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# Effects of curcumin on arginase enzyme activity, ornithine and nitric oxide levels in experimental breast cancer model

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**Abstract**

Breast cancer accounts for almost 30% of all cancer types, making it the most common type of tumor among women in the world. Arginase, an essential enzyme of the urea cycle, leads to the formation of urea and ornithine from L-arginine using the same substrate as nitric oxide synthase (NOS). Arginase has been reported to be higher in cancer patients and can be used as a useful biomarker. In this study, we aimed to investigate the effects of curcumin, an anticarcinogenic agent, on arginase enzyme activity, ornithine, and nitric oxide (NO) levels in experimental breast cancer model in mice. 43 male Balb/c mice were used, and Erhlich acid tumor model was created in the study. Mice were divided into five groups as healthy control group, curcumin treatment before tumor formation, curcumin treatment after tumor formation and cancer control groups. 100 mg/kg curcumin were given orally. Serum and tissue arginase enzyme activities, NO levels and tissue ornithine levels were determined spectrophotometrically. Increased serum arginase activity decreased with curcumin treatment, but this difference was not statistically significant. On the other hand, decreased NO levels were increased with curcumin treatment. In tumor tissue, arginase activity and ornithine levels decreased significantly with curcumin treatment and tissue NO levels increased significantly with curcumin treatment. In our study, we show that curcumin may have a protective effect on the development of breast cancer by inhibiting arginase enzyme activity and ornithine levels, which are the precursors of polyamines, as well as inducing NO production via NOS. As a promising anticancer agent, the net effects of curcumin in this mechanism should be supported by more advanced studies and new parameters.

**Keywords:** arginase, breast cancer, curcumin, nitric oxide, ornithine

## 1. Introduction

Breast cancer is one of the leading causes of cancer and cancer death, which is the most common diagnosis among women who constitute 23% of all cancer cases and 14% of cancer deaths (1). Therefore, it is very important to continue to develop the diagnostic and treatment methods currently used and to identify new prognostic variables (2). Arginase, (L-arginine amidinohydrolase; E.C.3.5.3.1) catalyzes the hydrolysis of L-arginine, urea and ornithine as the key enzyme (3) responsible for nitrogen metabolism (4). Nitric oxide synthase is the enzyme responsible for the catalysis of L-arginine, nitric oxide and L-citrulline oxidation (3).

Arginase enzyme has two isoforms, AI and AII. AI (hepatic arginase) is a cytosolic enzyme and is involved in the liver. AII (extrahepatic arginase) was localized to the mitochondrial matrix. While AI is mainly related to urea synthesis and detoxification of ammonia, AII is thought to be related to biosynthetic functions such as ornithine, proline, glutamate synthesis (5). Arginase can induce nitric oxide synthase (NOS) activity (6) and plays a role in wound healing, immune response, tumor biology and inflammation regulation in conjunction with NOS (7). Serum arginase activity is low in healthy individuals (8). The arginase enzyme is a potent immune inhibitor and is present in the cytoplasm of cancer cells in a much larger amount than in normal cells (9). It was found that serum arginase level was 4 times higher in breast cancer than healthy women in the preoperative period (10). Because of the polyamines, which are very important for cell proliferation, it is possible that high ornithine level due to increased arginase activity will lead to cancer development. Many different studies on serum and tissue arginase levels in various types of cancer indicate that arginase enzyme activity is related to cancer (8, 10). It was also stated that arginase enzyme activity might be an important determinant in breast cancer patients (11). Arginine is catalyzed by NOS and nitric oxide (NO) and citrullin are synthesized. Three types of NOS are mentioned: neuronal (nNOS or NOS1), inducible (iNOS or NOS2) and endothelial (eNOS or NOS3). eNOS and nNOS are thought to be constitutive (cNOS) (11). Numerous studies have shown that NO is associated with both tumor-inducing and tumor

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suppressing functions. Today, the contradiction continues (2). In addition to that, NO and NO metabolites have been shown to have an inhibitory effect on tumor tissue metastasis and cell development (12). Curcumin is a yellow-colored hydrophobic polyphenol extracted from the *Curcuma longa* roots. Experimental studies show that curcumin inhibits breast cancer cell growth through the nuclear factor kappa B (NF-κB) signaling pathway and induces breast cancer apoptosis by regulating the expression of apoptosis-related genes (13, 14). The aim of this study is to investigate the effects of curcumin on serum and cancer tissue arginase enzyme activities, NO levels and tissue ornithine levels for the first time in this study.

## 2. Materials and Methods

### 2.1. Animals and tumor cells

This study was performed in the Experimental Animal Breeding and Research Unit of the Trakya University upon approval by the local animal ethics committee. 43 male Balb / c mice with a mean weight of 25-30 grams (mean 27 grams) were used. Ehrlich ascites tumour cells which were derived from a spontaneous murine mammary adenocarcinoma were used obtaining the breast carcinoma (11).

### 2.2. Experimental design

In the eighth week, five groups were formed. The first group was healthy controls (Cont.). The other groups were breast cancer. To induce the formation of tumours, subcutaneous inoculation of Ehrlich ascites cells into the mice's left footpath was performed. Tumor growth was visible, and mice had difficulty walking. 7 days after injection of Ehrlich acid cells, the thickness of the footpad was measured and assessed. Group 2 (tumor cont. 1) received 100 µl of ethyl alcohol orally 5 days prior to the injection of Ehrlich acid tumor, which continued for 23 days. Group 3 (tumour cont. 2) One week after the injection of Ehrlich acid tumor, 100 µl of ethyl alcohol was given orally every other day for the whole experiment. Group 4 (Treatment 1), treatment was started 5 days before the injection of Ehrlich acid tumor and 100 mg/kg curcumin dissolved in 100 µl of ethyl alcohol and administered orally over the course of the entire experiment. Group 5 (Treatment 2) treatment was started 1 week after the Ehrlich acid tumor was injected and 100 mg/kg curcumin dissolved in 100 µl of ethyl alcohol and administered orally over the course of the entire experiment. At the end of the experimental studies, the animals were sacrificed under anaesthesia. Serum and cancer tissue samples were stored at -80 °C. Determination of Arginase enzyme activity in serum and cancer tissue Cancer tissue Arginase activity; The amount of urea produced as a result of the hydrolysis of arginine with arginase in the sample was determined by spectrophotometric determination with TDMU (Thiosemicarbazide-Diacetyl Monoxime Urea) method (15). Breast tissue ornithine Levels were measured colorimetrically with Chinard's Method (16). Breast tissue protein levels were determined by Lowry method (17). Serum arginase activity was determined by spectrophotometric determination with Munder method with some modifications (18). NO was measured spectrophotometrically using Cartos and Wakid method (19).

### 2.3. Statistical analysis

The statistical analysis of the data provided in our study was performed using STATISCA statistical program. The differences between arginase enzyme activity, ornithine and NO levels between tumor control and treatment groups were evaluated by Kruskal Wallis test and compared with Mann-Whitney U test. In addition, correlations between the groups were evaluated using Spearman's rank correlation coefficient analysis. P values below 0.05 were accepted to be statistically significant.

## 3. Results

Serum means and standard deviation values arginase enzyme activities and NO levels as well as tissue arginase enzyme activities, ornithine and NO levels were shown in Table 1. Serum arginase enzyme activities were significantly higher in tumor groups than healthy control groups. When treatment groups and tumor groups were compared, serum NO levels were significantly increased in treatment groups. Although there was a decrease in the serum arginase enzyme activity after treatment, this decrease was not statistically significant. It was found that arginase enzyme activity decreased significantly with curcumin treatment in tumor tissue samples. As a result of curcumin treatment, both decreasing arginase activity and ornithine levels were significantly decreased. On the other hand, curcumin treatment increased NO levels significantly. The effects of curcumin on cancer, arginase enzyme activity, ornithine and NO levels were found to be more effective than cancer after curcumin (Fig. 1-5). Also, Spearman's rank correlation coefficient analysis was performed and a significant difference was found between tissue arginase enzyme activities and ornithine levels only in the third group. The p value was determined to be 0.047.

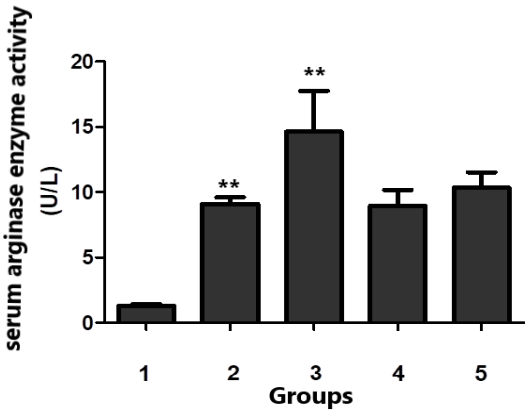


**Fig. 1.** Serum arginase enzyme activity, *: p<0.05, ** : p<0.001

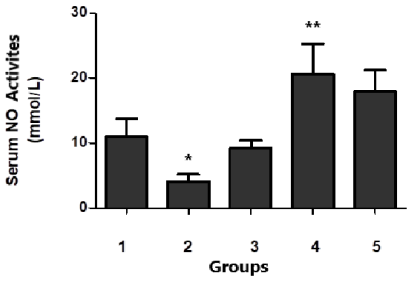


**Fig. 2.** Serum NO activity

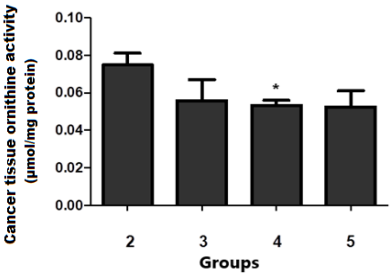


**Fig. 4.** Cancer tissue ornithine activity

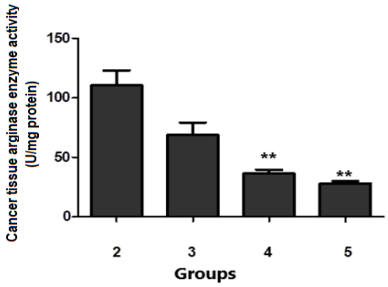


**Fig. 3.** Cancer tissue arginase enzyme activity

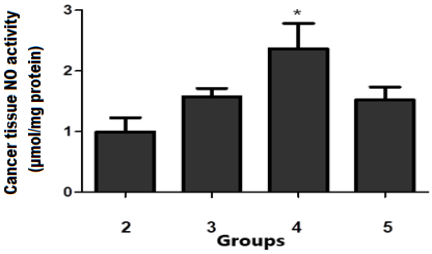


**Fig. 5.** Cancer tissue NO activity

**Table 1.** Arginase enzyme activity and NO levels of serum samples and arginase enzyme activity, ornithine and NO levels mean and standard deviation values of the tissue samples

| | Cont. (Group 1) | Tumor cont. 1 (Group 2) | Tumor cont. 2 (Group 3) | Treatment 1 (Group 4) | Treatment 2 (Group 5) |
|---|---|---|---|---|---|
| **Serum Arginase (U/L)** | 1.31±0.24 | 9.10±1.40c** | 14.63±8.84c** | 8.96±3.42 | 10.36±3.45 |
| **Serum NO (mmol/L)** | 10.95±7.39 | 4.06±3.19a* | 9.27±3.13 | 20.62±13.14b** | 17.96±9.79 |
| **Tissue Arginase (U/mg protein)** | | 110.48±35.66 | 68.98±28.40 | 36.28±9.09d** | 27.65±6.94e** |
| **Tissue Ornithine (µmol/mg protein)** | | 0.074±0.018 | 0.056±0.031 | 0.053±0.079f* | 0.056±0.026 |
| **Tissue NO (µmol/mg protein)** | | 0.99±0.65 | 1.57±0.39 | 2.36±1.17g* | 1.52±0.63 |

**a:** Comparison of serum NO levels between Group 1 and 2.
**b:** Comparison of serum NO levels between Group 2 and 4.
**c:** Comparison of serum arginase levels between Group 1, 2 and 3.
**d:** Comparison of tissue arginase levels between group 2 and 4.
**e:** Comparison of tissue arginase levels between group 3 and 5.
**f:** Comparison of ornithine levels between group 2 and 4.
**g:** Comparison of tissue NO levels between group 2 and 4.
* : p<0.05, ** : p<0.001.

## 4. Discussion

Curcumin may have a beneficial effect on cancer by inhibition of arginase enzyme. In addition to reducing arginase enzyme activity with curcumin, it is also possible to reduce ornithine levels, which are precursors of carcinogens and polyamines. The inclusion of NO in this mechanism and the demonstration that NO has anti-carcinogenic effect suggests that this system may be an important part of the non-carcinogenic curcumin mechanism. Both arginase and nitric oxide synthases enzymes competed for their common substrate; L-arginine. This relationship between the two enzymes represents one of the important factors in the regulation of NO production. Increased arginase activity may limit NO synthesis by reducing the availability of L-arginine for NOS (11). The arginase enzyme has a Vmax value at physiological pH, 1000 times more than the NOS enzyme Km value of arginase enzyme for L-arginine is 2-20 mM and it is 1-20 mM for NO (20, 21). Therefore, these two enzymes, even at low concentrations of L arginine are capable of using this substrate easily (5). It is stated that NO and NO metabolites inhibit cell growth and metastases in tumor tissues, inhibit tumor growth at high concentrations of NO and induce apoptosis of tumor cells in this way (12, 21, 22). At the same time, there are studies suggesting that NO can be used as an agent with chemical sensitivity and immune sensitivity and may be effective in immunotherapy or chemotherapy in cancer treatment by showing a synergistic effect (23). Up on constridation of serum NO levels in our study, NO values were decreased significantly in the tumor groups compared to the healthy group. This result gives us a meaningful understanding of the relationship between NO and cancer. In patients with breast cancer, significantly increased tissue ornithine levels and serum arginase enzyme activities were observed. These finding

will result in increased polyamine biosynthesis. In several studies, this is introduced as the triggering the mechanism for cancer development (3, 10, 24). In addition to this case, the decrease in NO level, which has been reported to play a protective role in the development of cancer, due to increased arginase enzyme activity, can be shown as a complement to the mechanism on the development of cancer (3, 23). In this study, the decreased NO level in the group of cancer patients against increased arginase enzyme activity supports this theory. In recent years, studies on the anticarcinogenic properties of curcumin have increased. In animal models, curcumin has been shown to be a potent antioxidant, anti-inflammatory molecule. It has been shown that it fights agonist carcinogenic DNA damage and inhibits tumorigenesis (25). Curcumin is one of the molecules used in the treatment of various diseases as an ethnic drug. Especially, curcumin has been acknowledged as an effective anticancer agent that regulates multiple intracellular signaling pathways, including transcription factors (e.g., STAT3, NF-κB, and AP-1), receptors (e.g., IL-8, HER2, and CXCR4), kinases (e.g., EGFR, ERK, and JAK), cytokines (e.g., TNF, IL, and MIP), enzymes (e.g., MMP, iNOS, and GST), and growth factors (e.g., EGF, NGF, and HGF) (13). Curcumin, p53, Ras, phosphatidylinositol-3-kinase, protein kinase B are targeted at numerous signaling pathways associated with cancer therapy and curcumin cell cycle regulation is closely related to PI3K / AKT / mTOR, a signaling pathway associated with cellular silence, proliferation, cancer and longevity. In addition, curcumin has been shown to cause anticancer effects by activating apoptotic pathways in cancer cells, inhibiting pre-cancerous processes including inflammation, angiogenesis and metastasis (26, 27, 28). Another study reveals the anti-metastatic and apoptosis-inducing potential of curcumin with increased Bax levels, decreased MMP-2, MMP-9 and Bcl-2 levels in breast cancer cells and erlich acid tumor cells (29). While curcumin treatment applied to breast cancer animals was found to increase serum NO levels significantly, a decrease in the same rate was not detected on arginase enzyme activity. However, even increasing the NO levels of curcumin can be considered as a positive effect in preventing cancer formation. Therefore, curcumin is an anticarcinogenic agent the action mechanism(s) as outlined above, due to inhibitory effects an arginase enzyme stimulatory effect on NO, as well as it suppressive effects on ornithine and polyamine levels. There is no study investigating the effects of curcumin on arginase enzyme activity which is reported to be associated with cancer and increased in cancer patients. In our study, interesting results were obtained in evaluating the effectiveness of time to start treatment of curcumin. Two different treatments were performed for this purpose. Although curcumin was administered to a group of animals (treatment group 1) before cancer cell injection, the second group (treatment group 2) was given curcumin after the cancer tissue was formed in the animal body. When these two groups were evaluated in terms of arginase enzyme activity, ornithine and NO levels; the curcumin intake was found to be much more effective before the cancer cell was given in the body. For this reason, curcumin, which is already used as a spice, can be considered as a factor that prevents the formation of cancer with daily diets. Unfortunately, we could not determine NOS levels in this study, and that was one of our study's limitations. As a result, curcumin, which is shown as an anticarcinogenic agent, exhibits this effect by inhibiting serum and tissue arginase enzyme activity in breast cancer patients and shifting the pathway towards from NO formation. This positive effect of curcumin can be more clearly shown by the fact that it starts to take a long time before the formation of cancer. Curcumin, a promising agent in cancer treatment, should be supported by further studies and new parameters.

**Ethics Committee Approval**

Ethics committee approval was received for this study from the ethics committee of Trakya University Animal Ethics Committee.

**Informed Consent:** N/A.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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