, N. and N. Iqbal, 2014. Human epidermal growth factor receptor 2 (HER2) in cancers: Overexpression and therapeutic implications. Mol. Biol. Int., Vol. 2014. 10.1155/2014/852748.
21. Pratama, D.A.O.A., A. Nur Cahyati, Ulayatul Kustiati, A.B. Hardian and F.S. Permata, 2024. Proteomics analysis of carcinogenesis in a rat model of mammary cancer induced by DMBA (7,12-dimethylbenz[a]anthracene). F1000Research, Vol. 12. 10.12688/f1000research.132524.2.
22. Papaharalambus, C.A. and K.K. Griendling, 2007. Basic mechanisms of oxidative stress and reactive oxygen species in cardiovascular injury. Trends Cardiovasc. Med., 17: 48-54.
23. Hu, Y.P., Y.B. Peng, Y.F. Zhang, Y. Wang, W.R. Yu, M. Yao and X.J. Fu, 2017. Reactive oxygen species mediated prostaglandin E_2 contributes to acute response of epithelial injury. Oxid. Med. Cell. Longevity, Vol. 2017. 10.1155/2017/4123854.
24. Abotaleb, M., S.M. Samuel, E. Varghese, S. Varghese, P. Kubatka, A. Liskova and D. Büsselberg, 2019. Flavonoids in cancer and apoptosis. Cancers, Vol. 11. 10.3390/cancers11010028.
25. Arfin, S., N.K. Jha, S.K. Jha, K.K. Kesari and J. Ruokolainen *et al*., 2021. Oxidative stress in cancer cell metabolism. Antioxidants, Vol. 10. 10.3390/antiox10050642.
26. Chaudhary, P., P. Janmeda, A.O. Docea, B. Yeskaliyeva and A.F. Abdull Razis *et al*., 2023. Oxidative stress, free radicals and antioxidants: Potential crosstalk in the pathophysiology of human diseases. Front. Chem., Vol. 11. 10.3389/fchem.2023.1158198.
27. Muchtaridi, M., F. Az-Zahra, H. Wongso, L.U. Setyawati, D. Novitasari, E.H. Khairul Ikram, 2024. Molecular mechanism of natural food antioxidants to regulate ROS in treating cancer: A review. Antioxidants, Vol. 13. 10.3390/antiox13020207.
28. Nesci, S., A. Spagnoletta and F. Oppedisano, 2023. Inflammation, mitochondria and natural compounds together in the circle of trust. Int. J. Mol. Sci., Vol. 24. 10.3390/ijms24076106.
29. Che, C.T., Z.J. Wang, M.S.S Chow and C.W.K. Lam, 2013. Herb-Herb combination for therapeutic enhancement and advancement: Theory, practice and future perspectives. Molecules, 18: 5125-5141.
30. Yun, Y., J. Son, K. Kim, B.H. Jang, I. Choi and S.G. Ko, 2017. Effectiveness and safety of combination treatment of herbal medicines and oral antihistamines for atopic dermatitis: A retrospective chart review. Intgr. Med. Res., 6: 19-25.