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The Effect of Zinc and Melatonin Administration on Lipid Peroxidation, IL-6 Levels, and Element Metabolism in DMBA-Induced Breast Cancer in Rats

Elif Gulbahce-Mutlu[1] · Saltuk Bugra Baltaci[2] · Esma Menevse[3] · Rasim Mogulkoc[2] · Abdulkerim Kasim Baltaci[2]

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Abstract
The purpose of this study was to investigate the effects of zinc and melatonin administration on interleukin-6, lipid peroxidation parameters, and element metabolism in DMBA-induced breast cancer in female rats. A total of 42 recently weaned Wistar rats were divided into 5 groups as follows: control (group 1), DMBA control (group 2), DMBA + zinc (group 3), DMBA + melatonin (group 4), and DMBA + melatonin and zinc (group 5). Malondialdehyde (MDA) and glutathione (GSH) levels in breast tissue and blood samples were determined via spectrophotometric methods. In addition, iron, magnesium, zinc, and copper levels in serum samples were determined by atomic emission, and plasma interleukin-6 levels were determined by ELISA method. The highest tissue and plasma MDA and the lowest tissue and erythrocyte GSH levels found in the study were in group 2; the highest tissue and erythrocyte GSH levels and the lowest tissue and plasma MDA levels are in group 5 ($P < 0.05$). Iron, magnesium, and zinc levels of groups 3, 4, and 5 were higher than the DMBA group without administration (group 2), but the copper values were significantly lower ($P < 0.05$). The highest IL-6 levels were determined in group 2 while IL-6 levels in the DMBA group (G5) treated with combined melatonin and zinc were lower than all other breast cancer groups ($P < 0.05$). According to the findings obtained in this presented study, combined zinc and melatonin therapy can contribute to the prevention of tumor growth by improving the disruption in element metabolism and suppressing IL-6 levels and reducing tissue damage that causes the cancer.

Keywords Breast cancer · DMBA · Lipid peroxidation · Trace element · IL-6 · Rat

Introduction

Breast cancer is a disease with high mortality and morbidity worldwide, especially affecting women. Although breast cancer is more common in developed countries, it also increases significantly in developing countries [1]. The production of the hormone melatonin, the main source of which is the pineal gland, is stimulated in the dark and is blocked by light [2]. It has been reported that melatonin hormone, a potent scavenger of free radicals, affects all stages of cancer, including carcinogenesis, tumor growth, progression, and the onset and elevation of metastasis [2–4]. As a result, it is well documented that melatonin hormone plays a critical role in preventing various types of cancer in most preclinical studies [5]. Zinc is an essential micronutrient that offers structural functions in zinc finger proteins [6]. This trace element is required for over 300 different cellular processes, including DNA repair, gene expression, enzyme activity, and intracellular signaling [7]. There is increasing interest in the role of zinc in preventing development of breast cancer [8]. Since zinc modulates various processes (i.e., oxidative stress, cell proliferation, and

✉ Abdulkerim Kasim Baltaci
baltaci@selcuk.edu.tr

Elif Gulbahce-Mutlu
elif.mutlu@karatay.edu.tr

Saltuk Bugra Baltaci
saltukbugrabaltaci@selcuk.edu.tr

Esma Menevse
emenevse@selcuk.edu.tr

Rasim Mogulkoc
mogulkoc@selcuk.edu.tr

[1] Medical Facultuy, Department of Medical Biology, KTO Karatay University, Konya, Turkey

[2] Department of Physiology, Faculty of Medicine, Selçuk University, Konya, Turkey

[3] Department of Biochemistry, Faculty of Medicine, Selçuk University, Konya, Turkey

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apoptosis) at the center of multi-stage carcinogenesis, altered zinc status may affect the development and prognosis of breast cancer [6, 8].

One of the best examples of cytokines that play an important role in inflammatory response and pathogenesis of cancer is interleukin-6 (IL-6) [9]. IL-6 is produced in response to tumor necrosis factor (TNF) and interleukin-1 (IL-1), which play a key role in the formation of immune and inflammatory events [10]. IL-6 deregulation may result in increasing the severity of inflammatory diseases and accelerating cancer development [10]. It is mainly produced by vascular endothelial cells, mononuclear phagocytes, fibroblasts, and activated T lymphocytes. The IL-6/JAK signal is important as it provides permanent activation of STAT-3 in patients with cancer. Permanent STAT-3 activation is effective in survival, angiogenesis, invasion, and tumor development [10]. Increased levels of IL-6, a multifunctional cytokine, have been associated with many cancers, especially prostate, bladder, colon, and breast cancer [9–11].

The aim of this study was to investigate the effects of zinc and melatonin administration on interleukin-6, lipid peroxidation, and element metabolism in DMBA-induced breast cancer, which is a strong carcinogenic substance in female rats.

Materials and Methods

Animal Material and Groups

The study was carried out on recently weaned Wistar breed female (40-day old) rats. The study protocol was approved by Selcuk University Experimental Medicine Research and Application Center, Animal Experiment Ethics Committee (2016-32). The study groups were formed as follows in the study conducted with a total of 42 female rats:

1. Group 1, (*n*: 6) control group: Group was fed with a normal diet with no administration.
2. Group 2 (*n*:6), DMBA control group: The animals in this group were administered 80 mg/kg 7,12-dimethyl[a]anthracene (DMBA) in colza oil (canola) through gavage to induce a tumor and fed on a normal diet.
3. Group 3 (*n*:10), DMBA + zinc group: The animals in this group were administered 80 mg/kg 7,12-dimethyl[a]anthracene (DMBA) in colza oil (canola) through gavage to induce a tumor, and supplemented with 5 mg/kg/day intraperitoneal (i.p.) zinc along with their normal diet for 4 weeks.
4. Group 4 (*n*:10) DMBA + melatonin group: The animals in this group were administered 80 mg/kg 7,12-dimethyl[a]anthracene (DMBA) in colza oil (canola) through gavage to induce a tumor, and supplemented with 5 mg/kg/day intraperitoneal (i.p.) melatonin along with their normal diet for 4 weeks.

Experimental Animals and Nutrition

The experimental animals were housed in a separate room at the Selcuk University Experimental Medicine Research and Application Center until the end of the study. They were kept in an environment with standard temperature and light (21 ± 1 °C and 12-h dark, 12-h light).

Experimental Procedures

Induction of Breast Cancer

To induce breast cancer, 7,12-dimethylbenz[a]anthracene (DMBA) supplied by Sigma-Aldrich company (St. Louis, MO, the USA) was used. For this purpose, a single dose of 80 mg/kg dimethylbenz[a]anthracene(DMBA) in colza oil (canola) was administered through gavage. One week after the administration, the animals' breast tissues were examined by palpation to check the enlargement of breast tissue. One week after the application, the animals were palpated and the control of breast tissue growth started. After the growth in the breast tissue were significantly detected, 6 rats from among the 36 rats which were administered DMBA were randomly chosen and their breast tissue samples were collected under general anesthesia. After the presence of the tumor was pathologically detected by light microscopic examination, zinc and melatonin supplementation started (Fig. 1). Tumor development was pathologically detected in the 10th week after DMBA administration.

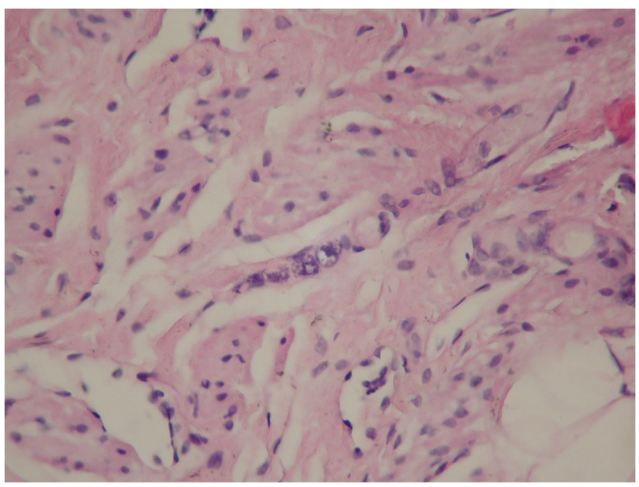

Fig. 1 Malignant changes were determined in breast tissue (HE: ×40)

Zinc Administration

Zinc (Merck, 7446-20-0) was dissolved in distilled water in the form of zinc-sulfate and i.p. injected with 0.5 mL saline solution (5 mg/kg of body weight/day). Zinc sulfate treatment was carried out between 9.00 and 10.00 am for 4 weeks.

Melatonin Administration

After melatonin (Sigma M 5250) was dissolved in pure ethanol, it was stored in the dark with the lid closed at 4 °C until the time of use. Melatonin was taken from the stocked solution at a dose of 5 mg/kg/day and was injected i.p. into rats in 0.5 mL saline. Melatonin administration was performed at the same hours for 4 weeks as in zinc administration.

Obtaining Samples

After 4 weeks of experimental applications, blood samples were taken from animals' heart tissue under anesthesia for IL-6, elements and lipid peroxidation measurement, and then euthanized. Breast tissue samples were also taken for lipid peroxidation analysis of euthanized animals.

Biochemical Analysis

Determination of Tissue Malondialdehyde Levels

Breast tissue malondialdehyde (MDA) level measurements were performed on the SPECTROstar (BMG Labtech, Germany) device using the Mihara and Uchiyama [12] method and the results were defined as nmol/g tissue.

Tissue Glutathione Analysis

The breast tissue glutathione (GSH) levels were measured in the SPECTROstar (BMG Labtech, Germany) device using the Ellmann method and the data were presented as mg/g tissue [13].

Determination of Plasma Malondialdehyde Levels

Plasma MDA levels were determined spectrophotometrically on the SPECTROstar (BMG Labtech, Germany) device using the method of Draper and Hadley [14], and data were presented in nmol/mL [14].

Erythrocyte Glutathione Analysis

Erythrocyte GSH analysis was spectrophotometrically read in SPECTROstar (BMG Labtech, Germany) device according to the methods of Atroshi et al. [15] and the results were recorded as mg/dL protein.

Determination of IL-6 Levels in Plasma

Plasma interleukin-6 levels were performed with SunRed Elisa commercial test kit (cat no: 210-11-0136). RAYTO trademark elisa washer (Indian) was used in the analysis and read on BMECT-LABTECH brand SPECTROstar Nano Elisa device (Germany) with 450 nm wavelength. Values were given in pg/mL.

Serum Trace Element Analysis (Magnesium, Iron, Copper, Zinc)

Magnesium and iron analyzes were performed on the ARCHITECT c8000 instrument, while Zn and Cu analyzes were performed on the ARCHITECT i2000 SR instrument with the Abbott ClinicalChemistry kits. Copper and iron were calculated in μg/dL. Mg mg/dL and Zn are given in μmol/L.

Statistical Analysis

The statistical analyses of data were conducted using SPSS 21.0 computer software and arithmetic means and standard deviations of all parameters were calculated. Shapiro–Wilk test was applied to determine the homogeneity of data, and it was seen that the data displayed a normal distribution. One-way variance analysis (ANOVA) test was employed to identify differences among groups, and the origin of the differences was detected using Duncan, a multiple comparison test. Differences for which $P < 0.05$ were accepted as significant.

Results

Tissue and Plasma MDA Levels of Study Groups

The highest tissue and plasma MDA levels in the study belong to the DMBA (G2) group ($P < 0.05$). Tissue and plasma MDA levels of zinc (G3) and melatonin (G4) administered DMBA groups were lower than DMBA (G2) group, and significantly higher than control (G1) group and combined zinc and melatonin administered DMBA (G5) group ($P < 0.05$). The lowest tissue and plasma MDA levels were obtained in the control (G1) group and the DMBA group (G5) in which combined zinc and melatonin administration ($P < 0.05$, Table 1).

Tissue and Erythrocyte GSH Levels of the Study Groups

The highest tissue and erythrocyte GSH levels in our study were obtained in the DMBA (G5) group in which zinc and

Table 1 Tissue and plasma MDA Levels of the Study Groups

Groups	Tissue MDA (nmol/g tissue)	Plasma MDA (nmol/mL)
G1 Control	19.73 ± 3.53c	2.96 ± 0.58c
G2 DMBA	42.21 ± 6.89a	5.94 ± 0.81a
G3 DMBA + zinc	29.33 ± 4.52b	4.14 ± 0.68b
G4 DMBA + melatonin	33.43 ± 5.16b	4.16 ± 0.62b
G5 DMBA + zinc + melatonin	21.66 ± 4.98c	2.80 ± 0.60c

There was a significant difference between groups in the same column and carrying different letters ($P < 0.05$)

melatonin were combined, and in the control (G1) group ($P < 0.05$).

Tissue and erythrocyte GSH levels of zinc (G3) and melatonin (G4) administered DMBA groups were higher than DMBA (G2) group, and significantly lower than control (G1) group and combined zinc and melatonin administered DMBA (G5) group ($P < 0.05$). The lowest tissue and erythrocyte GSH levels in our study were obtained in the DMBA (G2) group ($P < 0.05$, Table 2).

Serum Element Levels of Study Groups

The lowest iron, magnesium, and zinc levels and the highest copper values in the study were obtained in the DMBA (G2) group ($P < 0.05$). The iron, magnesium, and zinc levels of all DMBA groups (G3, G4, and G5) were higher than the DMBA (G2) group, while the copper values were significantly lower ($P < 0.05$, Table 3).

Plasma IL-6 Levels of the Study Groups

The highest IL-6 levels were in the control group (G2) with DMBA-induced breast cancer ($P < 0.05$). IL-6 values of DMBA + zinc (G3) and DMBA + melatonin (G4) groups were significantly lower than G2 ($P < 0.05$). IL-6 levels of DMBA (G5) group, which was treated with melatonin and zinc combination, were lower than all other groups with breast cancer (G2, 3, 4) ($P < 0.05$, Table 4).

Discussion

This study investigated the effects of zinc and melatonin administration on lipid peroxidation, element metabolism, and IL-levels in female rats with DMBA-induced breast cancer.

One of the most commonly used parameters as an indicator of oxidative stress and tissue damage is MDA levels. Therefore, in our study, tissue and plasma MDA levels were determined as an indicator of oxidant stress. The highest tissue and plasma MDA levels in the study were obtained in the DMBA (G2) group. Increased oxidative stress parameters have been reported in many experimental studies with DMBA-induced cancer [16–18]. In the current study, MDA levels in both breast tissues and plasma of rats with DMBA-induced cancer were significantly higher when compared with other groups. Carcinogenesis is a complex process involving a series of cellular and molecular events that support the transformation of a normal cell into a cancer cell. It is pointed out that reactive oxygen species may be associated with basic molecular mechanisms involved in all stages of the carcinogenesis process [19]. It is already known that increased reactive oxygen species and oxidative stress contribute to the advancement of breast cancer [20]. It is critical to investigate the preventability of increased oxidative stress in tumoral events by various factors that enable the antioxidant system [20]. Zinc is an important element with antioxidant properties. It is known that women with breast cancer have changes in zinc metabolism. These changes are closely related to the poor prognosis of the disease and the increase in the carcinogenic process [21]. It has been suggested that zinc may be effective in preventing tissue damage in tumoral events [22]. The

Table 2 Tissue and erythrocyte GSH levels of the study groups

Groups	Tissue GSH (mg/g tissue)	Erythrocyte GSH (mg/dL)
G1 Control	379.26 ± 34.19ab	14.53 ± 1.20b
G2.DMBA	178.60 ± 54.88c	11.37 ± 1.26c
G3 DMBA + zinc	327.14 ± 100.09b	14.95 ± 0.79b
G4 DMBA + melatonin	303.24 ± 43.36b	14.76 ± 0.58b
G5 DMBA + zinc + melatonin	438.25 ± 106.03a	16.79 ± 2.09a

There was a significant difference between groups in the same column and carrying different letters ($P < 0.05$)

Table 3 Serum element levels of study groups

Groups	Iron Fe (μg/dL)	Magnesium Mg (mg/dL)	Zinc Zn (μmol/L)	Copper Cu (μg/dL)
G1 control	337.83 ± 34.44a	2.90 ± 0.24a	18.17 ± 2.17b	122.16 ± 6.61b
G2 DMBA	256.17 ± 35.49c	2.16 ± 0.40b	13.58 ± 1.68c	134.50 ± 6.92a
G3 DMBA + zinc	319.90 ± 31.86ab	2.81 ± 0.22a	20.11 ± 2.09ab	111.71 ± 8.57b
G4 DMBA + melatonin	297.30 ± 37.14b	2.83 ± 0.46a	18.41 ± 2.79b	98.06 ± 12.02c
G5DMBA + zinc + melatonin	350.70 ± 25.65a	3.27 ± 0.63a	21.12 ± 1.99a	95.80 ± 12.91c

There was a significant difference between groups in the same column and carrying different letters ($P < 0.05$)

hormone melatonin is a powerful antioxidant that is mainly released from the pineal gland. There is a particularly inverse association between melatonin production and breast cancer incidence [23]. Therefore, treatment of melatonin is considered a strong candidate for the prevention of tissue damage in tumoral events [23]. In our study, the breast tissue and plasma MDA levels of both the zinc-treated DMBA group (G3) and the melatonin-treated DMA group (G4) were significantly lower than the same values of the DMBA (G2) group. The findings indicate that zinc and melatonin can prevent tissue damage in tumoral events. In our study, the decreased MDA levels we obtained with the supplementation of zinc and melatonin in breast cancer are consistent with the reports of studies suggesting that both zinc [22] and melatonin [23] may be an effective candidate in the prevention of tissue damage in tumoral events. In our study, we obtained the lowest MDA values in the DMBA group (G5) where combined zinc and melatonin were administered. We could not find a study on how the combined zinc and melatonin treatment affected tissue damage in breast cancer. However, Baltaci et al. [8] reported that combined zinc and melatonin administration activated immune parameters in breast cancer-induced rats, which supports, albeit indirectly, the reduced MDA values we obtained when we administered combined zinc and melatonin.

The lowest tissue and erythrocyte GSH levels in the study belonged to the DMBA (G2) group. It is known that antioxidant pathways, especially GSH, are suppressed in breast cancer [24, 25]. Therefore, our findings are consistent with reports showing that antioxidant activity is suppressed in breast cancer. In our study, the tissue and erythrocyte GSH levels of zinc (G3) and melatonin (G4) administered DMBA groups were higher than the DMBA (G2) group. Metallothionine, a metal-carrying protein in breast and prostate cancer, has been shown to increase antioxidant activity by increasing GSH levels together with zinc and consequently prevents oxidative damage in breast and prostate cancer [26]. It has already been reported that zinc is involved in key processes associated with breast carcinogenesis, and the incidence of breast cancer increases in zinc deficiency [27]. The fact that zinc [28] supplementation known as an antioxidant element increased the GSH levels in DMBA-induced breast cancer rats in our study is consistent with the research reports presented above. Epidemiological studies have shown a possible oncostatic feature of melatonin on different tumor types [29]. Melatonin hormone is already defined as a breast cancer inhibitor today [30]. One of the mechanisms underlying the cancer preventive effect of melatonin hormone is to prevent tissue damage by increasing antioxidant activity [29]. In our study, the increase of GSH levels in both tissue and erythrocytes with melatonin supplementation in rats with DMBA-induced breast cancer may be considered as an expected result considering the reports above. In our study, the highest tissue and erythrocyte GSH levels were obtained in the DMBA (G5) group, in which zinc and melatonin were combined. We have not been able to find a report on how the combination of zinc and melatonin hormone affects antioxidant activity in rats with breast cancer. The combined administration of zinc [28] and melatonin [29], known as antioxidants, significantly increased GSH levels when compared with DMBA-induced breast cancer group. This finding shows that the combined zinc and melatonin treartment may be important in preventing tissue damage in tumoral events.

Table 4 Plasma IL-6 findings of study groups

Groups	Interleukin-6 (pg/mL)
G1 control	31.54 ± 4.28d
G2 DMBA	140.80 ± 11.97a
G3 DMBA + zinc	59.67 ± 9.76b
G4 DMBA + melatonin	53.33 ± 8.41b
G5 DMBA + zinc + melatonin	42.40 ± 4.38c

There was a significant difference between groups in the same column and carrying different letters ($P < 0.05$)

Iron has been shown to stimulate free radical production of estrogen metabolites through a redox cycle and contribute to breast carcinogenesis [31]. It has been reported that patients with breast cancer have high iron levels and low serum iron levels in tumoral breast tissues [32]. Rajizadeh et al. [33] reported that iron deficiency anemia was more common in patients with breast cancer than in patients without breast cancer. However, in contrast to the evidence summarized above,

findings from population-based epidemiological studies on iron status and cancer risk have been contradictory [34]. Zinc is considered an essential nutrient that can prevent tumor development, with its role in maintaining antitumor immunity and its antioxidant effect [35]. Zinc levels differ significantly between breast cancer patients and their controls. It has been reported that serum-plasma and hair zinc levels in women with breast cancer are lower than those in healthy controls [35, 36]. It has been shown that the deficiency of magnesium required for DNA replication and repair increases DNA mutations leading to carcinogenesis [37]. In women with breast cancer, decreased serum magnesium has already been reported compared to their controls [37]. Choi et al. [38] reported that the serum copper levels of breast cancer patients were significantly higher than the controls. In the study, the lowest iron, magnesium, and zinc levels and the highest copper values were obtained in the DMBA (G2) group. Decreased iron, magnesium, and zinc levels and increased copper values that we obtained in the DMBA group are in line with the findings of the researchers who emphasize impaired element metabolism in breast cancer and presented above. The most striking result to be emphasized here is the improvement of element metabolism, which is impaired in breast cancer, as a result of the administration of zinc, melatonin, and combined zinc and melatonin. This finding shows us that the preventive effect of zinc and melatonin on tumoral events is not only limited to antioxidant activity but also has a regulatory effect on impaired element metabolism.

Breast cancer, one of the most dangerous tumors, is globally recognized as the main cause of cancer-related death in women [39]. Interleukin-6 (IL-6) is a proinflammatory cytokine released by various cells around the tumor microcirculation, including cancerous cells. IL-6 plays a critical role in the growth and differentiation of tumor cells [39]. Increased levels of IL-6 in the serum and tumor regions have been shown in many cancers, including breast cancer [39, 40]. Especially in patients with breast cancer, IL-6 induction is associated with poorer prognosis, and serum IL-6 levels increase at pathological degrees [39, 40]. In our study, the DMBA-induced breast cancer control (G2) group had the highest IL-6 levels. The high IL-6 levels we achieved are consistent with the findings of researchers reporting IL-6 levels that have increased pathologically in breast cancer. Similarly, in our study, IL-6 values of DMBA + zinc (G3) and DMBA + melatonin (G4) groups were significantly lower than G2. This finding is critical. Because IL-6, which increases in tumoral events, plays a critical role in the expansion and differentiation of tumor cells [39]. Zinc (G3) and melatonin (G4) supplementation given to DMBA-induced breast cancer groups suppressed IL-6 levels. Famurewa et al. [22] have shown that zinc supplementation inhibits increased liver-induced IL-6 synthesis in breast cancer. It has been reported that docetaxel and vinorelbine, used in the treatment of breast cancer, and melatonin supplementation in radiation therapy increase the decrease in the expression of IL-6 levels [41]. The decreased IL-6 levels obtained with zinc and melatonin supplementation in DMBA-induced breast cancer groups are consistent with the results of both reports presented above. In our study, the levels of IL-6 in the DMBA-induced group (G5), in which melatonin and zinc were combined, were lower than all other groups (groups 2, 3, 4) with breast cancer. This finding indicates that zinc and melatonin suppress IL-6 levels more strongly in breast cancer. We could not find a publication that investigated how combined melatonin and zinc treatment in breast cancer affects IL-6 levels in med-line scans. We could not even find a study investigating the relationship between IL-6 and combination of melatonin and zinc regardless of breast cancer. Based on this point, it can be said that tracking the levels of IL-6 in serum or plasma can be used as one of the cancer biomarkers and can also help in monitoring the cancer treatment process [42]. This finding of our study is the first report to show that the combination of melatonin and zinc strongly suppresses the increased IL-6 levels in breast cancer.

When the results of our study are evaluated as a whole,

1. Based on the findings obtained in our study, it could be concluded that the combined supplementation of zinc and melatonin may be beneficial in preventing increased IL-6 levels and repairing the impaired element metabolism in DMBA-induced breast cancer.
2. The current study is the first study to show that the combination of melatonin and zinc strongly suppresses increased tissue damage and increased IL-6 levels in breast cancer.

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Data Combined zinc and melatonin treatment may contribute to prevent tumor growth by improving impairment in element metabolism and suppressing IL-6 levels and increased tissue damage that causes acceleration of cancer development.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no confict of interest.

Informed Consent This study was performed on Wistar type adult male rats.

Ethical Approval Research meets ethical guidelines is a required field. The study protocol was approved by Selçuk University Experimental Medicine Research and Application Center Laboratory Animals Ethics Board (No: 2016-32).

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