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EFFECT OF TARAXASTEROL ON THE EXPRESSION OF VEGF AND CXCR4 IN THE PRECANCEROUS LESIONS OF BREAST CANCER IN RATS

H.M. Liu, Z.P. Wang*, C.S. Wang, H. Feng, Z.G. Liu and M. Guo

Department of Traditional Chinese Medicine, Zhongda Hospital Affiliated to Southeast University, Nanjing, China.
*Corresponding author's email: wangzhipengst@yeah.net

ABSTRACT

The aim of study was to explore the effect of Taraxasterol on VEGF and CXCR4 expression in the precancerous lesions of breast cancer (BC) in rats.One hundred and eighty Sprague-Dawley (SD) rats were randomly divided into control group, model group, low-dose Taraxasterol group, moderate-dose Taraxasterol group and high-dose Taraxasterol group and tamoxifen group. Dimethylbenz[a]anthracene (DMBA) was used to induce the model of dysplasia of BC in rats. After the model was successfully established, different doses of Taraxasterol were used for intervention, and tamoxifen was used as the intervention control. Finally, protein and mRNA levels of VEGF and CXCR4 were detected.The number of rats with atypical hyperplasia in high-dose Taraxasterol group and the tamoxifen group was less than that in model group (P<0.05 or P<0.01). After intervention for atypical hyperplasia rats, VEGF and CXCR4 expression levels in the Taraxasterol groups and Tamoxifen group were better than those in model group (P<0.01). In addition, VEGF and CXCR4 levels in each Taraxasterol groups differed drastically from those in the Tamoxifen group (P<0.01). The mRNA expression intensity of VEGF and CXCR4 of rats in each Taraxasterol group was superior to that of Tamoxifen group (P<0.05 or P<0.01). Taraxasterol can effectively reduce levels of VEGF and CXCR4 in DMBA-induced rat BC precancerous lesions, which may be an effective mechanism for inhibiting angiogenesis and blocking BC.

Keywords: Taraxasterol; breast cancer; Precancerous lesions; Vascular endothelial growth factor; CXCR4

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INTRODUCTION

Breast cancer (BC) has a band-like gradual continuous process of "normal epithelium-simple hyperplasia-atypical hyperplasia-carcinoma in situ-invasive carcinoma". (Barzaman *et al*., 2020) Intervention and treatment of precancerous lesions can effectively reduce the incidence of BC. Tumor growth, invasion, metastasis and treatment are related to vascular endothelial growth factor (VEGF), Cxcchemokin receptor 4 (CXCR4) closely, Fas (Factor associated suicide) and FasL (factor associated suicide ligand) improving these indicators can interfere with tumor growth and metastasis, and achieve the purpose of improving precancerous progression. (Li *et al*., 2018; Liu *et al*., 2017) VEGF and CXCR4 are two key regulatory factors. VEGF acts crucially in tumor angiogenesis by promoting new blood vessel formation, thus supplying the tumor with the oxygen and nutrients required for growth. (Al Kawas *et al.,* 2022) CXCR4, on the other hand, plays a crucial role in the distal metastasis of tumors, mediating interactions between tumor cells and other cells, and promoting the invasion and migration of tumor cells. (Yang *et al.,* 2023) Therefore, targeting VEGF, CXCR4, and other related factors may provide new strategies for the prevention and treatment of BC.

Studies have found that dandelion has antibacterial, anti-inflammatory, choleretic, hepatoprotective and immune enhancement effects. (Zhou *et al*., 2023; Giri *et al*., 2022) In recent years, studies have found that dandelion has good anti-tumor activity, and its flavonoids, phenolic acids, phytosterols and other ingredients have shown obvious advantages in anti-oxidation, anti-tumor, anti-bacterial and anti-inflammatory. (Qu *et al*., 2022; Gui and Fan, 2024; Zhang *et al*., 2021) Taraxasterol, an important component of dandelion, has a certain therapeutic effect on a variety of mastitis, bladder cancer, and colorectal cancer. (Movahhed *et al*., 2023; Wang *et al*., 2024; Yang and Wang, 2024) Studies noted that Taraxasterol significantly inhibits the migration and invasion abilities of TGF-β1-treated papillary thyroid carcinoma (PTC) cells, reduces matrix metalloproteinases (MMP-2 and MMP-9), and affects the expression of EMT-related markers. (Zhu *et al.,* 2021) However, it remains unclear whether Taraxasterol exhibits biological activity in precancerous lesions of BC, particularly regarding its regulatory effects on VEGF and CXCR4 and the underlying molecular mechanisms.

Although Taraxasterol exhibits certain antitumor activity, its specific effects on VEGF and CXCR4 expression, as well as the underlying mechanisms, during the premalignant stage of BC remain unclear. Existing studies lack a systematic investigation of the effects of different doses of Taraxasterol on VEGF and CXCR4 expression in BC premalignant models, and have not clarified its differences versus commonly used BC therapeutic agents in regulating these key factors' expression. Therefore, this study aimed to fill this research gap by thoroughly investigating the impact of Taraxasterol on VEGF and CXCR4 expression in BC premalignant lesions. In this study, rats with BC were given Taraxasterol to demonstrate the mechanism of Taraxasterol on BC precancerous lesions, and provide a new direction for the drug development of BC precancerous lesions.

MATERIALS AND METHODS

Medicines and reagents: Dimethylbenz[a]anthracene (DMBA) (Sigma-Aldrich, USA); Primary Antibody of VEGF From Rabbit and second antibody (HRP-Goat Anti-Rabbit IgG)(Abcam, USA); Taraxasterol (Chengdu Refines Biotechnology Co., Ltd., China); Tamoxifen (Liaoning Yicheng Pharmaceutical Co., Ltd., China); Horseradish peroxidase (HRP) kit (Sigma, USA); Horseradish peroxidase labeled goat anti-rabbit secondary antibody (Beijing Bersee, China); VEGF antibody (Abcam, USA); CXCR4 antibody (Abcam, USA); GAPDH antibody (Abcam, USA); RNA extraction kit (Easy Pure RNA kit, Beijing Quanshijin Biotechnology Co., Ltd., China); cDNA synthesis kit (Beijing Quanshijin Biotechnology Co., Ltd., China); PCR detection Kit (Thermo Scientific, USA).

Animals: One hundred and eighty specific pathogen-free (SPF) Sprague-Dawley (SD) rats (6-7-week-old, with body weight ranging from 221 to 310g) were used in this study. Rats were purchased from Western Biotech, Chongqing, China and were housed in Southeast University Experimental Animal Center (License No. was SYXK(SU)2016-0014). All animals were kept in a temperature-controlled room (20-24 ℃, 45%-50% humidity) in stainless steel cages with pellet feed diet and tap water. This study followed the guidelines of the Care and Use of Laboratory Animals at HNI and was approved by the Independent Ethics Committee of Zhongda Hospital Affiliated to Southeast University (Nanjing, China).

Animal grouping and establishment of model: After 7 days of adjustment, these rats were randomly rolled into control group, model group, low-dose Taraxasterol group, moderate-dose Taraxasterol group, high-dose Taraxasterol group, and Tamoxifen group (each n=30). Rats in control group were fed normally. Except for control group, all other groups of rats were induced with DMBA to establish a rat model of BC precursors. After rats were weighed, they were administered DMBA at a dose of 100 mg/kg (10 mL/kg) by gavage once. Each rat received the same dose of solvent or DMBA by gavage every three weeks, for a total of three doses, while rats in control group received the same amount of solvent. During the experiment, rats were observed daily, and behavioral performance, including food and water intake, activity levels, mental state, as well as physiological indicators such as body weight and fur condition, were recorded. Any abnormal behaviors, such as loss of appetite, reduced activity, lethargy, or changes in aggressiveness, were immediately documented, and appropriate measures were taken to alleviate these symptoms.

Treatments: Treatment started on the 2nd day after DMBA gavage, and rats in control and model groups received distilled water by gavage at a dose of 1mL/100g once a day for 4 consecutive weeks. (Li *et al*., 2018) Rats in low-dose, moderate-dose, and high-dose Taraxasterol groups received Taraxasterol by gavage at a dose of 1 mg/ rat, 2 mg/ rat and 4mg/ rat once a day for 4 weeks, respectively. (Ren *et al*., 2022) Rats in Tamoxifen group were given tamoxifen by gavage at a dose of 2mL/100g, once a day for 4 weeks.

Sample collection: During the study, a rat died in the Tamoxifen group. After the 4-week intervention, the 4th, 5th, and 6th pairs of breast tissue and surrounding soft tissues were fixed in 4% paraformaldehyde for 24 hours before being used for detection. The samples were divided into two parts: one part was stored in 4% paraformaldehyde solution for other use; the other part was frozen and stored in liquid nitrogen.

Immunohistochemistry: After these tissue samples were dehydrated with different concentrations of ethanol, the dehydrated samples were soaked in xylene. Then the processed samples were embedded in paraffin and cut into ultra-thin slices using a microtome. Next, the Samples were laid flat on the slices and baked on a slicer at 55°C. The paraffin sections were immersed in xylene to deparaffinize, and then these sections were immersed in gradually diluted ethanol. The slices were put into a container filled with citrate buffer solution and heated in a microwave oven to keep the liquid temperature in the container between 92°C and 98°C for 10-15 minutes. 0.5% TritonX-100 was applied dropwise to the sliced tissue and kept at 25°C for 20 minutes, and then TritonX-100 was washed off. Next, the sample tissue was soaked in with 3% h_2O_2 for 10 min to inactivate endogenous enzymes. The primary antibody (dilution ratio 1:250) was dropped onto the sample section and incubated overnight at 4°C. Sections were rinsed thrice with PBS, 5 min each time and then the working solution of the secondary

antibody (dilution ratio 1:70) was incubated at 37°C for 30 min. The well-washed sections were stained with 3,3 N-diaminobenzidine tetrahydrochloride and hematoxylin, and then dehydrated with series graded alcohol. Finally, the sections were sealed with neutral gum and used for observation and the mean integrated optical density (IOD) of images were measured with Image-Pro Plus soft (version 6.0).

Real-time quantitative PCR for Expression of mRNA: The breast tissue in liquid nitrogen was prepared and the total RNA in the breast tissue was extracted. After reverse transcription, GAPDH was used as the internal reference to perform PCR amplification (shown **Table 1** sequence of primer on internal reference of CXCR4). After PCR amplification was completed, the Ct value was obtained by data processing based on the fluorescence signal generated by the real-time quantitative PCR instrument; the relative quantitative $2^{-\Delta\Delta CT}$ methodology analyzed the results.

Table 1. Sequences of primers on CXCR4, VEGF and internal reference.

Gene	Primer sequence	
VEGF	Upstream	5'-ATC ATC TCC AAG CTG TCA CAC TCC-3'
	Downstream	5'-GTG ATG GAG ATC CAC TTG TGC AC-3'
CXCR4	Upstream	5'-ACG TCG GAG AGC AAC GTC AC-3'
	Downstream	5'-ACC GGG ATT TCT TGC GCT TTC GT-3'
β-actin	Upstream	5'-AGG GAA ATC GTG CGT GAC AT-3'
	Downstream:	5'-AAC CGC TCA TTG CCG ATA GT-3'

Statistical analysis*:* The data was analyzed with SPSS 20.0. One-way analysis of variance (ANOVA) compared measurement data between groups and LSD (Least-Significant Difference) compared data between any two groups. Inspection level was α=0.05. Chi-square test was conducted for comparison of count data between groups.

RESULTS

Appearance of rats: After modeling, rats in control group performed as usual, and rats in the other five groups showed a series of abnormal biological signs: loose and dim fur, decreased body mass, low skin temperature, curled up and less movement, fatigue, indifference, slow response, etc. After treatment, rats in control group showed no difference. Rats in model group performed the same as before. Rats in control group had their fur upright and were in a state of alert. They were curled up and stood in an arch shape. Their physique was slightly decreased; they were irritable, irritable, fighting, occasionally a little active, and slightly sensitive to stimuli. The changes in body weight of rats are shown in Table 2. The mean and standard deviation of the initial body weights across the groups were similar. ANOVA revealed slight difference in the initial body weights between the groups (F=0.128, P>0.05). After modeling, the body weight of control group increased to 258.75 ± 18.40 g, while the body weights of model group, three Taraxasterol groups, and tamoxifen group were markedly lower versus control group (F=5.610, P<0.05). Following treatment, the body weight of control group further increased to 290.33 ± 15.01 g, whereas the body weights of model group, three Taraxasterol groups, and tamoxifen group remained greatly lower versus control group (F=12.087, P<0.01). The body weights of three Taraxasterol groups, as well as the tamoxifen group, were superior to those of model group (P<0.05), with neglectable difference in body weight among three Taraxasterol groups, and tamoxifen group (P>0.05).

Table 2. Statistical changes in body weight of rats

Group	N	Initial weight (g)	Weight after modeling (g)	Weight after treatment (g)
Control group	30	258.75±18.40	280.01±18.45	290.33±15.01
Model group	30	260.14±19.31	239.96±18.03 [a]	228.87±16.48 [a]
Low-dose Taraxasterol group	30	261.19±18.29	241.54±15.29 [a]	250.42±12.99 [ab]
Medium-dose Taraxasterol group	30	259.85±12.69	240.23±13.47 [a]	254.51±10.25 [ab]
High-dose Taraxasterol group	30	260.57±15.22	238.18±17.82 [a]	260.17±11.86 [ab]
Tamoxifen group	30	261.02±16.63	240.42±15.55 [a]	265.20±12.97 [ab]
F		0.128	5.610	12.087
P		0.994	0.025	0.007

Notes: [a]P<0.05 vs. control group; [b]P<0.05 vs. model group.

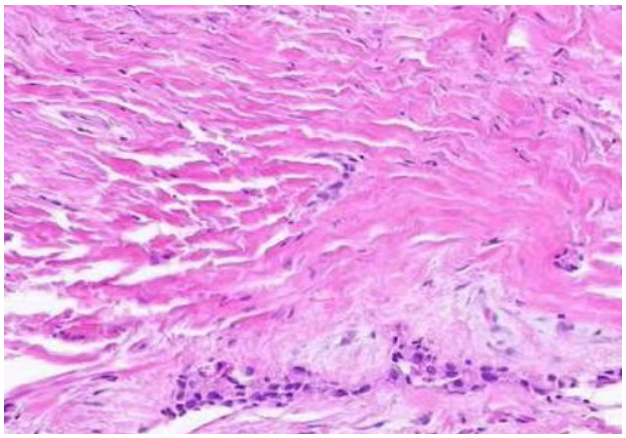
Pathological morphology of mammary gland tissue in rats: Control group consisted of 30 rats, all of which exhibited normal mammary tissue. In model group, only 2 rats (6.67%) had normal mammary tissue, 7 rats (23.33%) showed mild hyperplasia, 20 rats (66.67%) exhibited atypical hyperplasia, and 1 rat (3.33%) developed invasive carcinoma. The proportion of atypical hyperplasia rats in high-dose Taraxasterol group and the tamoxifen group contains was dramatically less than that in model group (P<0.05). The proportion of atypical hyperplasia rats differed slightly between high-dose Taraxasterol group and the tamoxifen group (P>0.05) (**Table 3**).

Results of immunohistochemical staining: Control group has roughly normal glands. In addition, the proliferative cells of model group were of different sizes, diverse shapes, large and densely stained nuclei, and increased nucleoplasm ratio. The cells of atypical hyperplasia have reached 2/3 of the epithelium, mainly type Ⅱ-Ⅲ atypical hyperplasia, and a small number of rats are accompanied by carcinoma in situ. Control group samples were mainly moderate hyperplasia and type I atypical hyperplasia, and the treatment group samples were mainly general hyperplasia and type I atypical hyperplasia. The above content is shown in **Fig. 1**.

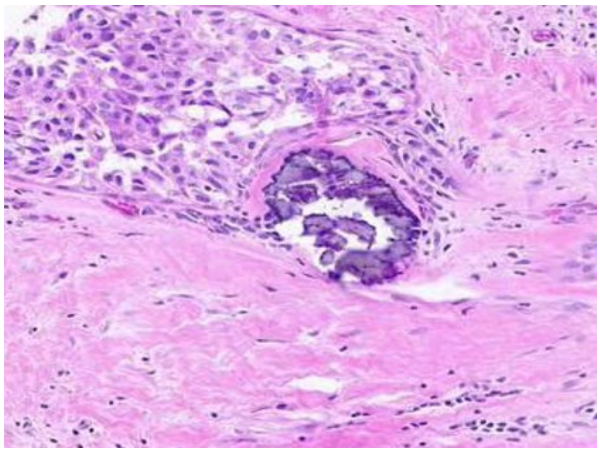
Table 3. Comparison of pathological conditions of the mammary gland tissues of rats.

Group	N	Normal n (%)	Ordinary hyperplasia n (%)	Atypical hyperplasia n (%)	Invasive carcinoma n (%)
Control group	30	30 (100)	0 (0.00)	0 (0.00)	0 (0.00)
Model group	30	2 (6.67)	7 (23.33)	20 (66.67)	1 (3.33)
Low -dose Taraxasterol group	29	4 (13.79)	7 (24.14)	16 (55.17)	2 (6.90)
Moderate-dose Taraxasterol group	30	5 (16.67)	10 (33.33)	14 (46.67)	1 (3.33)
High-dose Taraxasterol group	30	4 (13.33)	16 (53.33)	8 (26.67) [b]	2 (6.67)
Tamoxifen group	29	5 (17.24)	14 (48.28)	9 (31.03) [a]	1 (3.45)

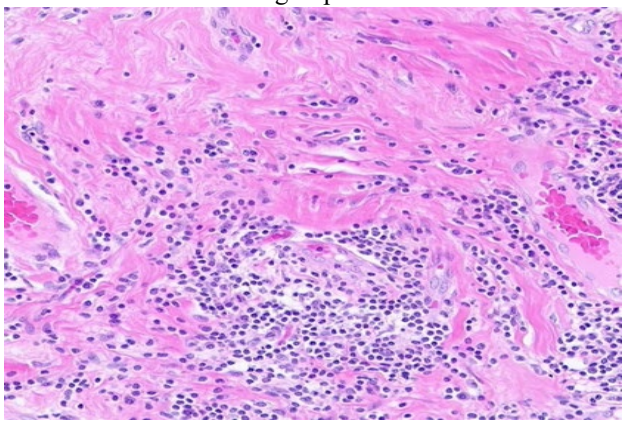
Notes: [a]P<0.05 vs. model group; [b]P<0.01 vs. model group; [c]P<0.05 vs. Taraxasterol group; [d]P<0.01 vs. Tamoxifen group.



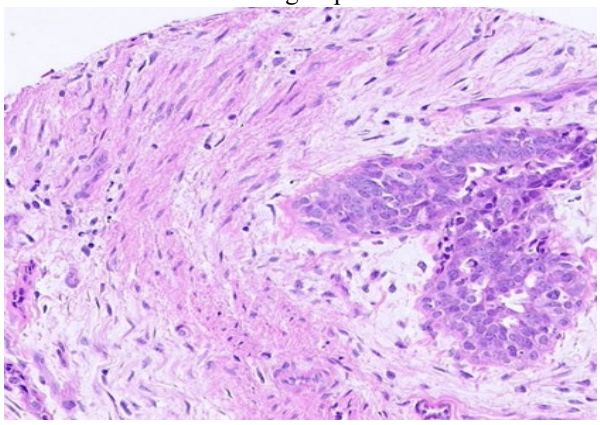
Control group ×100



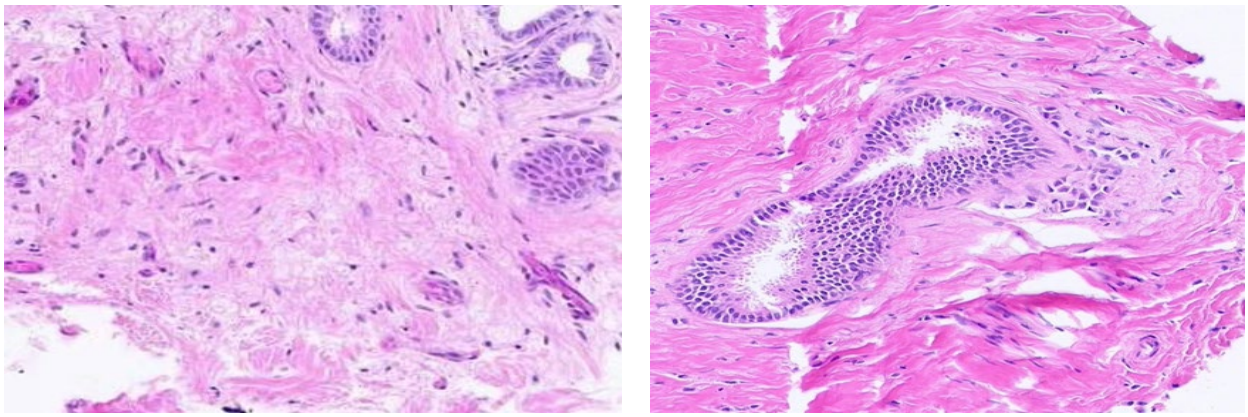
Model group ×100



Low-dose Taraxasterol group ×100



Moderate-dose Taraxasterol group ×100



High-dose Taraxasterol group ×100 Tamoxifen group ×100

Fig.1. VEGF expression in the mammary gland tissues of rats after immunohistochemical staining (VEGF-positive cells appeared brownish yellow, and the cell nuclei appeared blue.

The levels of VEGF and CXCR4: After intervention for atypical hyperplasia rats, VEGF and CXCR4 levels in the Taraxasterol groups and Tamoxifen group were superior to model group (P<0.01). In addition, VEGF and CXCR4 levels in each Taraxasterol groups differed markedly from those in the Tamoxifen group (P<0.01). The relevant values are shown in **Fig. 2** and **Fig. 3**. In addition, the stained smear is shown in **Fig. 4**.

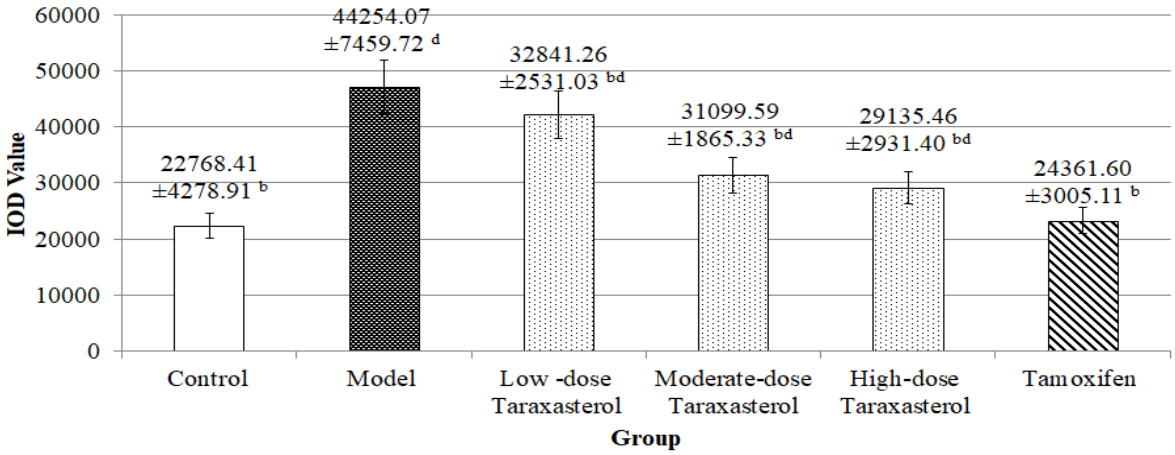


Fig. 2. IOD value reflects VEGF level of rats. ^{b}P<0.01 vs. model group; ^{d}P<0.01 vs. Tamoxifen group.

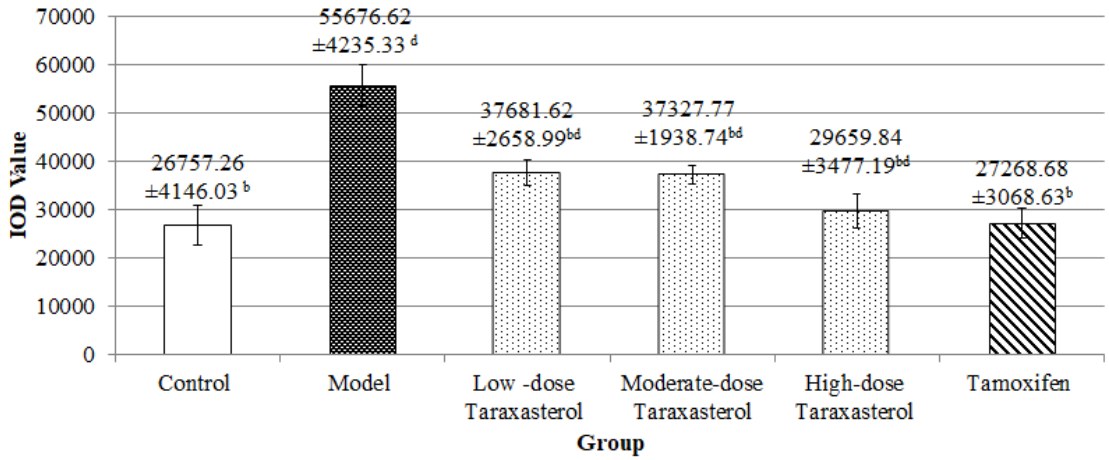
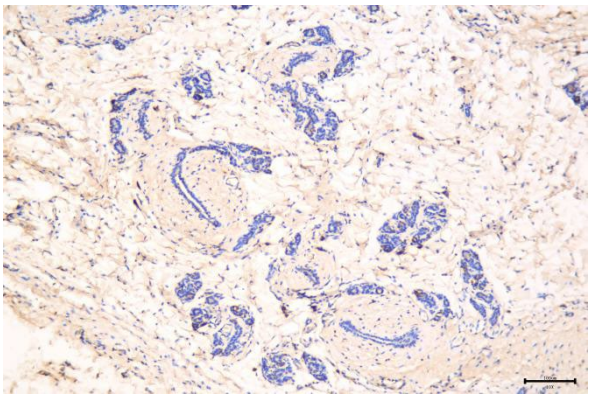
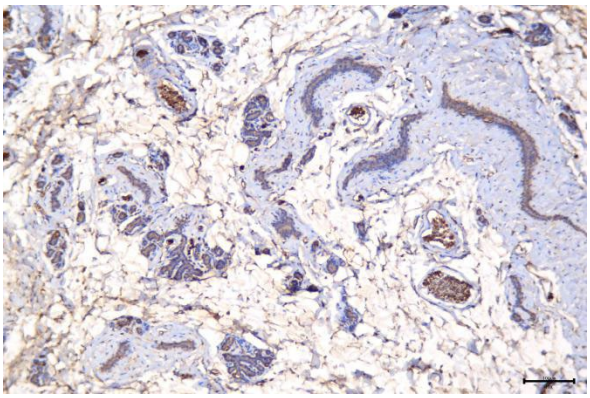


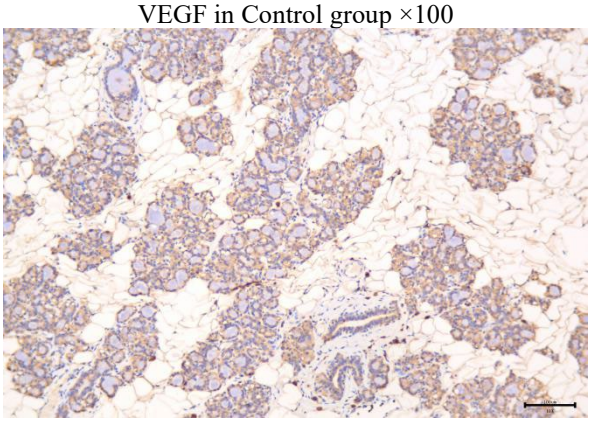
Fig.3. IOD value reflects CXCR4 level of rats. ^{b}P<0.01 vs. model group; ^{d}P<0.01 vs. Tamoxifen group.



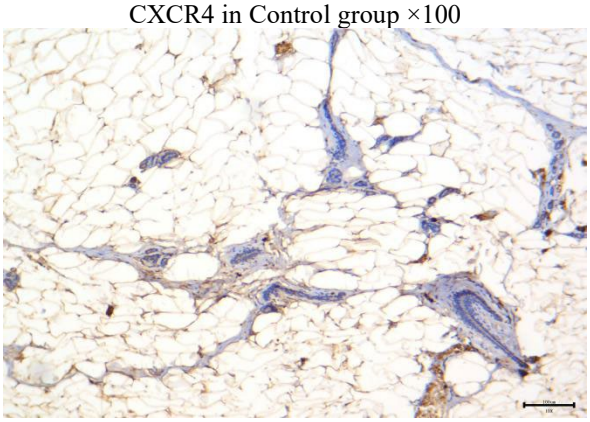
VEGF in Control group ×100



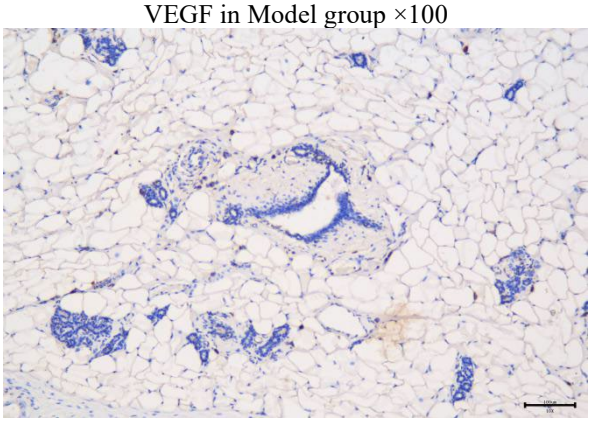
CXCR4 in Control group ×100



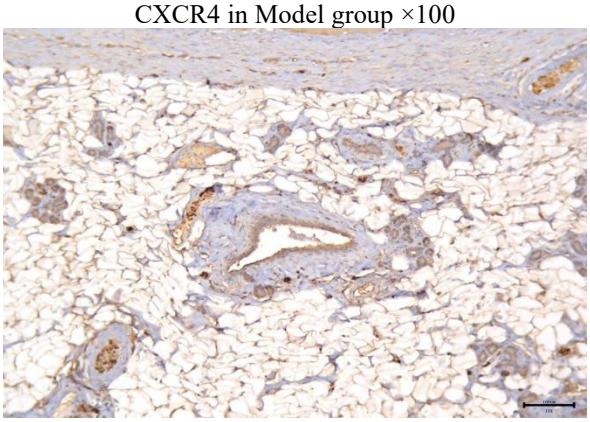
VEGF in Model group ×100



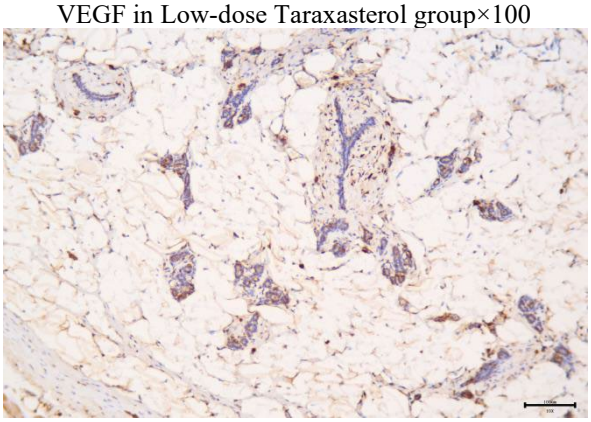
CXCR4 in Model group ×100



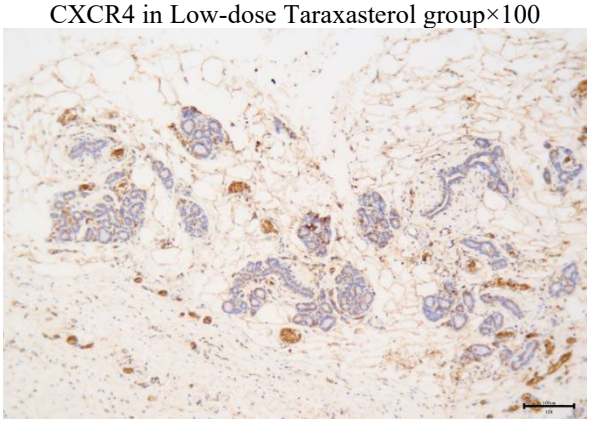
VEGF in Low-dose Taraxasterol group×100



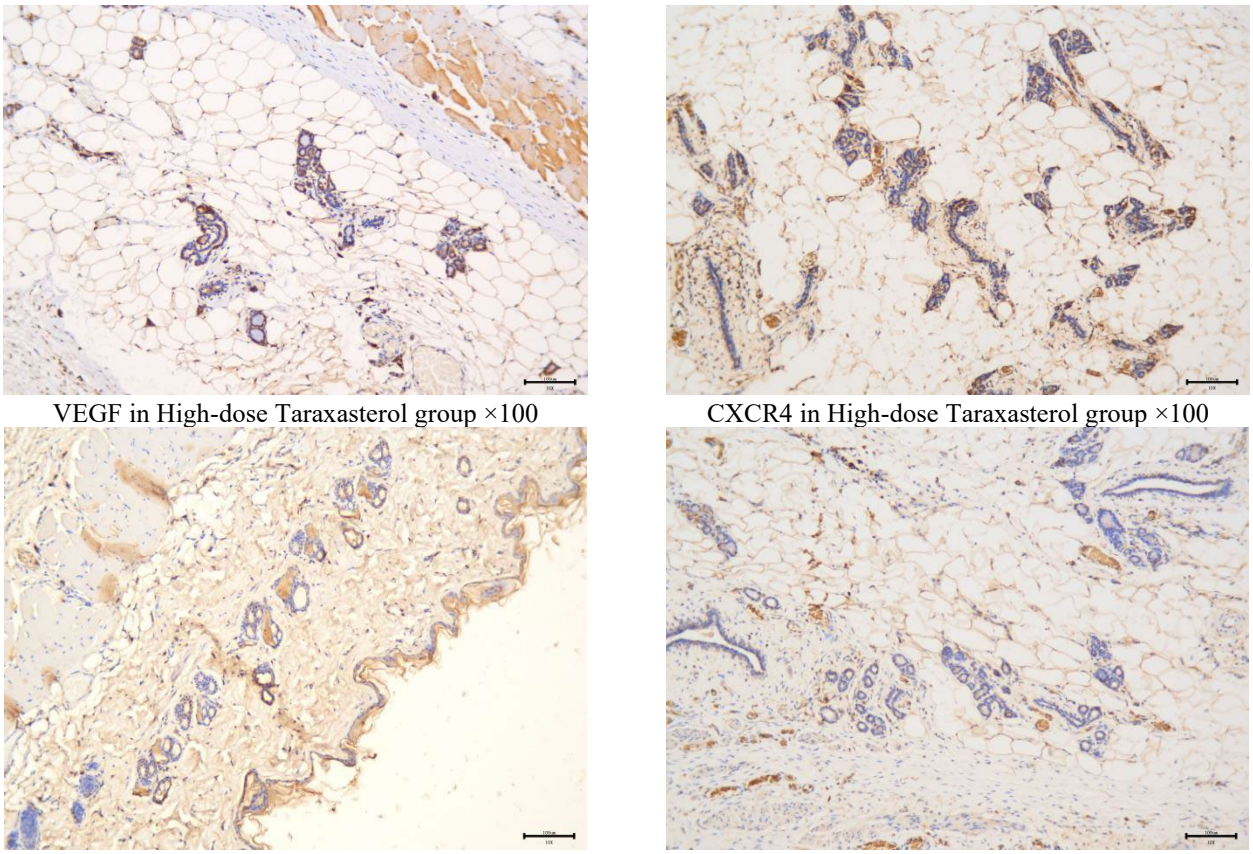
CXCR4 in Low-dose Taraxasterol group×100



VEGF in Moderate-dose Taraxasterol group ×100



CXCR4 in Moderate-dose Taraxasterol group ×100



VEGF in High-dose Taraxasterol group ×100 CXCR4 in High-dose Taraxasterol group ×100

VEGF in Tamoxifen group ×100 CXCR4 in Tamoxifen group ×100

Fig. 4. VEGF and CXCR4 expression in the mammary gland tissues of rats after immunohistochemical staining. The brown marks are positive cells and the blue is the nucleus.

Expressive intensity of VEGF and CXCR4 mRNA: After the treatment, the mRNA expression intensity of VEGF and CXCR4 in the model was superior to other groups (P<0.01). The mRNA expression intensity of VEGF and CXCR4 of rats in each Taraxasterol group was superior to Tamoxifen group (P<0.01 or P<0.05), as shown in **Fig.5** and **Fig. 6**.

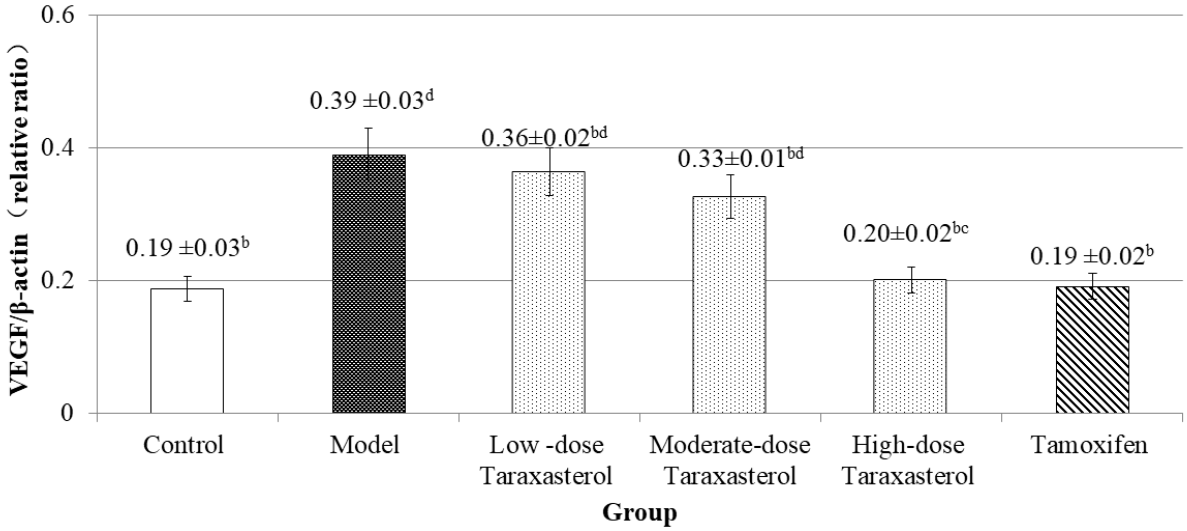


Fig. 5. Expressive intensity of VEGF mRNA in the breast tissue of rats in each group. [b]P<0.01 vs. model group; [c]P<0.05 vs. Taraxasterol group; [d]P<0.01 vs. Tamoxifen group.

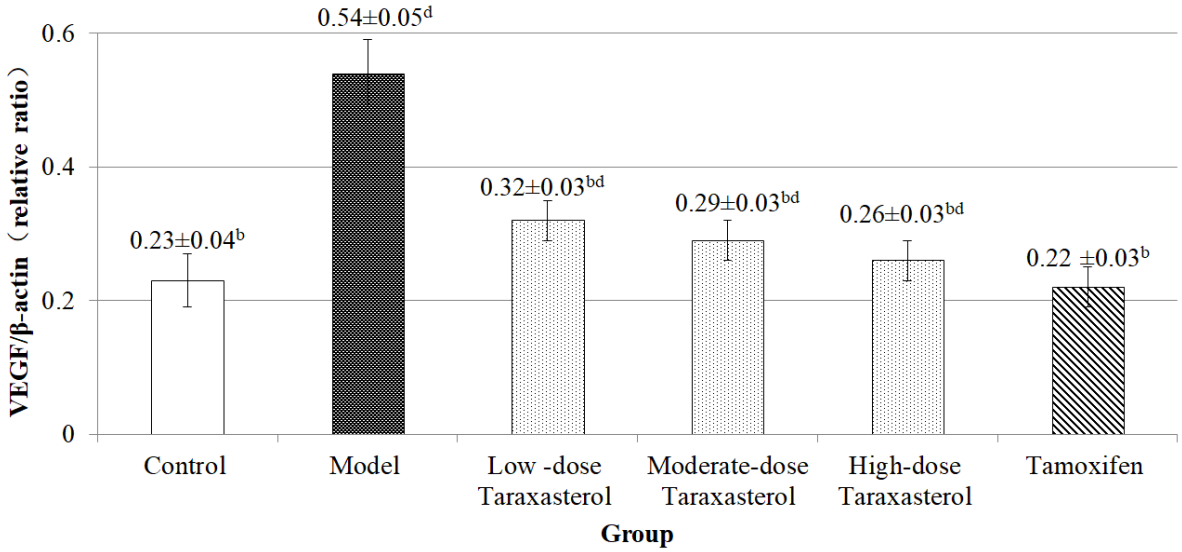


Fig.6. Expressive intensity of CXCR4 mRNA in the breast tissue of rats. $^{b}P<0.01$ vs. model group; $^{d}P<0.01$ vs. Tamoxifen group.

DISCUSSION

A rat model of BC precursors was fabricated by DMBA induction. During the acclimatization phase, rats with obvious health issues (such as infections and injuries) were observed and excluded to ensure the health status of rats entering the experiment was relatively consistent. Experimental conditions were controlled to minimize behavioral and health differences among rats due to environmental factors. All rats were treated at the same time points, using the same dose and administration method, to reduce the impact of procedural variability on the experimental outcomes. Throughout the experiment, the health status of rats was closely monitored, and if any group exhibited significant abnormal physiological indicators, the cause was promptly analyzed, and corresponding adjustments were made to ensure the health of rats remained stable across all groups. The results showed that, after modeling, all rats (except control group) exhibited a series of abnormal biological signs, including loose and dull fur, weight loss, reduced body temperature, decreased activity, and sluggish responses. This is primarily due to the significant damage caused by DMBA, a chemical carcinogen, during the induction of the rat BC precursor model. DMBA likely interferes with various physiological systems in rats, including the endocrine system, metabolic pathways, and immune system, leading to a deterioration of overall health and the manifestation of the aforementioned abnormal biological signs. After treatment, the body weight of control group continued to increase, while the body weight of the other groups remained markedly inferior to that of control group, with even more pronounced differences. This indicates that the impact of DMBA on rat body weight is persistent; despite treatment, the body weight has not fully recovered to the level of the normal control group. However, the body weights of low-dose, medium-dose, high-dose Taraxasterol groups, and the tamoxifen group were all superior to that of model group, suggesting that interventions with Taraxasterol and tamoxifen partially improved rats' physical condition. This improvement may be attributed to mechanisms such as metabolic regulation, appetite enhancement, or increased nutrient absorption, which alleviated the negative impact of DMBA on body weight.

Atypical hyperplasia of breast tissue is generally considered to be a precursor lesion of BC. (Vegunta *et al*., 2023) The study of BC precancerous lesions is closely related to the study of angiogenesis and cell apoptosis. (De Sousa *et al*., 2021) Angiogenesis is closely related to the malignant transformation of benign breast diseases, tumor occurrence, development, metastasis and prognosis. Past experiments on angiogenesis and BC precancerous lesions have suggested that, from mild atypical hyperplasia, moderate to severe atypical hyperplasia to BC, the blood vessel density of each group gradually increased. (Badodekar *et al*., 2021; Elayat and Selim, 2024) VEGF is also known as vascular permeability factor, a cytokine that can produce a variety of biological effects, which is widely expressed in embryonic tissues. (Smolanka *et al*.,2023; Wang *et al*., 2019) Research results confirmed that VEGF is the most important factor that promotes tumor angiogenesis, which is related to BC precancerous lesions and angiogenesis. (Li *et al*., 2021; Sohn *et al*., 2018) When blood vessels are formed, it is closed under normal physiological conditions. Therefore, VEGF is expressed at low levels in normal adult tissues. However, under pathological

conditions, abnormal expression of VEGF can occur in tissues. When tumor cells invade, macrophages and mast cells secrete high levels of VEGF, stimulating tumor vascular endothelial cells via paracrine signaling. This promotes endothelial cell proliferation, migration, and blood vessel formation, driving tumor growth. VEGF also increases vascular permeability, causing fibrin deposition and facilitating the infiltration of monocytes, fibroblasts, and endothelial cells. These processes support tumor stroma formation, enable tumor cells to enter new blood vessels, and promote metastasis. (Song *et al*., 2021; Mao *et al*., 2022) Therefore, blocking VEGF is one of the ways to fight tumor angiogenesis. CXCR4 is highly expressed in human BC cells and metastatic cancer cells, which has attracted much attention as a potential target for treating human BC. (Xu *et al*., 2015) It was reported that CXCR4 and VEGF expression in BC primary tumors and metastases are positively correlated, suggesting that CXCR4 and VEGF may have a synergistic effect in BC progression. (Zhou *et al*.,2018; Li *et al*., 2018)

Taraxasterol (an extract of dandelion) and tamoxifen were used to intervene the mRNA expression of VEGF in DMBA-induced rat pre-mammary lesion tissues to correct the imbalance of atypical hyperplasia from the perspective of anti-angiogenesis. This study believes that these two drugs can early intervention or reverse BC precancerous lesions and reduce the incidence of BC. Tamoxifen is currently a widely used first-line drug in the clinical therapy of estrogen receptor-positive BC. Selecting Tamoxifen as a control allows for a comparison of the efficacy of Taraxasterol with the existing standard treatment, thereby evaluating its potential clinical application value. Tamoxifen is a classic anti-estrogen drug that primarily exerts its effects by binding to the estrogen receptor, competitively inhibiting the activity of estrogen, and thus blocking the proliferative effects of estrogen on BC cells. The primary mechanism of action of Tamoxifen involves regulating ER-mediated signaling pathways to reduce cancer cell proliferation. (Xu *et al.,* 2023; Mishra *et al.,* 2021) In contrast, Taraxasterol, which is independent of the ER signaling pathway, acts primarily by modulating angiogenesis and the migratory capacity of cancer cells in the tumor microenvironment, thereby inhibiting tumor progression. (Bao *et al.,* 2018; Movahhed *et al.,* 2023) This mechanistic difference makes the use of Tamoxifen as a control more informative, as it clearly demonstrates the unique mode of action of Taraxasterol, highlighting its advantages in inhibiting angiogenesis and blocking cancer cell migration. It was found that the multi-target anti-tumor mechanism of Taraxasterol includes direct cytotoxicity, inhibition of tumor angiogenesis, inhibition of tumor cell DNA synthesis and nucleic acid metabolism, induction of cell apoptosis and enhancement of the body's immune protection effect. In addition, Taraxasterol can reduce the blood viscosity in the DMBA-induced rat BC precancerous lesion model and increase the microcirculation perfusion, and its effect is better than tamoxifen. The results demonstrated that the expression intensity of VEGF and CXCR4 mRNA in normal breast, general hyperplasia and atypical hyperplasia in each test group exhibited an increasing trend, and expression rate in cancer tissues was the highest. These suggest that the levels of VEGF and CXCR4 are related to cancer. This study found that Tamoxifen can inhibit VEGF and CXCR4 gene expression, and make the breast tissue of precancerous lesions in an ischemic state. In addition, Taraxasterol can regulate VEGF and CXCR4 gene expression, so that the levels of VEGF and CXCR4 tend to be normal. And this effect is a dose-dependent effect, that is, the effect of high-dose Taraxasterol group is notably better than that of low-dose group. In contrast, Taraxasterol have slightly weaker regulation of VEGF and CXCR4 expression.

In summary, it was revealed that Taraxasterol effectively reduced VEGF and CXCR4 levels in the DMBA-induced rat model of BC precursors. This finding suggests a potential mechanism for inhibiting angiogenesis and blocking the progression of BC. However, this research is primarily based on animal experiments and lacks a group treated only with Taraxasterol without DMBA exposure, making it difficult to fully assess the safety and baseline activity of Taraxasterol in the absence of carcinogenic interference. Furthermore, the study focused solely on VEGF and CXCR4, potentially overlooking other important factors related to the initiation and progression of BC. Future work will include a group treated with Taraxasterol but not exposed to DMBA to comprehensively evaluate its safety and baseline activity. Clinical trials will also be conducted to validate the efficacy and safety of Taraxasterol in humans, determining its potential for use in the prevention and treatment of BC. Additionally, expanding the scope of the research to measure more biomarkers associated with BC progression will provide a more comprehensive understanding of the anticancer mechanisms of Taraxasterol. In conclusion, these findings offer new insights and directions for the prevention and treatment of BC.

Conclusion: Taraxasterol can effectively reduce the expression intensity of VEGF mRNA in DMBA-induced rat BC precancerous lesions, which may be an effective mechanism for inhibiting angiogenesis and blocking BC.

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REFERENCES

Al Kawas, H., I. Saaid, P. Jank, C.C. Westhoff, C. Denkert, T. Pross, K.B.S. Weiler and M.M. Karsten (2022). How VEGF-A and its splice variants affect breast cancer development - clinical implications. Cell Oncol. (Dordr). 45(2): 227–239. https://doi.org/10.1007/s13402-022-00665-w.

Badodekar, N., A. Sharma, V. Patil, G. Telang, R. Sharma, S. Patil, N. Vyas and I. Somasundaram (2021). Angiogenesis induction in breast cancer: a paracrine paradigm. Cell Biochem. Funct. 39(7):860-873. doi: 10.1002/cbf.3663.

Bao, T., Y. Ke, Y. Wang, W. Wang, Y. Li, Y. Wang, X. Kui, Q. Zhou, H. Zhou, C. Zhang, D. Zhou, L. Wang and C. Xiao (2018). Taraxasterol suppresses the growth of human liver cancer by upregulating Hint1 expression. J. Mol. Med. (Berl). 96(7): 661–672. https://doi.org/10.1007/s00109-018-1652-7

Barzaman, K., J. Karami, Z. Zarei, A. Hosseinzadeh, M.H. Kazemi, S. Moradi-Kalbolandi, E. Safari and L. Farahmand (2020). Breast cancer: biology, biomarkers, and treatments. Int. Immunopharmacol. 84:106535. doi: 10.1016/j.intimp.2020.106535.

De Sousa, A.M., T.S. Dantas, P.G. Barros Silva, C.D.S. Martins, G.E. Freire, H.L.R. Junior, G.A.C. Brito, K.M.A. Pereira and R.F.C. Leitão (2021). Analysis of the immunoexpression of Opioid Receptors and their correlation with markers of angiogenesis, cell proliferation and apoptosis in breast cancer. Asian Pac. J. Cancer Prev. 22(2):633-640. doi: 10.31557/APJCP.2021.22.2.633.

Elayat, G. and A. Selim (2024). Angiogenesis in breast cancer: insights and innovations. Clin. Exp. Med. 24(1):178. doi: 10.1007/s10238-024-01446-5.

Giri, S.S., S.G. Kim, K.J. Woo, W.J. Jung, S.B. Lee, Y.M. Lee, S.J. Jo, J.H. Kim and S.C. Park (2022). Impact of dandelion polysaccharides on growth and immunity response in common carp Cyprinus carpio. Fish Shellfish Immunol. 128:371-379. doi: 10.1016/j.fsi.2022.08.008.

Gui, H. and X. Fan (2024). Anti-tumor effect of dandelion flavone on multiple myeloma cells and its mechanism. Discov. Oncol. 15(1):215. doi: 10.1007/s12672-024-01076-z.

Li, Q., R. Ma and M. Zhang (2018). $CoCl_2$ increases the expression of hypoxic markers HIF-1α, VEGF and CXCR4 in breast cancer MCF-7 cells. Oncol Lett. 15(1):1119-1124. doi: 10.3892/ol.2017.7369.

Liu, G., Z. Liu, Y. Yan and H. Wang (2017). Effect of fraxetin on proliferation and apoptosis in breast cancer cells. Oncol. Lett. 14(6):7374-7378. doi: 10.3892/ol.2017.7143.

Li, H., P. Wang, W. Gong, Q. Wang, J. Zhou, W. H. Zhu and Y. Cheng (2018). Dendron-grafted polylysine-based dual-modal nanoprobe for ultra-early diagnosis of pancreatic precancerosis via targeting a Urokinase-Type Plasminogen Activator Receptor. Adv. Healthc. Mater. 7(5), 10.1002/adhm.201700912. https://doi.org/10.1002/adhm.201700912

Li, Z., Y. Wang, C. Liu, Z. Wang, D. Wang, X. Liang and J. Tian (2021). Association between VEGF single nucleotide polymorphism and breast cancer in the Northern China Han population. Breast Cancer Res. Treat. 186(1):149-156. doi: 10.1007/s10549-020-06024-3.

Mao, G., S. Zheng, J. Li, X. Liu, Q. Zhou, J. Cao, Q. Zhang, L. Zheng, L. Wang and C. Qi (2022). Glipizide combined with ANP Suppresses breast cancer growth and metastasis by inhibiting angiogenesis through VEGF/VEGFR2 signaling. Anticancer Agents Med Chem.22(9):1735-1741. doi: 10.2174/1871520621666210910085733.

Mishra, A., A. Srivastava, A. Pateriya, M.S. Tomar, A.K. Mishra and A. Shrivastava (2021). Metabolic reprograming confers tamoxifen resistance in breast cancer. Chem. Biol. Interact. 347: 109602. https://doi.org/10.1016/j.cbi.2021.109602

Movahhed, M., M. Pazhouhi, H.E.G. Ghaleh and B.J. Kondori (2023). Anti-metastatic effect of taraxasterol on prostate cancer cell lines. Res. Pharm. Sci. 18(4):439-448. doi: 10.4103/1735-5362.378090.

Qu, J., F. Ke, X. Yang, Y. Wang, H. Xu, Q. Li and K. Bi (2022). Induction of P-glycoprotein expression by dandelion in tumor and heart tissues: impact on the anti-tumor activity and cardiotoxicity of doxorubicin. Phytomedicine. 104:154275. doi: 10.1016/j.phymed.2022.154275.

Ren, F., Y. Zhang, Y. Qin, J. Shang, Y. Wang, P. Wei, J. Guo, H. Jia and T. Zhao (2022). Taraxasterol prompted the anti-tumor effect in mice burden hepatocellular carcinoma by regulating T

lymphocytes. Cell Death Discov. 8(1): 264. https://doi.org/10.1038/s41420-022-01059-5

Smolanka, I.I., I.Y. Bagmut, O.V. Movchan, M.I. Sheremet, O.M. Bilyi, A.O. Lyashenko, I.V. Dosenko, A.D. Loboda, O.M. Ivankova and I.L. Kolisnyk (2023). Features of VEGF and IL-6 expression in patients with inflammatory breast cancer considering molecular-biological characteristics. J Med Life. 16(1):153-159. doi: 10.25122/jml-2022-0172.

Sohn, E.J., D.B. Jung, H. Lee, I. Han, J. Lee, H. Lee and S.H. Kim (2018). CNOT2 promotes proliferation and angiogenesis via VEGF signaling in MDA-MB-231 breast cancer cells. Cancer Lett. 412:88-98. doi: 10.1016/j.canlet.2017.09.052.

Song, Y., S. Zeng, G. Zheng, D. Chen, P. Li, M. Yang, K. Luo, J. Yin, Y. Gu, Z. Zhang, X. Jia, N. Qiu, Z. He, H. Li and H. Liu (2021). FOXO3a-driven miRNA signatures suppresses VEGF-A/NRP1 signaling and breast cancer metastasis. Oncogene. 40(4):777-790. doi: 10.1038/s41388-020-01562-y.

Vegunta, S., D.M. Mussallem, A.S. Kaur, S. Pruthi and C.L. Klassen (2023). Atypical hyperplasia of the breast: clinical cases and management strategies. Cleve. Clin. J. Med. 90(7):423-431. doi: 10.3949/ccjm.90a.22098.

Wang, L., H. Shi, Y. Liu, W. Zhang, X. Duan, M. Li, X. Shi and T. Wang (2019). Cystathionine-γ-lyase promotes the metastasis of breast cancer via the VEGF signaling pathway. Int. J. Oncol. 55(2):473-487. doi: 10.3892/ijo.2019.4823.

Wang, Q., R. Zhang, Y. He, G. Mao and Z. Kong (2024). Taraxasterol enhanced bladder cancer cells radiosensitivity via inhibiting the COX-2/PGE2/JAK2/STAT3/MMP pathway. Int. J. Radiat. Biol. 100(5):791-801. doi: 10.1080/09553002.2024.2324475.

Wang, F., S. Li, Y. Zhao, K. Yang, M. Chen, H. Niu, J. Yang, Y. Luo, W. Tang and M. Sheng (2016). Predictive role of the overexpression for CXCR4, C-Met, and VEGF-C among breast cancer patients: A meta-analysis. Breast. 28:45-53. doi: 10.1016/j.breast.2016.04.016.

Xu, C., H. Zhao, H. Chen, and Q. Yao (2015). CXCR4 in breast cancer: oncogenic role and therapeutic targeting. Drug Des Devel Ther. 9:4953-64. doi: 10.2147/DDDT.S84932.

Xu, Z., X. Wang, W. Sun, F. Xu, H. Kou, W. Hu, Y. Zhang, Q. Jiang, J. Tang and Y. Xu (2023). RelB-activated GPX4 inhibits ferroptosis and confers tamoxifen resistance in breast cancer. Redox Biol. 68:102952. https://doi.org/10.1016/j.redox.2023.102952

Yang, K. and Y. Wang (2024). Dandelion root extracts and taraxasterol inhibit LPS-induced colorectal cancer cell viability by blocking TLR4-NFκB-driven ACE2 and TMPRSS2 pathways. Exp. Ther. Med. 27(6):256. doi: 10.3892/etm.2024.12544.

Yang, Y., J. Li, W. Lei, H. Wang, Y. Ni, Y. Liu, H. Yan, Y. Tian, Z. Wang, Z. Yang, S. Yang, Y. Yang and Q. Wang (2023). CXCL12-CXCR4/CXCR7 Axis in Cancer: from Mechanisms to Clinical Applications. Int. J. Biol. Sci. 19(11): 3341–3359. https://doi.org/10.7150/ijbs.82317

Zhang, S., Z. Song, L. Shi, L. Zhou, J. Zhang, J. Cui, Y. Li, D.Q. Jin, Y. Ohizumi, J. Xu and Y. Guo (2021). A dandelion polysaccharide and its selenium nanoparticles: structure features and evaluation of anti-tumor activity in zebrafish models. Carbohydr. Polym. 270:118365. doi: 10.1016/j.carbpol.2021.118365.

Zhou, S., Z. Wang, Y. Hao, P. An, J. Luo, and Y. Luo (2023). Dandelion polysaccharides ameliorate high-fat-diet-induced atherosclerosis in mice through antioxidant and anti-inflammatory capabilities. Nutrients. 15(19):4120. doi: 10.3390/nu15194120.

Zhou, Y., F. Yu, F. Zhang, G. Chen, K. Wang, M. Sun, J. Li, and D. Oupický (2018). Cyclam-Modified PEI for combined VEGF siRNA silencing and CXCR4 inhibition to treat metastatic breast cancer. Biomacromolecules. 19(2):392-401. doi: 10.1021/acs.biomac.7b01487.

Zhu, J., X. Li, S. Zhang, J. Liu, X. Yao, Q. Zhao, B. Kou, P. Han, X. Wang, Y. Bai, Z. Zheng and C. Xu (2021). Taraxasterol inhibits TGF-β1-induced epithelial-to-mesenchymal transition in papillary thyroid cancer cells through regulating the Wnt/β-catenin signaling. Hum. Exp. Toxicol. 40(12_suppl): S87–S95. https://doi.org/10.1177/09603271211023792.