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Effects of curcumin on lipid peroxidation and antioxidant enzymes in kidney, liver, brain and testis of mice bearing Ehrlich Solid Tumor

Wpływ curcuminy na peroksydację lipidów i enzymy antyoksydacyjne nerek, wątroby, mózgu i jąder u myszy obarczonych guzem Ehrlicha

Mustafa Nisari[1,A,B,D,E,F,G], Seher Yılmaz[2,B,F], Tolga Ertekin[3,C,G], Arzu Hanım Yay[4,C,G], Dilek Ceylan[5,B,D], Neriman İnanç[1,E], Özge Al[6,F,G], Harun Ülger[6,E,G]

[1]Department of Nutrition and Dietetics, Faculty of Health Sciences, University of Nuh Naci Yazgan, Kayseri, Turkey
[2]Department of Anatomy, Bozok University School of Medicine, Yozgat, Turkey
[3]Department of Anatomy, Kocatepe University School of Medicine, Afyon, Turkey
[4]Department of Histology, Erciyes University School of Medicine, Kayseri, Turkey
[5]Genome and Stem Cell Center, University of Erciyes, Kayseri, Turkey
[6]Department of Anatomy, Erciyes University School of Medicine, Kayseri, Turkey

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Summary

Introduction: Cancer is the second most common cause of death in the world. Several natural products have been studied for anticancer activity and for the prevention or repair of oxidative injury. Curcumin is one of the natural products of high medicinal interest. This study was performed to investigate the effects of curcumin on lipid peroxidation and antioxidant enzymes in tissues of mice bearing Ehrlich solid tumor.

Materials/Methods: Forty mice were distributed to four groups as healthy control and treatments that received 1x106 Ehrlich ascites tumor (EAT) cells and EAT cells plus 25 mg/kg/day or 50 mg/kg/day curcumin with a single subcutaneous injection. The liver, kidney, brain and testis tissues were collected for the MDA, SOD and CAT analyses.

Results: Tumor development increased MDA levels in liver ($p = 0.001$), kidney ($p < 0.001$) and testis ($p < 0.01$) and curcumin reduced liver MDA. Liver and kidney SOD activities were increased by both levels of curcumin ($p = 0.001$), but 50 mg/kg/day curcumin increased brain SOD activity ($p < 0.001$). The kidney CAT activity was increased by 50 mg/kg/day curcumin ($p < 0.001$).

Discussion: This study showed that curcumin suppresses tumor progression, alleviates the lipid peroxidation, and improves antioxidant status in the tissues of solid tumor-bearing mice.

Keywords: curcumin, Ehrlich solid tumor, lipid peroxidation, antioxidant enzymes

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Author's address: Mustafa Nisari, PhD, Department of Nutrition and Dietetics, Faculty of Health Sciences, University of Nuh Naci Yazgan, Kayseri, 38090, Turkey; e-mail: mnisari@nny.edu.tr

Abbreviations: **BW** – body weight, **CAT** – catalase, **EAT** – Ehrlich ascites tumor, **GSH** – glutathione, **HAc** – acetic acid, **MDA** – malondialdehyde, **NBT** – nitrobluetetrazolium, **Nrf2** – nuclear factor 2- related factor, **PBS** – phosphate buffer saline, **ROS** – reactive oxygen species, **sc** – subcutaneously, **SDS** – sodium dodecyl sulfate, **SOD** – superoxide dismutase, **TBA** – thiobarbituric acid, **XO** – xanthine oxidase.

INTRODUCTION

Cancer is a major public health problem worldwide and is the second leading cause of mortality after cardiovascular diseases [9, 19, 32, 36]. The most commonly used alternative methods in cancer cases are herbal therapies. Nowadays, phytotherapy is defined as a complementary and alternative treatment method.

Several natural products have been investigated for their anticancer activities. A number of experimental cancer models have been developed for use in cancer-related studies and among them Solid Ehrlich carcinoma is a commonly used tumor model [17, 19]. Ehrlich ascites carcinoma is a spontaneous murine mammary adenocarcinoma adapted to ascites form and carried in outbred mice by serial intraperitoneal (i.p.) passages [15]. Solid Ehrlich carcinoma is an undifferentiated tumor [17].

Along with the chemotherapeutic agents, several natural products have been studied for anticancer activity and [5, 9, 15, 19] for prevention or repair of oxidative injury [15]. Curcumin is one of these natural products of high medicinal interest. Curcumin is derived from turmeric (*Curcuma longa*), a member of the *Zingiberaceae* family that has been used topically and orally in skin and gastro-intestinal diseases as well as wound healing, especially in Indian and Chinese traditional medicine. Curcumin has been seen as a promising compound due to its low toxicity [10, 25]. Many studies have shown the potential anti-carcinogen, anti-inflammatory, anti-allergic and anti-demential effects of curcumin. Curcumin suppresses the initiation, progression and metastasis of several tumors [10, 20, 25, 31] by inducing apoptosis in cancer cells without damaging healthy cells [18]. The degradation (autoxidation) of curcumin yields deoxygenated metabolites with electrophilic and nucleophilic moieties. Diverse biological effects of curcumin are attributed to its keto-ene moiety acting as a Michael acceptor, β-dicarbonyl as a metal chelator and the phenolic hydroxyl moiety as H donor and antioxidant [13, 15].

Reactive oxygen species (ROS) are related to the initiation and progression of tumors [20, 31]. The effects of curcumin on pro-inflammatory mediators have been intensively investigated in cancer cases. Several studies have claimed that curcumin is a free radical scavenger and also an antioxidant [1, 8, 16, 22, 28, 30, 34]. It clears O_2. OH and NO. radicals and play a role in the inhibition of lipid peroxidation [35, 37]. Bansal et al. [6] reported that curcumin also increases the concentration of other endogenous antioxidants via nuclear factor 2-related factor (Nrf2) pathway to improve the defense system of body against ROS.

Sandur et al. [29] reported that curcumin exerts its apoptotic and anti-inflammatory activities by modulating the redox status of the cells. These authors have also shown that curcumin leads to ROS production and changes the intracellular glutathione (GSH) level.

Although the anticancer effect of curcumin is widely accepted, curcumin plays a dual role i.e. both scavenging and generating ROS [4, 8, 25, 29]; thus, further studies are needed to highlight the antioxidant features of curcumin, which are responsible for its anti-inflammatory, anti-proliferative, proapoptic and chemo-preventive effects. Therefore, the present study was performed to determine the effects of different levels of the curcumin on tumor progression, malondialdehyde (MDA) level as a lipid peroxidation marker and antioxidant enzyme activities such as superoxide dismutase (SOD) and catalase (CAT) in different tissues of Balb/C mice bearing EAT.

MATERIALS AND METHODS

Animals, management, and experimental design

In this study, 52 Balb/C male mice aged 8–10 weeks and weighing 25–30 g were obtained from Erciyes University Experimental and Clinical Research Center (DEKAM). The study was held at DEKAM with the permission of Erciyes University Experimental Animals Local Ethics Committee, Approval No. 14/029, and dated 12/02/2014.

Animals were maintained in polycarbonate cages sized 42 x 26 x 15 cm at this center, which provides appropriate standard conditions (21 ±2°C room temperature, 50 ±5% humidity, environmental ventilation systems providing air flow rotation of 12 per hour and 12 hours light/dark light cycle) for highest health status throughout the study. Water and feed were supplied ad libitum during the study.

In the beginning of the study, all animals were weighted. Before starting the experiment, 12 mice were kept as cancer stocks to obtain sufficient EAT cells. The remaining 40 animals were assigned into four experimental groups consisting of 10 mice in each. Group I was kept as a healthy control and 0.1 ml of physiologic saline solution was administered subcutaneously (s.c.) for 15 days. On the first day of the experiment, a single dose of 1x106 EAT cells in 0.1 ml of phosphate buffer saline (PBS) was injected via s.c. route

through nape skin to each animal in Groups II, III and IV for solid tumor development. On the day of the experiment, the curcumin extract was dissolved in different volumes via dimethyl sulfoxide (DMSO) and phosphate buffer saline (PBS) in a way that it would provide the desired concentrations for each experimental group [38]. Mice in groups III and IV received a 0.1 ml of curcumin (Merck, CAS-No: 458--37-7, Merck KGaA, 64271 Darmstadt, Germany) in DMSO and PBS solution providing 25 mg/kg/day and 50 mg/kg/day curcumin via s.c. route through nape skin every day for 15 days.

Measurements of body weights and tumor volumes

The animals were weighed daily and the body weights (BW) were recorded throughout the experiment. At the same time, the tumor sizes were measured by a digital caliper with 0.01 mm sensitivity (Brand Digital Caliper 300: Qingdao Tide Machine Tool Supply Co., Ltd. Shandong, China) after tumors started to form. Tumor sizes were recorded every day from the day when tumors can be measured on the skin surface (Fig. 1). Tumor volumes were determined with the following formula: Tumor volume (mm^3) = $Width^2$ x Length x 0.52.

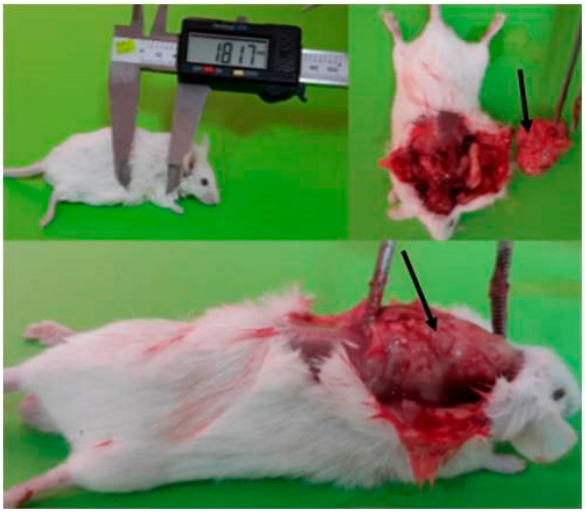

Fig. 1. Ehrlich Solid Tumor in the neck region in Mouse

Histopathologic evaluation

For histological examination, routine paraffin wax embedding procedures were used. The kidney tissues were removed, fixed in 10% formalin and processed by routine histological methods and embedded in paraffin blocks. Paraffin sections 5 µm in thickness were cut from each specimen and were put on poly-L-lysine slides. All sections were stained with hematoxylin-eosin staining to evaluate a morphological overview of the tissue and its structure. The images were captured using Olympus BX51 microscope (Olympus Corporation, Shinjuku-ku, Tokyo, Japan) and analyzed.

Sample collection and preparations

At the end of the experiment, all of the animals were sacrificed under general anesthesia and the liver, kidney, brain and testis tissues from each animal were collected in order to determine MDA levels, SOD and CAT activities.

Homogenization of tissues

Tissue samples (500 mg) were thawed and homogenized in a glass-glass homogenizer with physiological saline solution (pH = 7.4) (1/10, w/v). The homogenates were centrifuged at 12,000 rpm for 20 minutes in 4°C. Some parts of the supernatants were separated for MDA and CAT analyses. The remaining supernatants were mixed with ethanol /chloroform mixture [5/3 (v/v)] at a 1/1 ratio and were centrifuged again at 12.000 rpm for 20 minutes in a refrigerated centrifuge. The supernatants were separated for SOD enzyme activity.

Determination of MDA concentration, SOD and CAT activities

Malondialdehyde forms a pink-colored complex with thiobarbituric acid (TBA) under aerobic conditions at pH = 3.4 following the incubation at 95°C. The absorbance of this complex was measured at 532 nm by a UV-Visible spectrophotometer (Shimadzu, UV1601, Japan) using freshly prepared 10, 20, 40, 60, 80, 100 nMol/ml of 1,1,3,3-tetramethoxypropane (density: 0.99 g/ml) solutions as standards according to the method described by Ohkawa et al. [24]. Briefly, 100 µl of tissue homogenate was mixed with 8.1% of sodium dodecyl sulfate (SDS), 20% of acetic acid (HAc) (pH = 3.5) and 0.8% of TBA (pH = 3.5) and incubated at 95°C for 30 minutes. n-Butanol-pyridine (nBu-Pri) solution and distilled water were added following cooling and strongly vortex mixed. The supernatant was separated following the centrifugation at 4.000 rpm for 10 minutes and the absorbance was read. The result was expressed as nMol/mg protein.

The activity of SOD was measured spectrophotometrically according to the method described by Sun et al. [33]. This method is based on the reduction of nitrobluetetrazolium (NBT) by superoxide radicals, which is formed by the enzymatic reaction of xanthine oxidase (XO). The colorless NBT ion is transformed into a blue-colored formazan, giving maximum absorbance at 560 nm when reduced with the superoxide radical.

The tissue was homogenized with 1/10 of distilled water. The sample was mixed with the chloroform/ethanol mixture 1/1 (v/v) and centrifuged at 12.000 rpm for 2 hours at +4°C. The supernatant was separated to determine SOD activity. Fifty microliters of tissue supernatant and 50 µl of XO in 2 M ammonium sulphate solution (1/100, v/v) were added to 2.9 ml of the reagent mixture consisting of xanthine solution+ NBT+Na2CO3+BSA. After incubation at 25 °C for 20 minutes, 1 ml of 0.8 mM CuCl2 was added to the

Table 1. Body weights of curcumin treated mice bearing Ehrlich Solid Tumor

Days	Healthy control	Tumor control	Curcumin 25 mg/kg/day50mg/kg/day		p
1	28.69 ±3.25	*28.84 ±1.55*	*29.34 ±1.54*	*28.35 ±1.55*	*0.79*
2	29.67 ±2.37	28.97 ±1.42	29.29 ±1.70	28.59 ±1.65	0.365
3	29.63 ±2.47	29.43 ±1.34	28.59 ±1.67	28.01 ±2.41	0.202
4	29.59 ±1.80^{ab}	31.37 ±1.27^{a}	28.79 ±1.66^{b}	28.39 ±2.40b	0.012
5	30.03 ±1.58^{b}	33.70 ±1.07^{a}	29.83 ±1.28^{b}	29.21 ±2.31b	0.000
6	30.32 ±1.59^{b}	34.58 ±1.16^{a}	30.05 ±0.96^{b}	29.49 ±1.93b	0.000
7	31.26 ±1.84^{b}	35.31 ±0.97^{a}	30.40 ±1.12^{b}	30.73 ±1.91b	0.000
8	31.14 ±1.71^{b}	35.59 ±0.91^{a}	31.16 ±0.70^{b}	30.66 ±1.67b	0.000
9	31.57 ±1.4^{7b}	35.68 ±0.74^{a}	31.08 ±0.94^{b}	30.84 ±1.77b	0.000
10	31.62 ±1.82^{b}	36.31 ±0.89^{a}	31.55 ±0.52^{b}	31.17 ±1.86b	0.000
11	32.46 ±1.68^{b}	36.38 ±0.65^{a}	32.24 ±0.82^{b}	32.58 ±1.85b	0.000
12	32.89 ±1.81^{ab}	36.65 ±0.76^{a}	33.16 ±0,93^{b}	29.92 ±10.64b	0.079
13	32.14 ±1.78^{b}	36.15 ±2.05^{a}	32.25 ±1.02^{b}	32.40 ±1.66b	0.000
14	32.41 ±1.54^{b}	37.08 ±0.86a	32.70 ±1.07^{b}	32.53 ±1.65b	0.000
15	32.52 ±1.48^{b}	37.17 ±0.90^{a}	33.01 ±1.20^{b}	32.98 ±1.35b	0.000
Body weight change					
Days 1–15 (%)	0.11 ±0.08	0.22 ±0.03	0.11 ±0.05	0.13 ±0.05	

^{a,b} The values within the same row with different superscripts differ significantly.

Table 2. Body weights of curcumin treated mice bearing Ehrlich Solid Tumor

Days	Tumor control (n = 10)	Curcumin		p
		50mg/kg/day (n = 10)	50mg/kg/day (n = 10)	
7	60.42 (49.29–110.71)	–	–	–
8	60.42 (49.29–110.71)	72.54 (19.02–354.76)	159.98 (39.85–195.49)	0.614
9	384.57 (253.49–762.10)	138.07 (77.79–263.49)	309.62 (142.49–482.17)	0.118
10	1127.99 (345.56–2156.91)^{a}	388.91 (282.76–415.76)^{b}	604.96 (195.70–875.25)^{ab}	0.012
11	1763.52 (514.33–2574.21)^{a}	478.30 (335.68–677.29)^{b}	1379.30 (497.12–2451.48)^{ab}	0.037
12	1746.88 (494.36–3161.91)^{a}	606.99 (341.79–773.41)^{b}	1162.43 (462.50–2303.32)^{ab}	0.040
13	2728.83 (1363.49–3881.40)^{a}	810.26 (477.03–1323.69)^{b}	1857.10 (639.81–2447.20)^{ab}	0.012
14	4086.90 (2093.69–8049.06)^{a}	969.56 (579.21–1669.80)^{b}	2112.84 (859.81–2864.16)^{ab}	0.009
15	4603.99 (3196.06–8049.06)^{a}	1179.56 (607.81–1910.22)^{b}	2059.12 (855.16–2059.12)^{b}	0.002

^{a,b} The values within the same row with different superscripts differ significantly.

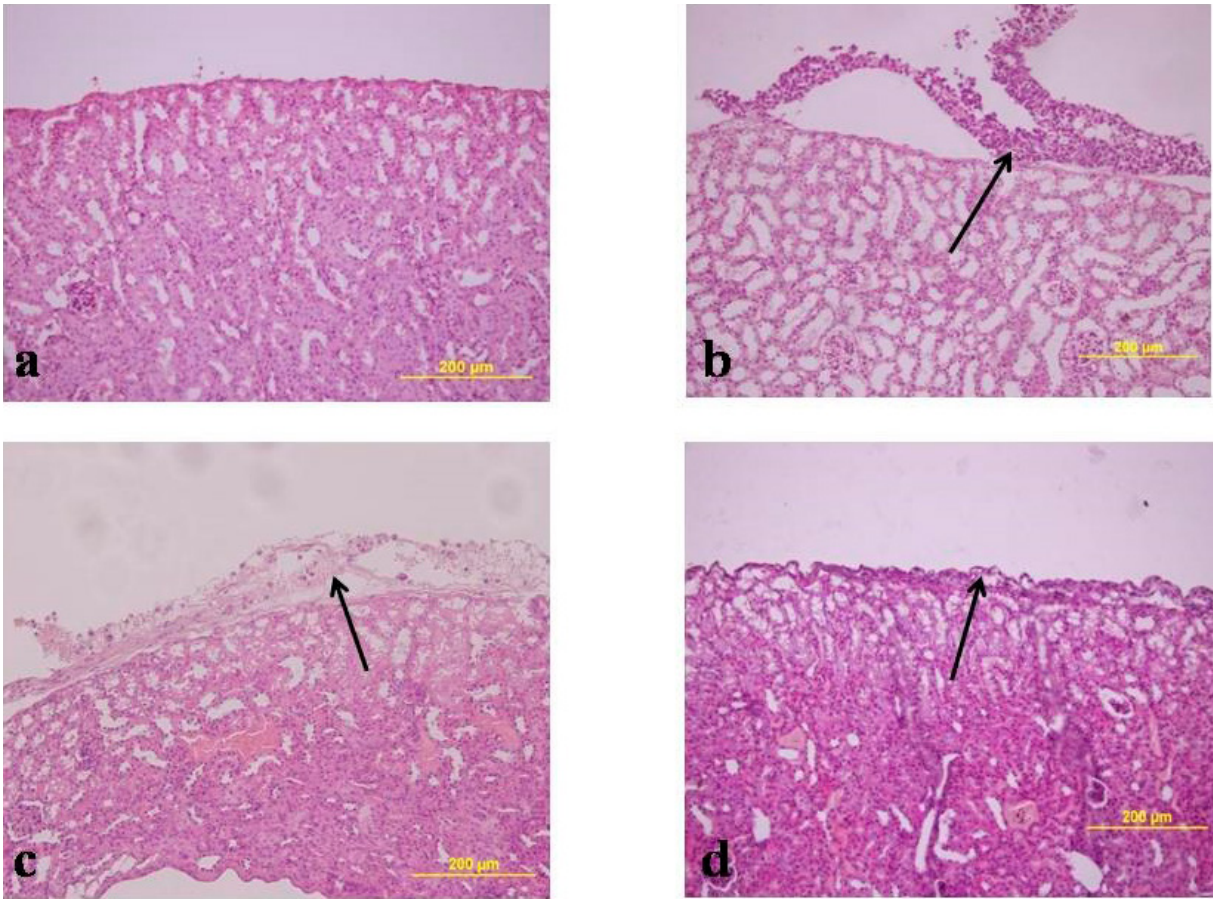

Fig. 2. The histopathological findings in the kidney tissues, a) Healthy control group b) Tumor control group c) The group to which tumor and 25 mg/kg curcumin were applied d) The group to which tumor and 50 mg/kg curcumin were applied (H&E, 20X)

tube and the optical density of the sample was read at 560 nm. The SOD activity was expressed as unit/mg protein (1 unit = 50% inhibition of NBT reduction) and % inhibition was calculated with the following formula: % inhibition = [(blank abs-tissue abs)/blank abs] x100.

Catalase enzyme catalyzes the conversion of H_2O_2 to H_2O. This conversion can be followed by a decrease in absorbance at 240 nm. The decrease in absorbance at 30 second is related to catalase activity. The CAT activity was determined as described previously by Aebi [3]. The CAT assay was performed as follows: tissue homogenate was mixed with H_2O_2 solution (30 mM) + freshly prepared PBS (50 mM, pH = 7.0), then the absorbance was measured spectrophotometrically at 240 nm after 30 second against blank. The extinction coefficient was 0.004 (0.0039) mM-1mm-1. The CAT activity was expressed as U/mg protein/min for tissue.

Analysis of the data

IBM SPSS Statistics 22.0 (IBM Inc., ILL, USA) program was used for statistical analysis of the data. The normal distribution of the data was evaluated by a histogram, q-q graphs and Shapiro-Wilk test. The variance homogeneity was tested by the Levene test. One-way ANOVA and Kruskal-Wallis test were used in the intergroup comparisons. Tukey and Dunn-Bonferroni tests were applied for multiple comparisons. The data were evaluated using the R 3.2.3 program (GNU Free Software). Data were presented as means ± standard deviation of the means and median (25–75%) where appropriate. Significance level was accepted as $p < 0.05$.

RESULT

Body weight changes, tumor development and tumor volumes

The BW of mice in all groups increased during the study. The BW of tumor control was higher than healthy control and curcumin treated mice. The body weight changes of the mice in curcumin treated groups were close to the mice in healthy control group (Table 1).

Tumor development started in solely EAT cell injected group (tumor control) on the 5th day of the experiment and reached to measurable size on day 7. In curcumin treated mice, no measurable tumor masses were determined until day 8. Therefore, statistical analyses for day 7 could not be performed. Statistically significant

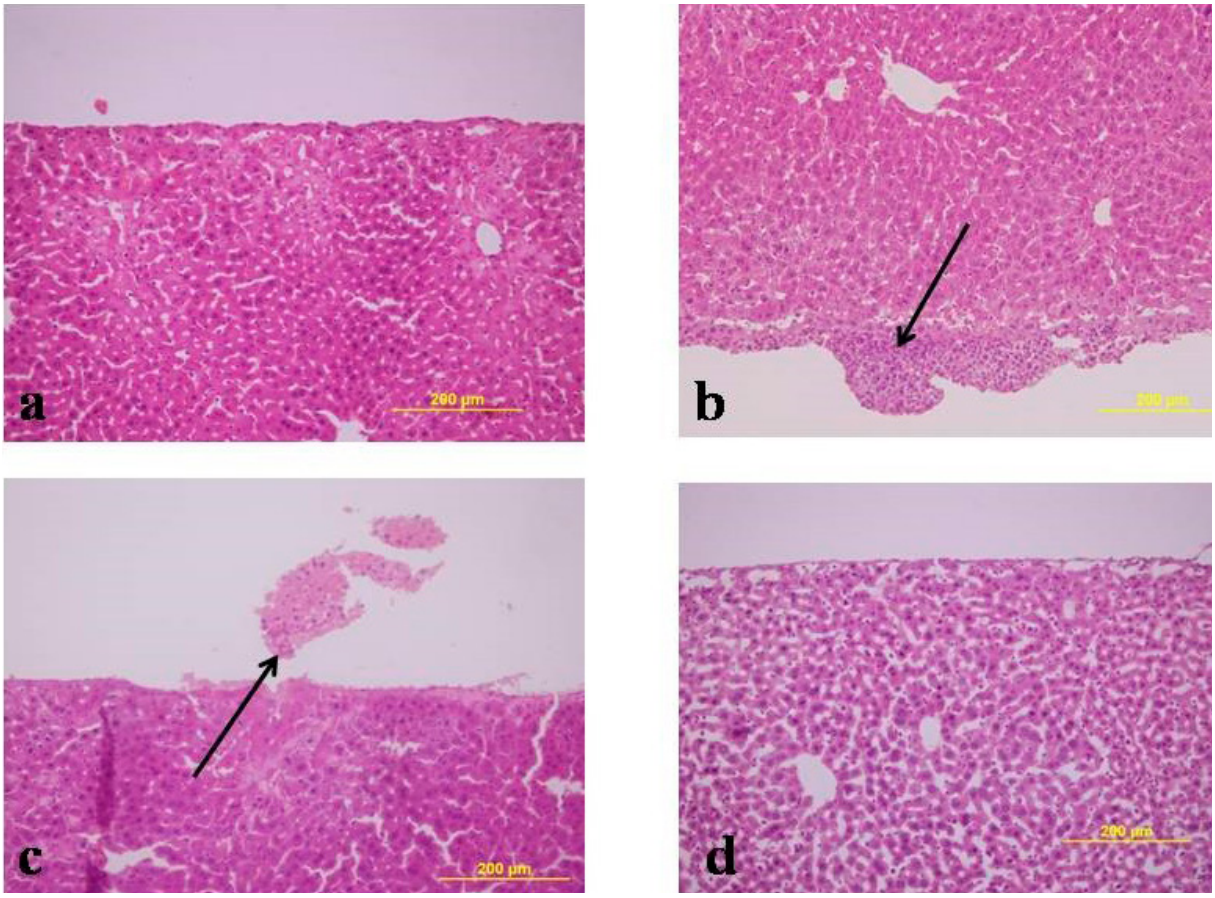

Fig. 3. The histopathological findings of the liver tissues, a) Healthy control group b) Tumor control group c) The group to which tumor and 25mg/kg curcumin were applied d) The group to which tumor and 50 mg/kg curcumin were applied (H&E, 20X)

differences ($p < 0.05$-$p < 0.01$) were determined between tumor control and curcumin treated groups on day 10 and thereafter. Tumor volumes in both curcumins treated mice were significantly lower than the tumor volumes measured in tumor control mice throughout the study (Table 2).

Histopathologic findings

In the kidney tissues of Group 1, the connective tissue capsule appeared normal. Also, kidney parenchyma, renal corpuscles, proximal and distal tubules, peritubular capillaries showed normal histological findings. In Group 2, EAT cells were observed attached to the connective tissue capsule. When the kidney sections of this group were examined, invasive tumor cells were observed at different densities in the tissue capsule. The presence of tumor cells in Group 2 was also detected in the kidney capsule of Group 3, but the density was lower. Group 4 had fewer clusters of EAT cells than Group 2 and Group 3 in the connective tissue capsule in the kidney sections, whereas EAT cells were not found in some sections (Fig. 2).

In the liver tissues of Group 1, normal parenchymal structure was observed. Liver cells progressing radially around the vena centralis in the lobules were normal. Liver sinusoids were observed to extend normally between the hepatocyte cords. In the portal areas, bile duct, hepatic artery and venous structures were visible in the connective tissue. In Group 2, EAT cells were observed attached to the connective tissue forming the glisson capsule whereas in Group 3 EAT cells were observed attached to the capsule of liver tissue. Group 4 had fewer clusters of EAT cells in the connective tissue capsule in the liver sections, whereas EAT cells were not found in some sections (Fig. 3).

Tissue MDA levels, SOD and CAT activities

Liver MDA level in tumor control group was higher than the MDA level determined in healthy control and both curcumin injected mice ($p = 0.001$). Compared the healthy control, kidney ($p < 0.001$) and testis ($p < 0.01$), MDA levels were significantly high in EAT cells injected and curcumin treated mice. Brain MDA level in tumor control group was slightly but not significantly lower than that in the healthy control and in the 25 mg/kg/day curcumin injected group. The MDA levels in 50 mg/kg/day curcumin injected mice were significantly higher than those in the tumor control mice ($p < 0.001$) (Table 3).

Table 3. Liver, kidney, brain and testis MDA levels, SOD and CAT activities in curcumin treated mice bearing Ehrlich Solid Tumor

Tissues /Parameters	Healthy Control	Tumor Control	Curcumin		p
			25 mg/kg/day	50 mg/kg/day	
Liver	n:10	n:10	n:10	n:10	
MDA	12.02^{ad} (11.69–12.47)	15.93^{be} (14.54–16.77)	11.91^{bd} (11.67–13.25)	11.63^{ac} (11.49–11.74)	0.001
SOD	6.50^{dfg} (6.40–6.90)	4.50a (4.30–4.50)	6.50^{bef} (6.35–6.50)	6.70^{ceg} 6.29–6.85)	0.001
CAT	47.85^{ce} (44.72–50.90)	43.90^{bd} (37.02–46.20)	31.71^{a} (27.36–33.58)	38.20^{ade} (37.23–40.45)	0.000
Kidney	n:10	n:10	n:10	n:10	
MDA	0.87 ±0.03^{a}	0.93 ±0.03^{b}	0.92 ±0.04^{b}	0.95 ±0.03^{b}	0.000
SOD	5.45^{abd} (5.38–5.68)	3.70^{a} (3.50–3.90)	5.75^{be} (5.38–6.13)	5.69^{cde} (5.53–5.88)	0.001
CAT	27.93^{ac} (27.42–32.32)	23.86^{a} (21.64–26.25)	27.54^{a} (26.32–28.26)	44.6^{9bc} (33.07–53.97)	0.001
Brain	n:10	n:10	n:10	n:10	
MDA	8.20^{ad} (7.80–8.42)	6.25^{ab} (6.07–6.68)	8.52^{bde} (7.73–8.84)	9.70^{ce} (9.15–10.24)	0.000
SOD	4.03 ±0.61^{a}	4.33 ±0.58^{a}	4.57 ±0.52^{a}	5.43 ±0.58^{b}	0.000
CAT	25.53 ±1.32^{a}	32.64 ±1.65^{b}	30.59 ±1.52^{b}	31.58 ±2.37^{b}	0.000
Testis	n:10	n:10	n:10	n:10	
MDA	0.79^{ab} (0.69–0.93)	0.97b^{de} (0.89–24.24)	0.84^{ad} (0.78–0.94)	0.99^{ce} (0.97–1.15)	0.002
SOD	2.45 (2.40–2.95)	2.75 (2.70–3.33)	3.20 (2.70–3.35)	2.50 (2.25–3.15)	0.151
CAT	12.95 ±0.64^{a}	15.97 ±0.39^{b}	15.39 ±0.63^{b}	15.42 ±1.01^{b}	0.000

$^{a-f}$ The values within the same row with different superscripts differ significantly.

Liver and kidney SOD activities were decreased by tumor development. Both of 25 mg/kg/day and 50 mg/kg/day curcumin injections increased the SOD activities (p = 0.001) in these tissues. Concerning brain SOD activity, there was no significant difference between healthy, EAT cells and 25 mg/kg/day curcumin injected mice, whereas 50 mg/kg/day curcumin increased brain SOD activity (p <0.001). There was no significant difference between controls and treatment groups for testis SOD activities (Table 3).

Compared to healthy mice, liver CAT activities were lower in EAT cells injected control and in both levels of curcumin treated mice (p <0.001). The CAT activities in the kidneys of EAT cells injected tumor control mice were slightly but not significantly lower than in healthy controls. Injection of 25 mg/kg/day curcumin had no effect on kidney CAT activity, but 50 mg/kg/day curcumin injection increased kidney CAT activity significantly (p <0.001). The CAT activities in brain and testis of EAT cells injected control mice were higher than healthy controls. Both levels of curcumin treatments did not affect brain and testis CAT activities (p <0.001) (Table 3).

DISCUSSION

The BWs of the animals in the control and treatment groups were measured during the 15-day experiment. When overall BW changes from day one to the last day of experiment were evaluated, compared to healthy control, the BW of tumor bearing control increased. The increase in body weight of the mice seems to be related to the high tumor volumes in EAT cell injected mice. However, increased BW was reduced almost to the BW of healthy control mice by both levels of curcumin treatments.

The EAT cells cause an undifferentiated carcinoma that resembles to human tumors, and they undergo rapid proliferation in almost any mouse host because they lack H-2

histocompatibility antigens [11, 19]. In the present study, the tumor masses showed rapid growth rate and reached the palpable size on day 5 following the s.c. injection of 0.1 ml of ascitic fluid containing 1 x 106 EAT cells. The volume of tumor masses became measurable on day 7 in mice kept as tumor control whereas on day 8 in both of curcumin injected groups similar to the results obtained in the study of Abou Zaid et al. [2] who induced solid tumor by intramuscular inoculation of mice with 0.2 ml of EAT cells, which contained 2.5 x 106 viable EAT cells. These authors reported that the tumor developed and became palpable in all injected animals on 7–10 days post inoculation. In another study by Sakr et al., [27] the tumor developed and became palpable in all injected animals 10 days after 3 × 106 cells/20 g body weight EAT cell inoculation. In the present study, the solid tumor was developed within a shorter time than previously reported duration of 10 [27] and 12 days [17, 23]. The diversity in the onset of the tumor among the studies may result from the inoculum size of the EAT cells.

During the experimental period, the tumor sizes increased in all groups. It has been reported that curcumin suppress initiation, progression and metastasis of several tumors [19, 31]. In the present study, on the 15th day of the experiment, tumor tissues in various sizes were observed in tumor control and curcumin treated mice. However, tumor volumes were lower in 25 mg/kg/day curcumin (1179.56 mm3) and in 50 mg/kg/day curcumin (2059.12 mm^3) injected mice than the tumor volume (4603.99 mm^3) measured in mice kept as tumor control ($p < 0.01$). Gururaj et al. [13] injected EAT cells via i.p route into mice to investigate the effects of curcumin on the growth of Ehrlich ascites tumor cells and found that curcumin effectively decreased the formation of ascites fluid by 66% in EAT bearing mice. Curcumin caused a reduction in the number of EAT cells without exerting cytotoxic effects. Belakavadi and Salimath et al. [7] also demonstrated the inhibition of proliferation of EAT cells and ascites formation (approximately 55%) with curcumin treatment in vivo.

It has been reported that the anti-cancer activity of curcumin may be attributed to its ability to block the transcription factor NF-κB, a regulator of inflammation, cell proliferation and apoptosis [20]. Biochemically, apoptosis is characterized by fragmentation of chromosomal DNA. Curcumin treatment causes DNA fragmentation, leading to the formation of DNA ladder in EAT cells, which is possibly necrotic to EAT cells [7]. The decreases in tumor volumes resulting from both levels of curcumin injections may be due to the suppression of the tumor progress by inducing apoptosis [13, 18, 20, 29, 31].

Previous studies have suggested that ROS are related to initiation and progression of tumors [21, 31]. The occurrence of malondialdehyde (MDA), a secondary end product of the oxidation of polyunsaturated fatty acids, is considered a useful index of general lipid peroxidation [14]. The increase in MDA level in sera or tissue is considered as the indicator of lipid peroxidation due to ROS, which are related to initiation and progression of tumors [20, 31]. Increased ROS in malignant cells may cause overexpression of antioxidant enzymes. The cells protect themselves against oxidative damage by enzymatic and nonenzymatic antioxidant systems. Superoxide dismutase and CAT are enzymatic antioxidants. Superoxide dismutase catalyzes the reaction of superoxide radicals to hydrogen peroxide, which is metabolized by CAT. Increased antioxidant enzyme activities may be related with the susceptibility of cells to carcinogenic agents and the response of tumor cells to the chemotherapeutic agents [26]. A common method for measuring MDA, referred to as the thiobarbituric acid-reactive-substances (TBARS) assay, is to react it with thiobarbituric acid (TBA) and record the absorbance at 532 nm [14]. In the present study, the elevated liver, kidney and testis MDA concentrations might result from tumor development as previously indicated by Kabel et al. [17], who also implanted EAT cells into mice via s.c. route and found increases in MDA levels in tumor tissue. Abou Zaid et al. [2] investigated the antitumor activity of basic curcumin nanoparticles modified with basic nano black seeds (Nigella sativa) and calcium ascorbate in EAT solid tumor bearing mice and determined a significant increase in the liver MDA concentration in solid tumor bearing mice and a reduction with curcumin complex. Similarly, in the present study, both levels of curcumin treatments decreased the elevated liver MDA levels in solid tumor bearing mice, even lower than the MDA levels of healthy controls. Sener et al. [30] reported that kidney MDA level was increased by intraperitoneal formaldehyde injection and reduced by 100 mg/kg intragastric curcumin treatment. In addition, Ugur et al. [37] investigated the effects of orally given 100 mg/kg curcumin on cisplatin induced nephrotoxicity in rat, and they have suggested that curcumin administration causes a reduction in the increased MDA level and curcumin pose a protective role in nephrotoxicity by preserving renal function and providing redox balance in mitochondria. In the present study both level of curcumin treatments had no effects on increased kidney and testis MDA levels. On the other hand, compare to healthy control mice, s.c. injection of EAT cells caused no statistically significant changes in MDA levels in brain of tumor control and 25 mg/kg/day curcumin injected mice. The brain MDA level in 50 mg/kg/day curcumin injected mice was nearly similar to the level of 25 mg/kg/day curcumin injected mice, which was also slightly higher than the level measured in solely EAT cells injected mice, but surprisingly higher than both healthy and tumor bearing control mice. The increased MDA level in the brain tissue with 50 mg/kg/day curcumin injection cannot be explained. However, it can be speculated that curcumin may show its effects in different manner in different tissues because it has the role in generating ROS as well as scavenging [4, 8, 25, 29]. In addition, although it has been reported that curcumin reaches the brain by crossing the blood-brain barrier, insufficient curcumin concentration may reach particular organs such as brain due to low serum concentration of curcumin in rodents [5].

Various treatments, such as irradiation to tumors, formaldehyde injection, chronic restraint stress, bile duct ligation, decreased antioxidant enzymes [30, 34].

Kabel et al. [17] has shown that the subcutaneous injection of EAT cells results in significant decreases in tissue antioxidant enzymes. Abou Zaid et al. [2] found a significant decrease in liver SOD concentration due to EAT cell injection, whereas treatment with nano composite group (curcumin) increased the activity of liver SOD. Similarly, in the present study, the decreased SOD activity in liver and kidney tissues caused by tumor development by subcutaneous injection of EAT cells were increased by both levels of curcumin injections. The 50 mg/kg curcumin injection resulted in a more pronounced elevation of SOD activity in liver and brain tissues. The elevated SOD activities in liver and kidney with curcumin treatment were consistent with the findings of Tokac et al. [34]. In line with our results, Sener et al. [30] also reported increased SOD activity in renal tissue by curcumin treatment. Jagetia and Rajanikant [16] found that curcumin treatment also increased SOD activity on mouse skin exposed to radiation. Abarikwu et al. [1] exposed the rats to gallic acid for inducing oxidative stress and they reported that diminished SOD activity in the liver of rat was reversed by curcumin treatment.

This study determined the low CAT activities in liver and kidney of EAT cells injected control mice compared to healthy mice, which confirms the results of Kabel et al. [17], who had reported that s.c. injection of Ehrlich tumor cells into mice decreased tumor tissue CAT activity as well as the findings of Abou Zaid et al. [2], who determined lower CAT activity in the liver of solid tumor bearing mice, and these authors reported improvements in liver CAT activity with curcumin nano particles. The EAT cells injection increased testis and brain CAT activities in the present study. Both levels of curcumin injection did not affect liver, brain and testis CAT activities in contrast to the finding of Samarghandian et al., [28] who found increased antioxidant defense mechanism in the brain and liver. On the other hand, determination of significantly increased kidney CAT activity ($p<0.001$) with 50 mg/kg/day curcumin injection confirms the findings of previous studies [28, 30, 34, 35].

It can be speculated that the more pronounced effects of curcumin on liver indicate that the liver, as a central organ for many metabolic processes, is more prone to oxidative stress [1]. The antioxidant features of the curcumin may result from either its ability to react with ROS or its ability to induce the expression of cytoprotective proteins, such as superoxide dismutase, catalase and others [35].

The results of this study have shown that curcumin suppresses tumor progression, alleviates the lipid peroxidation and improves antioxidant status in solid tumor-bearing mice. Because of its safe nature, curcumin could be a promising agent with therapeutic efficacy.

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