



Original Article

## Effect of Octopine on Oxidative Stress Indices and Serum Levels of Lipids and Trace Elements in Mice with Breast Cancer

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### ABSTRACT

**Background and objectives:** The aim of this study was to evaluate the effectiveness of octopine (phytogenic-derivative of arginine) on antioxidant indices, trace elements and lipid profiles of a mouse model of breast cancer.

**Methods:** In this study, 48 Balb/c mice were divided into six groups: healthy control, cancer control, cancer group receiving 50 mg of octopine, cancer group receiving 100 mg of octopine and cancer group receiving 150 mg of octopine. The octopine treatment was carried out for three weeks. The 4T1 cell line was used to induce cancer. Fasting blood samples were taken from mice to evaluate lipid profile, copper and zinc levels. Malondialdehyde, superoxide dismutase and glutathione peroxidase activity in breast tumor tissues was evaluated. Data were analyzed by SPSS 18 software using one-way ANOVA and t-test.

**Results:** Octopine had no significant effect on superoxide dismutase and glutathione peroxidase activity in the treatment group compared with the control cancer group. However, it significantly increased total antioxidant capacity and decreased malondialdehyde activities. Furthermore, treatment with octopine significantly decreased serum zinc, copper, TG, cholesterol and low-density lipoprotein levels but significantly increased high-density lipoprotein compared with the untreated cancer group.

**Conclusion:** Octopine administration is effective in reducing some oxidative stress indices and improving trace elements abnormalities and lipid profile in mouse models of breast cancer.

**Keywords:** Octopine, Lipids, Oxidative stress, Trace elements, Breast neoplasms

## INTRODUCTION

Breast cancer is one of the most common malignancies and a main cause of death in women (1). Of every nine women, one suffers from life-threatening mammary carcinoma, and more than 130,000 women die of breast cancer every year (2). There is a well-established relationship between oxidative stress, carcinogenesis and different types of cancer (3). Due to oxidative stress, active oxygen species in cancer cells is increased and the level of antioxidants is decreased (4). Glutathione peroxidase (GPX), superoxide dismutase (SOD) and malondialdehyde (MDA) are clinically important oxidative stress biomarkers of tissues, blood, urine and other body fluids (5). Generally, oxidative damage is often associated with a decrease in the activity of antioxidant enzymes, which itself is dependent on the level of trace elements (6). In this regard, a significant difference in the level of some rare elements including copper and zinc has been reported in patients with cancer (7). Some studies have used the zinc/copper ratio to estimate prognosis of cancer (8). Several studies have shown that plasma levels of copper can be used as a useful indicator of cancer progression or response to treatment (9). Lipoproteins play an important role in the progression of cancer by delivering lipids to tumors. Patients with various cancers show irregular patterns of lipid profiles. However, these patients often have significantly increased serum triglyceride (TG) and decreased cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels (10). It has been proposed that dietary supplementation of amino acids, such as arginine can have beneficial effects (11). L-arginine is a cationic amino acid with anticancer and anti-inflammatory effects (12). Free radical gas-nitric oxide is a main antioxidant agent produced from L-arginine (13). One of the derivatives of arginine is octopine, a phytochemical compound composed of alanine and arginine (14). It belongs to the opine family and generates from the interaction of pyruvate and arginine under the catalytic activity of octopine dehydrogenase (15). To our knowledge, no study has investigated the clinical and anti-cancer effects of opines. Therefore, we aimed to evaluate the effects of octopine on oxidative stress, trace elements and lipid profiles of a mouse model of breast cancer.

## MATERIALS AND METHODS

### *Cell culture*

The 4T1 cells were cultured in RPMI 1640 medium (Sigma-Aldrich, USA) with 10% fetal bovine serum, 100 mg/ml streptomycin, 2 mM L-glutamine and 4.5 g/L glucose. The cell culture medium was renewed every 2-3 days. Cells were passaged with 0.25% trypsin after reaching confluency of 80-90%. The cultures were maintained at 37 °C and 5% CO<sub>2</sub>.

### *Tumor induction*

Methods: In this study, 48 Balb/c mice were divided into six groups: the healthy control, the cancer control, the cancer group receiving 50 mg of octopine (as a solution and purchased from Sigma), the cancer group receiving 100 mg of octopine and the cancer group receiving 150 mg of octopine. The octopine treatment was carried out for three weeks. The 4T1 cell line was used to induce cancer. Fasting blood samples were taken from mice to evaluate lipid profile, copper and zinc levels. Malondialdehyde, superoxide dismutase and glutathione peroxidase activity in breast tumor tissues was evaluated. Data were analyzed by SPSS 18 software using one-way ANOVA and t-test. First, a cell suspension containing 10 million cells/ml was prepared in phosphate buffer saline. Then, one million cells were infused adjacent to the mouse lowest left mammary gland (16). Tumor was generated after two weeks and treatment with octopine was initiated on 14th day and continued for three weeks. After appearance of breast tumor, octopine was administered as a solution (prepared in deionized water), once a day for three weeks. The treatment groups received octopine solution by gastric gavage and the control groups received serum physiology by gavage (17).

On 21st day, blood samples were taken the heart of the mice and breast tumor samples were collected and stored at -70 °C. After lysate preparation, activity of SOD, GPX and MDA as well as total antioxidant capacity (TAC) were measured.

### *Tumor volume measurement*

Tumor volume was determined using the following formula:  $V = (L \times W \times W) / 2$ , where V is the tumor volume, L is the tumor length and W is the tumor width (18).

*Antioxidant indices measurement*

The antioxidant capacity was determined by Ferric Reducing Ability of Plasma (FRAP). In this method, colorless ferric-TPTZ complex (Tripyridyl-S-Triazine) is converted to the purple  $Fe^{+2}$ -TPTZ by antioxidants whose maximum absorption is at 593 nm. Increased concentration of the complex and increased absorbance correlates with antioxidant activity (19).

*Assessing superoxide dismutase activity*

The activity of superoxide dismutase enzyme was assessed using a commercial kit (Manual/Rx Monza-Ransod-Sd 125, Randox Laboratories Ltd. Co., Antrim, UK) according to a method described by L'Abbé and Fischer (20).

*Assessing GPX activity*

The GPX enzyme activity was measured using a commercial kit (Gpx Manual/Ransel Kit, Randox Laboratories Ltd. Co., Antrim, UK) based on a method described by Paglia and Valentine (21).

*Measurement of MDA*

Lipid peroxidation level was measured using the thiobarbituric acid method. As the end product of lipid peroxidation, MDA reacts with aqueous thiobarbituric acid at low pH and high temperature and produces a red colored complex with a maximum absorbance at 532 nm (22).

*Measuring lipid profile and trace elements*

Serum zinc and copper levels were measured by atomic absorption using commercial kits and the Shimadzu AA-670 apparatus (Kyoto, Japan) (23). Before testing, all samples were diluted with Triton X-100 (1%) and measured by flame atomic absorption method. Deionized water was used as standard for drawing the proper curve. After selection of proper curve by the apparatus, all samples were injected to apparatus and absorptions were measured. Finally, serum lipid profile was assessed using Pars Azmoon Co. (Iran) kits.

*Data analysis*

All data were analyzed using SPSS software version 18. One-way analysis of variance was used to compare the means within groups and the t-test was used to compare means between groups. All statistical analyses were performed at significance level of  $<0.05$ .

**RESULTS**

As shown in figure 1, 150 mg of octopine significantly reduced the tumor volume ( $P=0.00$ ).

Results of one-way ANOVA and t-test showed that octopine treatment caused a non-significant decrease in blood cholesterol compared to untreated cancer group. However, 1500, mg of octopine significantly decreased TG compared to untreated cancer group ( $P=0.01$ ). Treatment with 100 and 150 mg of octopine compared resulted in a significant increase in blood HDL ( $P=0.05$  and  $p=0.00$  respectively) compared to the untreated cancer group, and the highest effect was achieved at a concentration of 150 mg octopine. Moreover, consumption of 100 and 150 mg octopine significantly reduced blood LDL ( $p=0.05$  and  $p=0.04$  respectively) compared to the untreated cancer group, and the highest effect was observed at concentration of 150 mg octopine. As shown in figure 3, octopine treatment (150 mg) significantly reduced blood copper ( $P=0.04$ ) and zinc ( $P=0.01$ ) levels compared to the untreated cancer group. Also, octopine treatment significantly reduced MDA compared to the untreated cancer group, with the highest effect observed at concentration of 150 mg octopine. A significant increase in blood TAC was observed following octopine treatment with different doses ( $P=0.05$ , 0.04 and 0.01), while the highest effect was observed at concentration of 150 mg octopine. Furthermore, all doses of octopine caused an insignificant increase in GPX and SOD activity (Figure 3 and Table 1).

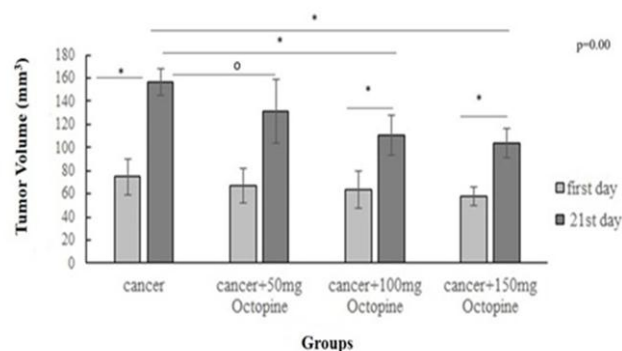


Figure 1. Results of tumor volume measurement in the study groups. \*:  $P < 0.001$ , °:  $P < 0.05$

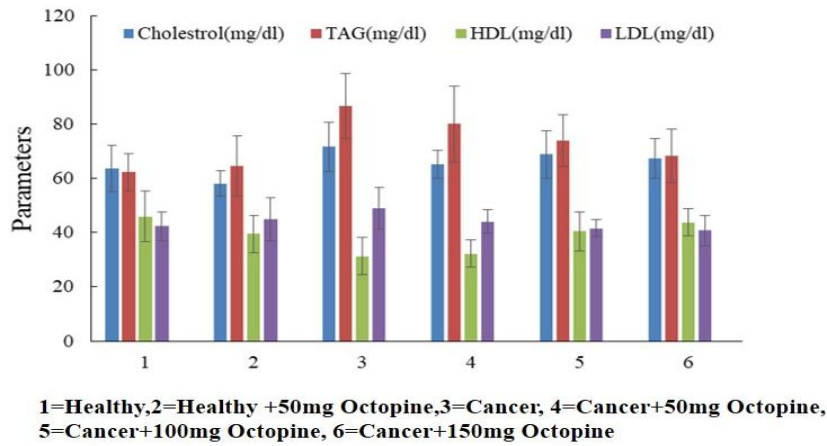


Figure 2. Mean level of serum cholesterol, TAG, HDL and LDL in different study groups

Table 1. Comparison of SOD activity between the groups

Groups	SOD (U/grPro)
Healthy control	175.49±1170.00
Healthy + 50mg octopine	86.89±1195.00
Cancer control	86.12±1091.66
Cancer + 50mg octopine	170.37±113.33
Cancer + 100mg octopine	129.09±1103.45
Cancer + 150mg octopine	159.30±1131.66

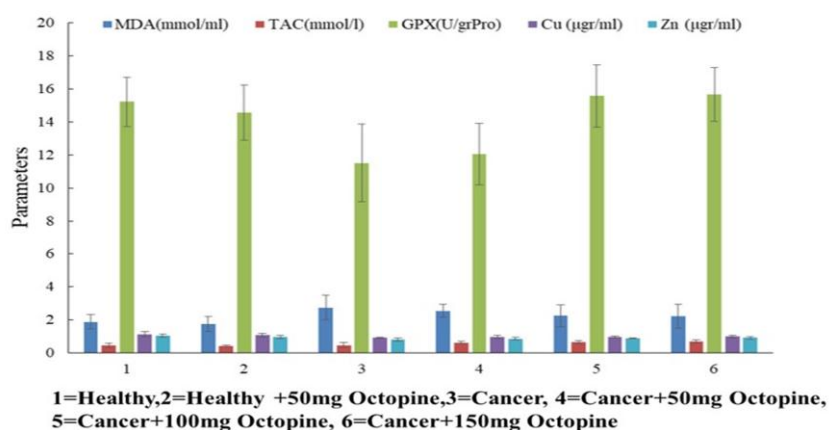


Figure 3. Comparison of trace elements and oxidative stress indices among the study groups

**DISCUSSION**

Many studies have investigated the changes in the concentrations of different bioelements in body tissue or fluids during cancer. Zinc is an antioxidant and anti-tumor element that naturally inhibits copper, which has a pro-oxidant property (24). Zinc is involved in the synthesis of metallothionein, an important factor in inhibiting free radicals. Furthermore, it has been demonstrated that zinc chloride reduces DNA breaks in human fibroblasts exposed to ultraviolet radiation (25). These findings infer that there may be an inverse relationship between zinc concentration and cancer risk (26). For example, serum zinc levels were lower in breast cancer patients compared to healthy subjects (27). Skrajnowska et al. reported that copper level in breast cancer tissue is higher than that in normal tissue of rats with breast cancer (28). A study by Koso et al. revealed that high copper concentrations in the serum are likely due to the copper release from necrotic cells (29). On the other hand, cancer cells are under constant oxidative stress and free radicals are recognized as key factors in cancer biology (30). Very few clinical reports are available regarding the anti-cancer activity of octopine. In this study, the use of octopine did not have a significant effect on the antioxidant activity of GPX and SOD. However, octopine treatment significantly decreased and increased the level of MDA and TAC, respectively. This can be due to the presence of arginine in the octopaine structure. Jabecka et al. showed that 2 g/day L-arginine supplementation for four weeks significantly increases TAC levels in patients with atherosclerosis (31). On the other hand, Lucotti et al. showed that daily consumption of L-arginine (8 g/day) for 21 days results in weight loss and a significant increase in SOD activity (32). In a study, the effect of L-arginine on the oxidative stress in diabetes resulted in decreasing the level of MDA in the gastrointestinal tract of rats and the increasing of activity of antioxidant enzymes (33). The serum lipid profile of different tumor types varies widely. It is not clear how increased serum cholesterol is associated with increased mortality (34). Previous studies have reported contradictory results regarding the TG levels in patients with malignancies (35-37). It is clear that serum lipoprotein patterns vary among different tumor types. However, a single tumor type can have different lipoprotein levels based on the disease stage

(38). A study by Sato et al. showed that octopine administration in cholesterol-fed rats significantly reduces LDL and VLDL levels and increases HDL level (39).

**CONCLUSION**

Octopine administration is effective in reducing some oxidative stress indices and improving trace elements abnormalities and lipid profile in mouse models of breast cancer.

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**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest regarding the publication of this article.

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