**Disease and Diagnosis**

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**Abstract**

**Background:** Cancer metastasis is the leading cause of death among cancer patients, but there are numerous treatment options available, including drugs such as albendazole (ABZ) and complementary therapies such as cannabis-based medicines, making them important targets for therapeutic interventions. The present study aimed to assess the impact of ABZ on the protein expression of Tau and Stathmin, as well as cell migration, in patients with metastatic breast cancer (BC), where these proteins play critical roles.

**Materials and Methods:** MCF-7 and MDA-MB-231 cell lines were split into a treatment group that received varying concentrations of a standardized extract of ABZ for 48 hours and a control group that received no treatment in this study. The relative gene expression was measured using a quantitative reverse transcription-real-time polymerase chain reaction assay and the ΔΔct method. A migration assay was also performed to assess cancer metastasis.

**Results:** Tau and Stathmin gene expression and cell migration were significantly decreased compared to the control group.

**Conclusion:** The results of the study demonstrated that ABZ reduced both Tau and Stathmin gene expression, as well as cancer metastasis. Nonetheless, evidence suggests that we can use ABZ as an anti-tumor drug for BC treatment.

**Keywords:** Metastasis, Albendazole, Tau, Stathmin, Breast cancer

**Introduction**

Metastasis is the primary cause of mortality in cancer disease, and it is a complex phenomenon (1). Factors increasing the risk of contracting breast cancer (BC) include age, gender, genetic factors, and environmental factors such as smoking, alcohol, and food (2). Various strategies, including surgery, gene therapy, radiotherapy, hormone therapy, and chemotherapy, can be used to treat cancer (3). Several studies have demonstrated that patients with metastases have a shorter lifespan compared to those without metastases (4). The composition of microtubules is based on a singular type of globular protein known as tubulin (5). Tubulin is regarded as the dimeric subunit of microtubules and is the primary protein in the cell, playing a crucial role in cell division (6). The sensitivity of chemotherapy and the growth of BC tumors can be affected by the interactions between tubulin and microtubule-associated proteins (MAPs) (7). Tau protein is part of the (MAPs) family that increases the effects of microtubules (8). The growth of neurites is significantly impacted by the Tau protein, an indispensable member of the neuronal MAP family (9).

There is a possibility that Tau protein may be involved in the development of acquired resistance to taxanes (10). The reason for the competition between Paclitaxel and Tau protein is that Paclitaxel adheres to the Tau protein (11). The occurrence of hyperphosphorylation and neurodegenerative disorders after translation can be attributed to incorrect mRNA splicing resulting from mutations in the Tau gene (12). According to recent data, it is evident that the phosphorylation of Tau can result in the abrupt death of neurons (13). Previous studies have indicated that Stathmin is a conserved protein (14). The function of microtubules, which are major components of the cytoskeleton, is significantly influenced by Stathmin, a regulatory protein (15). While Stathmin plays a significant role in regulating the cell cycle, it is also considered an oncoprotein (oncoprotein 18) because mutations in the Stathmin gene can lead to uncontrolled cell proliferation (16). Stathmin expression has been observed in different human cancers, including ovarian cancer, as per previous research (17). Elevated levels of the Stathmin protein have been detected in various malignant tumors such as acute leukemia, lymphoma, lung cancer, thyroid cancer, ovarian cancer, and prostate cancer based on research (18). Albendazole (ABZ), which

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is a derivative of Benzimidazole, was initially identified as an anthelmintic medication in 1982 according to earlier research (19). Many studies have shown that ABZ possesses anti-tumor activities. One example is its ability to destroy factor-1-α activity and prevent the growth of lung cancer cells (20).

**Objectives**
The objective of this study was to investigate the impact of ABZ on Tau and Stathmin expression in a BC cell line (MCF-7 and MDA-MB-231) and its effect on cancer invasion.

**Materials and Methods**

**Drug**

After being dissolved in dimethyl sulfoxide at a concentration of 0.01% V/V, ABZ (Sigma-Aldrich, USA) was added to the corresponding culture medium for each cell line.

**Cell Culture**

MCF-7 and MDA-MB-231 cell lines were chosen for this project due to their accessibility and reproducibility. They were obtained from the National Cell Bank of Iran, which is affiliated with the Pasteur Institute in Tehran. The cells were cultured in Dulbecco’s Modified Eagle Medium supplemented with 10% fetal bovine serum, 1 g/L glucose, 1% L-glutamine, and 1% penicillin-streptomycin at 37 °C in a CO₂ atmosphere with 5% CO₂ and 95% O₂ concentration. The medium was changed on a daily basis, and the third passage cells were utilized for experiments once they reached 80% confluency.

**Real-Time Polymerase Chain Reaction Procedure**

To prepare cDNA, two primers, a random primer and the Oligo-dT primer, were utilized following the instructions of the Quantic Transcription Kit. In this project, the real-time polymerase chain reaction (RT-PCR) was employed using the Rotor-Gene 6000 system (Corbett Research, Australia) to measure the expression of Tau and Stathmin genes. The PCR was performed in two stages with an annealing and extension temperature of 60 °C over 55 cycles. By binding SYBR Green dye to dsDNA, it generated fluorescence, which was measured to directly detect PCR products. The reference gene glyceraldehyde-3-phosphate dehydrogenase was employed to analyze all samples in triplicate. By employing the ΔΔCt formula, (ΔΔct) was calculated for each gene with ABZ tested at different concentrations.

\[ \Delta \Delta C_t = \Delta C_t_{\text{case}} - \Delta C_t_{\text{control}} \]

**Invasion Assay**

An in vitro experiment was conducted using uncoated transwell inserts with an 8-µm pore size (SPL, Germany).

To treat cancer, different dosages of standardized ABZ were administered for 48 hours, while the control group received no treatment. The experiment was repeated three times in duplicate, and cells that did not migrate through the membrane after a 48-hour incubation period were removed from the top of the membrane.

**Results**

**The Effect of Albendazole on Tau Gene Expression in MDA-MB-231**
The results (Figure 1) revealed that the expression of the Tau gene in the MDA_MB_231 cell line was significantly reduced by ABZ at concentrations of 0.1, 1, 10, and 100 µm.

**The Effect of Albendazole on Stathmin Gene Expression in MDA-MB-231**

Based on the findings (Figure 2), ABZ did not significantly affect the expression of the Stathmin gene.

**The Effect of Albendazole on Tau Gene Expression in MCF-7**
The obtained results demonstrated that ABZ had no
significant impact on the expression of the Tau gene (Figure 3).

The Effect of Albendazole on Stathmin Gene Expression in MCF-7

According to the findings (Figure 4), the expression of the Stathmin gene was reduced by different concentrations of ABZ.

Discussion
The growing resistance of cancers to standard treatments is a current issue (21). Cancer can be treated using various approaches such as chemotherapy, radiation therapy, surgery, gene therapy, hormone therapy, and the inhibition of angiogenesis, among others. Several drugs have been evaluated for their effectiveness in treating cancer (22). Accordingly, the research and development of effective drugs are crucial (23). In this study, it was found that ABZ has therapeutic potential for MCF-7 and MDA-MB-231 tumors and reduces the gene expression of Tau and Stathmin proteins. Several drugs were examined for this purpose. ABZ, a benzimidazole derivative, has been clinically tested and reported to possess anti-tumor properties (24). Additional studies demonstrated that cells that are resistant to paclitaxel exhibit sensitivity to ABZ (25). Research studies indicated that a lower expression of the Tau protein leads to improved function (26). The effect of Taxanes drugs such as paclitaxel is associated with their interaction with tubulins, resulting in the suppression of microtubule dynamics (27). Several studies examined the usefulness of Tau expression as a prognostic and predictive marker in the treatment of BC (28). According to researchers, the expression of the Tau protein significantly decreased in patients with advanced BC who received paclitaxel chemotherapy. Additionally, by measuring the extent of Tau expression reduction, the drug dosage can be adjusted accordingly (29). Stathmin’s effect on the migration and invasion of cancer cells has been investigated in several studies. Its promotion of tubulin polymerization and microtubule-actin association facilitates these processes (30). According to earlier studies, the ratio of Tau-to-Stathmin protein expression is a more dependable prognostic marker for BC patients than other tested biomarkers. Elevated Stathmin and Tau expression enhances microtubule stability, leading to a better prognosis for BC (31). The RT-PCR results of the present study demonstrated that ABZ reduced Tau gene expression significantly at concentrations of 0.1, 1, 10, and 100 µm in the MDA-MB-231 cell line, with a reduction of approximately 90%. In contrast, ABZ significantly decreased Stathmin gene expression but did not have a significant effect on the expression of the Tau gene in the MCF-7 cell line (Figure 3). In both cell lines, there was a significant decrease in gene expression with an increase in drug concentration.

Conclusion
The findings of this study revealed that ABZ possesses anti-tumor properties by suppressing the gene expression of Tau and Stathmin in BC and metastatic cells. Therefore, there is a growing interest in developing new drugs for cancer treatment, and ABZ is a promising candidate that could be used alongside existing anti-cancer medications.

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Effect of albendazol on Tau and Stathmin in breast cancer

Competing Interests
The authors declare that there are no conflicts of interest.

Ethical Applicable
Not applicable.

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References


