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Effect of Curcumin on Testis in Mice with Ehrlich Ascites Tumor

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Abstract: In the current work, the effects of curcumin on testicular tissues in Ehrlich Ascites Tumor (EAT) model developed in Balb/C mice. EAT cells ($1x10^6$) received from stock of animals were injected intraperitoneally to animals. 25 mg/kg and 50 mg/kg of curcumin were administered intraperitoneally. Testicular tissues obtained after all the experiment were evaluated for histopathological and biochemical parameters. Histopathological results showed that 50 mg/kg curcumin group had less EAT cells around testicular tissues than tumor control group. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) activities and reduced glutathione (GSH), oxide glutathione (GSSG), total antioxidant status (TAS), total oxidant status (TOS), interleukin-1beta (IL-1β), interleukin-6, interleukin-17, tumor necrosis factor-alpha (TNF-α) levels were measured in testis tissues. Oxidative stress index (OSI) and GSH/GSSG ratio were calculated. Findings clearly suggested that antioxidant parameters (except CAT and GPx) had higher value in animal models treated with 25 and 50 mg/kg curcumin groups associated to tumor control group. There was a statistically noteworthy variance between the groups in all parameters. Antitumor effect of curcumin on ascites tumor cells produced by EAT cells evidenced with histopathological while antioxidant and anti-inflammatory biochemical parameters evidenced with biochemical parameters.
Keywords: Testis, EAT, Mice, Curcumin, Antioxidant.

1. Introduction

Testicular cancer is a common case in young people and is increasing day by day. As a common tumor, testis cancer is influenced by genetic and epigenetic factors. Additionally, oxidative stress played central role in development and progression of cancer. In addition to chemotherapy and radiotherapy, complementary medicine is also used in cancer treatment and the use of plant extracts is increasing gradually (1, 2).

One of these plant extracts is curcumin. It is a yellow herbal product with anti-inflammatory, anti-carcinogenic and anti-oxidant properties, used as spice, food coloring and preservative among the public. Curcumin, also called Curcuma longa, is a perennial plant native to South Asia (3, 4). There are many articles in the literature that demonstrate the antineoplastic mechanism of curcumin. It is stated that

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curcumin has anticarcinogenic effects on various tumors with a variety of mechanisms.

Many studies show that curcumin suppresses many tumor genesis types including skin, mammary gland, mouth, esophagus, stomach, intestine, colon, lung and liver (5). Curcumin has captured appreciation because of its extra-ordinary pharmacological properties and experimentally verified ability to inhibit and/or prevent cancer. Wealth of information has highlighted potential of curcumin as an effective antioxidant, anti-inflammatory and an antidiabetic agent. Curcumin has been reported to exert inhibitory effects on interleukin-1β, TNF-α, superoxide ion and hydrogen peroxide production (6). It has been reported that curcumin significantly enhanced apoptosis in drug-resistant cancer types (7). Plant extracts have been tested on many cancer models while, one of which is the EAT model. EAT first appeared as a spontaneous breast adenocarcinoma in a female mouse, and tumor fragments were transplanted subcutaneously from mouse to mouse into an experimental tumor. After obtaining the liquid growing form in the peritoneum of mice, ascitic fluid was formed in addition to cells in the peritoneum, therefore the tumor was named as EAT (8, 9). In the current literature, the efficacy of curcumin on EAT tumor model was examined histologically but not in terms of biochemical parameters.

Copper, zinc-superoxide dismutase (Cu, Zn-SOD), Catalase (CAT) and Selenium-dependent glutathione peroxidase (Se-GSH-Px) are the main antioxidant enzymes of all aerobic cells (10). Cu, Zn-SOD (EC 1.15.1.1) catalyzes the dismutation of superoxide anions to hydrogen peroxide (11). CAT (EC 1.11.1.6) catalyzes the degradation of H_2O_2 to H_2O and O_2 (11). Se-GSH-Px (EC 1.11.1.9) is a biocatalyst that metabolizes H_2O_2 and lipid hydroperoxides (12). During this reaction, glutathione (GSH) is used as hydrogen donor and GSH is oxidized (GSSG) (13). Glutathione is the most important nonenzymatic antioxidant molecule inside the cell (14). GSH is also a hydroxyl radical and singlet oxygen scrounger (15). GSH (reduced glutathione)/GSSG (oxidized glutathione) ratio is one of the important determinants of oxidative stress in the body (10). The amounts of total antioxidant status (TAS) and total oxidant status (TOS) were useful for calculation of oxidative status (16). IL-1β, IL-6, IL-17 and TNF-α are among the pro-inflammatory cytokines (17, 18).

In this study we hypothesized that curcumin could play critical role on normalizing oxidative and inflammatory parameters which upregulated by EAT cells. Therefore, we aimed to evaluate the effect of low and high dosages of curcumin on testis tissue after investigated with EAT cells.

2. Material and Methods

2.1. Animals, management and experimental design

Studies with experimental animals were carried out in accordance with the decision of Ethics Committee of Animal Ethics Local in Erciyes University (2014 HADYEK-29). 25-30 grams and 8-10 weeks old Male Balb/C type mice were used for each group. The number of groups was 4, with 7 mice in each group. 4 animals were also used to create stock animals outside the groups. Mice were maintained in specially prepared, automatically air-conditioned rooms with constant temperatures of 21 °C and 12 hours of light/dark periods during the study. Before the groups were formed in the study, we first created the stock mouse to obtain enough EAT cells. The ascitic fluid from the stock animal was suspended in 0.1 ml PBS and counted on the thoma slide and $1x10^6$ EAT cells were injected intraperitoneally to form a liquid tumor in mice.

2.2. Dissolution and sterilization of curcumin extract

Curcumin was supplied as powder from Sigma Aldrich and curcumin was dissolved in different volumes to provide the desired concentrations for the experimental groups and sterilized by filtration. Curcumin was freshly prepared on each injection day to being dissolved completely.

2.3. Formation of Experimental Groups

Group 1/Negative control group: Cancer formation and animals were fed with normal diet for 10 days. 0.1 ml of physiological saline solution (PSS) was administered intraperitoneally for 10 days.

Group 2/Positive control group: In this group, 0.1 ml of ascitic fluid containing $1x10^6$ EAT cells was administered intraperitoneally to the abdomen on day 0. Mice were injected intraperitoneally with 0.5 ml of physiological saline solution (PSS) for 10 days from day 0.

Group 3/Treatment group (25 mg/kg curcumin): In this group, 0.1 ml ascitic fluid containing $1x10^6$ EAT cells was administered intraperitoneally to the abdomen on day 0. Mice were injected with curcumin 25 mg/kg; day intraperitoneally for 10 days from day 0

Group 4/Treatment group (50 mg/kg curcumin): In this group, 0.1 ml ascitic fluid containing $1x10^6$ EAT cells was administered intraperitoneally to the abdomen on day 0. From day 0, the mice were injected with Curcumin 50 mg/kg per each day intraperitoneally during 10 days.

2.4. Sample collection and preparations

All animals were sacrificed on day 10 under general anesthesia by ketamine and xylazine with concentrations of 75 mg/kg and 15 mg/kg respectively. One testis from each subject was fixed in 10% cold formaldehyde for routine histopathological examination, the other was transferred to sterile plastic bags and immediately transferred to the laboratory in cold conditions and stored at -80 °C temperature until biochemical experiments.

2.5. Preparation of testis homogenates

Testis tissue of each mouse were arranged and studied distinctly. Tissues were homogenized in 2 mL +4 °C

temperature phosphate-buffer saline in ice. At the end of the homogenization process, the mixture was centrifuged at 3000 g for 10 min and the supernatant was composed. Supernatant was deposited at -80 ºC until analysis.

2.6. Biochemical Analysis

The protein content of each tissue was determined in each sample with Bio-Rad reagents (Bio-Rad laboratories GmbH, München, Germany) using bovine serum albumin as the standard. Activities of CAT and Se-GSH-Px and the levels of GSH and GSSG were measured in tissues by the modified methods of Ozturk et al." (18). TOS, TAS levels and SOD activity were measured using commercially available kits (Relassay, Turkey). OSI was defined as TOS to TAS ratio was calculated as follows: OSI (arbitrary unit) = [(TOS, μmol H_2O_2 equivalent/mg protein)/(TAS, μmol Trolox equivalent/mg protein] × 100). The levels of IL-1β, IL-6, IL-17, TNF-α were studied from the blood samples collected with enzyme linked immunosorbent assays (ELISA) method (Elabscience, Wuhan, China) as specified in the protocol by the manufacturer.

2.7. Statistical analysis

Data were analyzed using the statistical package program SPSS for Windows® 23.0 (SPSS, Chicago, IL, USA). Normality of the all data was analyzed with Kolmogorov-Smirnov D test to determine a test type from both the parametric and non-parametric tests. Distribution of OSI, GPx and IL-1β were non-parametric. Data have a normal distribution (Kolmogorov–Smirnov D test, p≥0.05), parametric test ANOVA (post-hoc: Tukey's HSD and Tamhane) was used for multiple comparisons. Kruskall-Wallis test was used for non-parametric distribution data and pairwise comparisons of groups were made. Experimental data were expressed as the Mean±SD. The P <0.05 were considered statistically significant.

3. Results

3.1. Histopathological Findings

According to histopathological experiments, control groups' tissues showed normal histological properties. In the EAT cell groups positive control groups which has no treatment has more EAT cells as compared to the 25 and 50 mg/kg curcumin treated groups (Figure 1). Around the testicular capsule dispersed EAT cell assemblies were observed.

3.2. *Biochemical Results*

The results of oxidative stress parameters of all groups are represented in figure 2-4. SOD activity, GSH level, GSH/GSSG ratio and TAS levels, which are among the antioxidant markers, were meaningfully lower in the tumor group compared to group 1 (p<0.05). In groups 3 and 4 there was a significant dose-dependent increase compared to the tumor group. However, the increase in antioxidant activity in groups 3 and 4 did not reach the control group (p<0.05). GPx activity was expressively higher in group 2 and 3 compared to group 1 and pointedly lower in group 4 than group 2 (p<0.05). CAT activity and GSSG and TOS levels were significantly higher in the tumor group compared to group 1, and dose-dependent significantly lower in group 3 and 4 compared to the tumor group (p <0.05). While the same parameters were found to be significantly higher in group 3 than in group 1, CAT activity was lower in group 4 and GSSG and TOS levels were higher in group 4 (p<0.05). OSI values were significantly higher in group 2 and 3 than in group 1 and meaningfully lower in group 4 than in tumor group (p<0.05) (Figure 2,3).

IL-17 and TNF-α levels were suggestively lower in the treatment groups compared to the tumor group (p<0.05) The values of group 3 were lower than group 4 (p<0.05). IL-6 and IL-1β levels were lower in the treatment groups than in the tumor group (p<0.05). There was no statistically significant difference between the treatment groups (Figure 4).

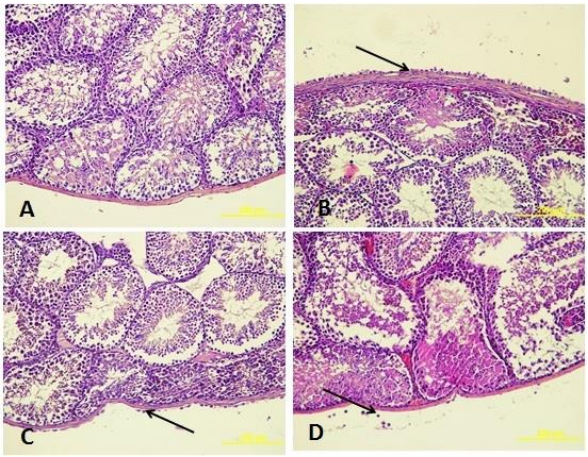

Figure 1: Histopathological findings (H&E, 20X) of the negative control, treatment and positive control groups in testicular tissue. A) Healthy control group. B) Tumor control group C) Tumor and 25 mg/kg curcumin treated group D) Tumor and 50 mg/kg curcumin treated group.

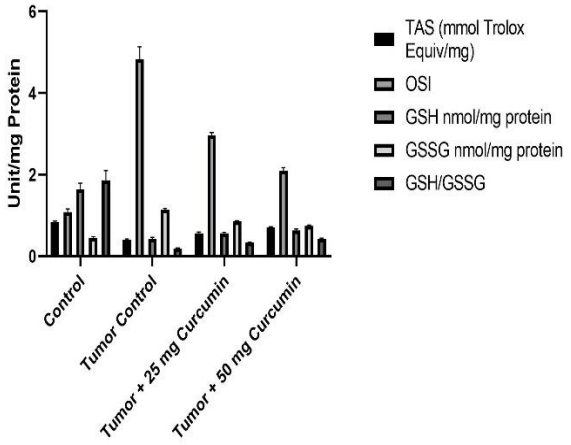

Figure 2: Measurements of oxidative stress and inflammatory markers in the testis.

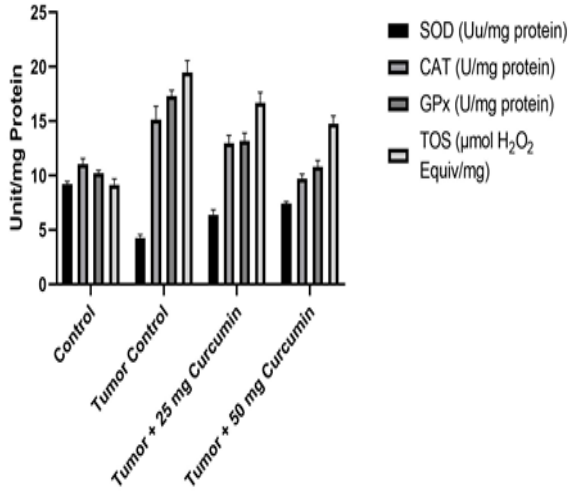

Figure 3: The results of oxidative stress parameters of all groups.

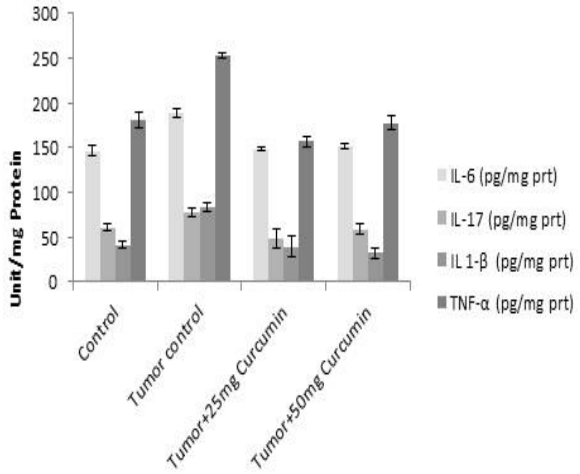

Figure 4: Assessment of IL-6, IL-17, IL1β and TNF-α levels of three groups.

4. Discussion

The prevalence of cancer types causing deaths in men and women varies. Testicular cancer has an increasing rate in young men aged 15-35 years. Drug resistance and metastatic spread have been recognized as major stumbling blocks in decreasing the quality of clinical outcome of wide ranging therapeutics. The presence of frequent metastases in other organs has accelerated the search for treatment in testicular cancer. In recent years, complementary medicine has been used in addition to medical treatments for cancer treatment. Some plants have been shown to be useful in cancer treatment. Bioactive molecules present in different Plants and vegetables used by the people in various countries have documented health promoting effects. More importantly, certain high-quality bioactive constituents have been shown to induce regression of tumors in xenografted mice (19, 20). It has been shown that the natural chemicals contained in some of these plants (resveratrol in red grapes, genistein in soybean and curcumin in turmeric) have anti-cancer properties (21). Curcumin has been reported to reduce toxicity caused by anti-cancer agents, sensitize resistant cancer cells, suppress proliferation, and induce apoptosis in tumor cells (22-24). In the current study, anti-inflammatory and antioxidant effects of curcumin in EAT model mice were investigated histopathological and biochemically on testicular tissue. Kanter et al. (25) investigated the protecting properties of curcumin and amifostine against gamma radiation-induced jejunal mucosal damage and reported that curcumin's efficacy diminished in high-dose radiation. Lopez (26) showed that curcumin at a dose of 20 µg / mL stopped growth of 50% in human chronic myelogenous leukemia cells. Pan et al. (27) examined the therapeutic effect of curcumin by forming an Alzheimer's model in mice. They reported that Bax levels did not change in hippocampal mice. Facchini et al. investigated the effects of Pleurotus ostreatus (fungus) polysaccharide fractions intraperitoneally in $5x10^6$ EAT cells and gave high tumor inhibition (28). Yilmaz et. al. (2019) studied the antioxidant properties of curcumin and Cornus mas L in mice forming EAT model and showed antioxidant properties in tissues such as kidney and liver in vivo and in vitro (29,30).

Activated monocytes/macrophages allow pro-inflammatory cytokines, such as TNF-α, IL-1, and IL-6 that play an important role in inflammatory reaction. Ketanserin has been shown BY Yank et al. (2019) in literature to inhibit TNF-α and IL-1 production in endotoxic shock (31). Ueki et al. examined the therapeutic effect of curcumin in mice with cisplatin-induced renal inflammation (32). They indicated that serum TNF-α concentration decreased by 30% in the group which has been given cisplatin (100 mg/kg) and curcumin (100 mg/kg); decreased as well as healed renal dysfunction. Cho (2007) found that curcumin inhibited the expression of TNF-α-induced IL-1β, IL-6, and TNF-α in TNF-α treated keratinocytes (33).

In this study, IL-1β, IL-6, TNF-α and as a pro-inflammatory cytokine IL-17 decreased in curcumin treated groups compared to tumor control group. The decrease in IL-17 and TNF-α was found to be lower in the dose-dependent group compared to the 25 mg administered group. IL-6 and IL-1β levels have been found lower independent of dose. These findings suggest that curcumin may have dose-dependent anti-inflammatory effects in mouse testes with EAT. Additionally, curcumin has been exposed to prevent the production of superoxide and nitric oxide by inflammatory cells, which could also donate to its anti-inflammatory motion because of the significant role of free radicals in inflammatory processes (34, 35). In literature, we could not confirm a detailed study investigating the effect of curcumin on oxidant and antioxidant system in testicular tissues with EAT

tumor. Khopde *et al.* (1999) showed that curcumin is a more effective antioxidant than α-tocopherol and inhibits lipid peroxidation (36). While Venkatesan *et al*. (1997) investigated the effects of curcumin on bleomycin-induced lung injury (BILI) in rats, they showed that curcumin had anti-inflammatory and antioxidant effects in BILI in the curcumin treated group (37). Kuhad *et al.* (2008) found that curcumin antioxidant parameters such as SOD, GSH and CAT increased compared to diabetic rats in their study (38).

5. Conclusions

In this study, TAS and GSH levels, which are antioxidant parameters, decreased in the testicular tissues of the mice, while dose-dependent increase was detected in the curcumin-treated groups likened to the tumor group. Likewise, the oxidant parameters TOS and GSSG levels increased in the testicular tissues of the mice, while dose dependent decrease was detected in the curcumin groups in proportion to the tumor group. When the enzyme activities responsible for oxidant defense were evaluated, it was evaluated that as the cancer was formed, SOD enzyme activity, which removes superoxide in the environment, decreased. We observed that CAT and GPx activities, which are responsible for the neutralization of H_2O_2 to water and oxygen, were increased in the next step. Dose-dependent SOD activity increased while CAT and GPX activity decreased with curcumin effect. The resulting high amount of H_2O_2 inhibits product inhibition on SOD, while CAT and GPx may have shown more activity to remove accumulated H_2O_2. Each tissue has diverse volume against stress response for each oxidant or antioxidant parameter (38). As a result of increased GPx activity in cancer, GSH reserve in the environment decreased and GSSG amount increased. In the curcumin group, this was vice versa. These findings support the antioxidant effects of curcumin in mouse testicular tumor tissue in accordance with the literature.

Conflict of interest statement

There is no conflict of interest.

References

1. Kayaalp SO. Tıbbi Farmakoloji, Hacettepe Taş Kitapçılık. Ankara. **1996**, pp. 731-779.
2. Weiss RB, Henney JE, DeVita VT. Multimodal treatment of primary breast carcinoma. analiysis of accomplishments and problem areas. *Am J Med* **1981**; 70: 844-851.
3. Pei Y, Ai T, Deng Z, et al. Impact of plant extract on the gastrointestinal fate of nutraceutical-loaded nanoemulsions: phytic acid inhibits lipid digestion but enhances curcumin bioaccessibility. *Food Funct* **2019**; 106: 3344-3355.
4. Toraya S, Uehara O, Daichi H, et al. Curcumin inhibits the expression of proinfammatory mediators and MMP-9 in gingival epithelial cells stimulated for a prolonged period with lipopolysaccharides derived from Porphyromonas gingivalis. *Odontology* **2020**; 108(1); 16-24.
5. Bhaumik S, Anjum R, Rangaraj N, et al. Curcumin mediated apoptosis in AK-5 tumor cells involves the production of reactive oxygen intermediates. *FEBS Lett* **1999**; 456: 311-314.
6. Basham SA, Hunter S. W, Ben M. K, et al. Effect of Curcumin Supplementation on ExerciseInduced Oxidative Stress, Inflammation, Muscle Damage, and Muscle Soreness. *J Diet Suppl* **2019**; 26: 1-14.
7. Kocaadam B, Şanlıer N. Curcumin, an active component of turmeric (curcuma longa), and Its effects on health. *Crit Rev Food Sci Nutr* **2015**; 3: 123-127.
8. Hatcher H, Planalp R, Cho J, et al. Curcumin from ancient medicie to current clinical trials. *Cell Moll Life* **2008**; 65: 1631-1652.
9. Gevorkyan L, Gambashidze K. Anticancer efficacy of hydroxyethylthiamine diphosphate in vivo. *Exp Oncol* **2014**; 36: 48-49
10. Birben E, Sahiner UM, Sackesen C, et al. Oxidative Stress and Antioxidant Defense. *WAO J* **2012**; 5: 9–19.
11. Limon-Pacheco J, Gonsebatt ME. The role of antioxidants and antioxidant related enzymes in protective responses to environmentally induced oxidative stress. *Mutat Res* **2009**; 674(1-2): 137-147.
12. Young IS, Woodside JV. Antioxidants in Health and Disease. *J Clin Pathol* **2001**; 54(3): 176-186.
13. Reiter RJ, Melchiorri D, Sewerynek E, et al. A review of the evidence supporting melatonin‘s role as an antioxidant. *J Pineal Res* **1995**; 18(1): 1-11.
14. Gürdöl F. Tıbbi biyokimya. 3. Baskı. 2018, pp. 644.
15. Joanisse DR, Storey KB. Oxidative damage and antioxidants in Rana syvatica, the freeze-tolerant wood frog. *Am J Physiol* **1996**; 271: 545-553.
16. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clin Biochem* **2004**; 37: 277–285.
17. Mohan ML, Vasudevan NT, Naga Prasad SV. Proinflammatory Cytokines Mediate GPCR Dysfunction. *J Cardiovasc Pharmacol* **2017**; 70(2): 61-73.
18. Oztürk O, Gümüşlü S. Changes in glucose-6-phosphate dehydrogenase, copper, zinc-superoxide dismutase and catalase activities, glutathione and its metabolizing enzymes, and lipid peroxidation in rat erythrocytes with age. *Exp Gerontol* **2004**; 39(2): 211-216.
19. Scott MS, William T. Epidemiology and Diagnosis of Testis Cancer. *Urol Clin North Am* **2015**; 42(3): 269-275.
20. Raju A, Christina MJA, Murali A. Antitumor activity of ethanol and aqueous extracts of Drosera Burmannii vahl. İn EAC Bearing Mice. *Spatula DD* **2012**; 2: 83-88.
21. Azab ME, Hishe H, Moustapha Y, et al. Anti-angiogenic effect of resveratrol or curcumin in

ehrlich ascites carcinoma-bearing mice. *Eur J Pharmacol* **2011**; 652: 7-14.
22. Maheshwari RK, Singh AK, Gaddipati J, et al. Multiple biological activities of curcumin: A short review. *Life Sci* **2006**; 78: 2081-2087.
23. Kohli K, Ali J, Ansari MJ, et al. Curcumin a antiinflammatory agent. *Indian J Pharmacol* **2005**; 37: 141-147.
24. Chainoglou E, Hadjipavlou-Litina D. Curcumin analogues and derivatives with anti-proliferative and anti-inflammatory activity: Structural characteristics and molecular targets. *Expert Opin Drug Discov* **2019**; 16: 1-22.
25. Kanter P, Tarladaçalışır YT, Akpolat M, et al. Gamma radyasyona bağlı oluşan jejenum mukoza hasarına karşı curcumin ve amifostinin koruyucu etkilerinin incelenmesi. *Tıp araştırmaları dergisi* **2008**; 6(3): 128-135.
26. Lopez LM. Anticancer and carcinogenic properties of curcumin. Considerations for its clinical development as a cancer chemopreventive and chemotherapeutic agent. *Mol Nutr Food Res* **2008**; 52: 103-127.
27. Pan HM, Huang TM, Lın JK. Biotransformation of curcumin through reduction and glucuronidation in mice. *Am Soc Pharmacol Exp Ther* **1998**; 27:486-493.
28. Facchini JM, Alves EP, Aguilera C, et al. Antitumor activity of pleurotus ostreatus polysaccharide fractions on ehrlich tumor and sarcoma. *Int J Biol Macromol* **2014**; 68: 72-77.
29. Yılmaz S, Ülger H, Ertekin T, et al. Investigating the anti-tumoral effect of curcumin on the mice in which Ehrlich ascites and solid tumor is created. *Iran J Basic Med Sci* **2019**; 22: 418-425.
30. Yılmaz S, Alpa Ş, Nisari M, et al. Examining the Antitumoral Effect of Cornelian Cherry (Cornus mas) in Ehrlich Ascites Tumor-induced Mice. *J Anat Soc India* **2019**; 68: 16-22.
31. Yang W, Zhang J, Zhang B. The role of ketanserin in maintaining cırculatıon stability in endotoxic shock rats. *Acta Medica Mediterr* **2019**; 35: 2871.
32. Ueki M, Ueno M, Morishita J, et al. Curcumin ameliorates cisplatin-induced nephrotoxicity by inhibiting renal inflammation in mice. *J Biosci Bioeng* **2013**; 115: 547-551.
33. Cho JW, Lee KS, Kim CW. Curcumin attenuates the expression of IL-1beta, IL-6, and TNF-alpha as well as cyclin E in TNF-alpha-treated HaCaT cells; NF-kappaB and MAPKs as potential upstream targets. *Int J Mol Med* **2007**; 19(3): 469-474.
34. Bhaumik S, Jyothi MD, Khar A. Differential modulation of nitric oxide production by curcumin in host macrophages and NK cells. *FEBS Lett* **2000**; 483: 78-82.
35. Brouetand I, Ohshima H. Curcumin, an anti-tumour promoter and anti-inflammatory agent, inhibits induction of nitric oxide synthase in activated macrophages. *Biochem Biophys Res Commun* **1995**; 206: 533-540.
36. Khopde M S, Priyadarsini KI, Venkatesan P, et al. Free radical scavenging ability and antioxidant efficiency of curcumin and its substituted analogue. *Biophys Chem* **1999**; 80(2): 85-91.
37. Venkatesan N, Punithavathi V, Chandrakasan G. Curcumın protects bleomycın-ınduced lung ınjury ın rats. *Life Sci* **1997**; (61): 5l-58.
38. Kuhad A, Chopra K. Curcumin attenuates diabetic encephalopathy in rats: behavioral and biochemical evidences. *Eur J Pharmacol* **2008**; 576: 34-42.

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