The effect of curcumin on lipid peroxidation and selected antioxidants in irradiated rats

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Abstract

The effect of irradiation on oxidants and antioxidants in selected tissues and a possible protective effect of curcumin on these indices were investigated. A total of 28 rats were divided into 3 groups; group 1 was control; group 2 was the irradiation group, saline was administered intraperiotenally (i.p.) for three days and then, 9 Gy gamma irradiation was applied; group 3 was the irradiation + curcumin group: curcumin was given i.p. for three days at 200 mg/kg body weight and then the same dose of irradiation was applied. A significant increase in malondialdehyde (MDA) was detected in the liver, kidney, and brain tissues of the rats as a result of irradiation ($P < 0.01$). Glutathione peroxidase (GSH-Px) activity in all the tissues (except for kidneys) decreased ($P < 0.01$), liver SOD (superoxide dismutase) activity decreased ($P < 0.05$), and GSH (glutathione) levels in kidney and ovary tissues ($P < 0.001$) significantly increased. While curcumin administration returned the increased MDA levels in the kidneys and brain in result of irradiation to normal ($P < 0.01$), it did not return the increased MDA levels in the liver tissue to normal ($P < 0.001$) despite significantly reducing them. While decreased GSH-Px and SOD activity in the liver in result of irradiation increased with the addition of curcumin ($P < 0.05$), increased GSH levels in the kidneys and ovaries returned to control levels ($P < 0.001$). When MDA values were examined, it was found that the addition of curcumin protected the liver, kidneys and brain from the oxidative damage caused by irradiation.

Gamma-ray, phenolic compound, oxidative stress

Radiation is widely used for radiotherapy, medical diagnosis, dental radiography and several imaging protocols. Its exposure is also in question in accidental radiation releases (Radwan and Mohamed 2018). It has been determined that ionizing radiation causes oxidative stress by generating various reactive oxygen species (ROS) molecules such as superoxide, singlet oxygen, and hydrogen peroxide that have the potential to damage critical cellular elements like DNA, proteins, and membrane lipids and consequently lead to physical and chemical damage in cells, resulting in cell death (Koc et al. 2003).

Cells have an important antioxidant defense against ROS. The antioxidant system involves low molecular weight antioxidant molecules like glutathione (GSH) as well as a number of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). Excessive production of oxygen metabolites is associated with imbalance between oxidants and antioxidants, causing oxidative stress (El-Gazzar et al. 2016). Detrimental effects of irradiation on healthy tissues can be reduced due to the capacity of antioxidants (Okunieff et al. 2008). Antioxidant supplements decrease the side effects induced by the radiation treatment by inhibiting the oxidative damage to normal cells (Lawenda et al. 2008).

It has been stated that several dietary antioxidants reduce free radical attack on biomolecules (Duthie et al. 1996). Being a major bioactive compound in turmeric,

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curcumin is a yellow pigment phenolic compound obtained from the roots of turmeric used as spice. It has a wide spectrum of anti-oxidant, anti-carcinogenic, anti-mutagenic, and anti-inflammatory properties (Tawfik et al. 2013).

Apoptosis is a biological event based on morphological changes that occurs physiologically or pathologically and is regulated by genes (Kerr et al. 1972). The enzymes that control this type of death which is also known as programmed cell death, are known as caspases (Metzstein et al. 1998). Among them, caspase 3 has an important place in the apoptosis mechanism (Korsmeyer 1999; Hugle and Fulda 2015). A correlation between irradiation and caspase 3 expression has been reported in some studies (Ozyurt et al. 2014; Li et al. 2015; Ismail et al. 2016).

In liver cancer, hepatic radiotherapy causes irradiation-associated liver damage which can be life-threatening (Radwan and Mohamed 2018). Cancer radiotherapy and accidental exposure to ionizing radiation require new safe and effective radioprotective agents for the purpose of treating the damage to normal tissue induced by exposure to ionizing radiation. This study investigated how oxidants and antioxidants in the kidney, spleen, heart, brain and ovary tissues along with the liver were affected as a result of irradiation, and whether or not the antioxidant curcumin had a protective effect on these indicators.

Materials and Methods

Animals

Female fertile Wistar albino rats (200 ± 10 g) aged 4–5 months were obtained from the Animal Care Unit of Fırat University and were kept in plastic cages with stainless-steel grid tops. The experimental conditions were environmentally controlled in terms of temperature (23 ± 2 °C), humidity (50 ± 5%), and light (12 h of light and dark cycle). The animals were fed with pellet diet and water *ad libitum*. Three rats were kept together in polypropylene cages containing sterile husk bedding during the experiment. The experiments were carried out after the approval of the Local Ethics Committee of the Veterinary Research Institute (Official form date and number: 18.04.2013 and 2013/4-1) in Elazıg.

Irradiation of the rats

Aγ-ray source was used to perform whole-body irradiation. The animals were placed in Plexiglass® cages and irradiated in groups of seven rats, simultaneously. The source-to-skin distance was 291 cm with a dose of 0.0233 Gy/s (Benkovica et al. 2008) and absorbed dose of 9 Gy. They were irradiated by a 160 MLC LINAC (Siemens Artiste linear accelerator, using 6 MV photons, Belgium). The rats were irradiated under continuous isoflurane anaesthesia in a specially fabricated plexiglas chamber radiating out from the center.

Experimental design

The rats were divided into three groups including 7 rats in each.

Group 1: rats were included in the negative control group and did not receive any treatment.

Group 2: rats were included in the positive control group and treated with intraperitoneal injection (i.p.) containing saline for 3 days. All rats in this group were irradiated with gamma-rays at a dose of 9 Gy.

Group 3: rats were included in the curcumin group and treated with i.p. injection containing curcumin at a dose of 200 mg/ kg body weight (Yılmaz et al. 2013; Xie et al. 2014) for 3 consecutive days. Then, all rats were irradiated with gama-rays at a dose of 9 Gy.

Ketamine (ketamine hydrochloride, 50 mg/kg [Ketalar® 5%, Parke-Davis] and xylazine 8 mg/kg [Rompun® 2%, Bayer]) mixture was used intraperitoneally in order to anaesthetize the rats. All rats were sacrificed at 24 h after irradiation. After decapitation, whole liver, spleen, kidney, brain, heart and ovary tissues were rapidly resected. The tissues were stored at -80 °C.

Biochemical analysis in tissues

The tissues were homogenized by using a Teflon-glass homogenizer with 1.15% KCl in order to obtain 1:10 (w/v) homogenate. Malondialdehyde (MDA) concentration of tissue homogenates expressed as the thiobarbituric acid reactive substances (TBARS) was assayed spectrophotometrically according to the method of Placer et al. (1966). The MDA concentrations were expressed as nmol/g protein. Concentration of GSH of tissue homogenates was measured by an assay using the dithionitrobenzoic acid recycling method by Sedlak and Lindsay (1968). The GSH-Px activity was determined according to the method of Lawrance and Burk (1976) which records the decrease of NADPH at 340 nm. The SOD activity was performed based on the method by Sun et al. (1988). Tissue protein contents were determined in accordance with the method of Lowry et al. (1951).

Immunohistochemical examination

The liver was removed immediately, fixed in 10% neutral formalin for 24–48 h, and then processed to obtain paraffin blocks. Paraffin-embedded blocks were routinely processed; 5 µm thick sections were stained with cleaved caspase 3. After deparaffinization, the slides were immersed in antigen retrieval solution (pH 6.0) and heated in microwave for 15 min to unmasked antigens. The sections were then dipped in 3% H_2O_2 for 10 min to block endogenous peroxidase. Sections were incubated at room temperature with polyclonal rabbit active/cleaved caspase 3 antibody (cat no. NB600-1235, dilution 1/200; Novus Biological, USA) for apoptosis. Expose mouse and rabbit specific HRP/DAB detection IHC kit was used as follows: sections were incubated with goat anti-mouse antibody, with streptavidin peroxidase, and finally with 3,3′ diaminobenzidine + chromogen. The slides were counterstained with haematoxylin. Immunoreactivity in the sections was rated as 0 (none), 1 (mild), 2 (moderate), and 3 (severe).

Statistical analysis

The SPSS statistical software (SPSS for windows, version 20.0) was used for all statistical analyses. All the data were presented as mean ± and S.E. (standard error). Nonparametric Kruskal-Wallis test was used to analyze differences in measured indicators among the groups. Mann-Whitney U-test was used to assess dual comparisons among groups having significant values ($P < 0.05$).

Table 1. The effect of curcumin on MDA and some antioxidant levels in the tissues of the irradiated rats.

Groups	Control	Irradiation	Irradiation + Curcumin	*P*
Liver				
MDA (nmol/g prot)	4.56 ± 0.38^{c}	13.57 ± 0.56^{a}	8.22 ± 0.56^{b}	***
GSH-Px (U/g prot)	2.12 ± 0.33^{a}	0.75 ± 0.16^{b}	1.05 ± 0.22^{b}	**
GSH (nmol/g prot)	0.59 ± 0.04	$0.35 \pm 0,03$	0.48 ± 0.10	NS
SOD (U/g prot)	1.90 ± 0.16^{a}	1.14 ± 0.03^{b}	1.33 ± 0.19^{a}	*
Kidney				
MDA (nmol/g prot)	10.10 ± 0.33^{b}	22.57 ± 2.86^{a}	13.92 ± 2.82^{b}	**
GSH-Px (U/g prot)	2.54 ± 0.27	3.19 ± 2.18	2.47 ± 0.89	NS
GSH (nmol/g prot)	0.41 ± 0.02^{b}	1.92 ± 0.43^{a}	0.58 ± 0.05^{b}	***
SOD (U/g prot)	1.52 ± 0.10	1.64 ± 0.09	1.29 ± 0.14	NS
Heart				
MDA (nmol/g prot)	12.24 ± 0.98^{a}	15.02 ± 1.37^{a}	5.98 ± 0.60^{b}	***
GSH-Px (U/g prot)	45.29 ± 1.58^{a}	22.61 ± 1.56^{b}	7.52 ± 0.46^{c}	***
GSH (nmol/g prot)	0.73 ± 0.06^{a}	0.82 ± 0.09^{a}	0.42 ± 0.04^{b}	**
SOD (U/g prot)	3.36 ± 0.33^{a}	2.76 ± 0.24^{a}	1.23 ± 0.11^{b}	***
Spleen				
MDA (nmol/g prot)	14.09 ± 1.47^{a}	16.76 ± 0.70^{a}	6.78 ± 1.40^{b}	***
GSH-Px (U/g prot)	18.35 ± 1.12^{a}	11.23 ± 1.11^{b}	3.07 ± 0.63^{c}	***
GSH (nmol/g prot)	0.41 ± 0.01	0.55 ± 0.05	0.43 ± 0.07	NS
SOD (U/g prot)	1.4 ± 0.05^{a}	1.5 ± 0.13^{a}	0.93 ± 0.13^{b}	**
Brain				
MDA (nmol/g prot)	13.07 ± 0.86^{b}	21.65 ± 2.02^{a}	10.25 ± 1.60^{b}	***
GSH-Px (U/g prot)	38.41 ± 1.98^{a}	21.36 ± 1.77^{b}	21.06 ± 3.03^{b}	***
GSH (nmol/g prot)	0.5 ± 0.10	0.67 ± 0.04	0.5 ± 0.10	NS
SOD (U/g prot)	1.97 ± 0.14^{a}	1.97 ± 0.11^{a}	1.17 ± 0.16^{b}	**
Ovary				
MDA (nmol/g prot)	12.93 ± 0.82^{a}	14.64 ± 1.40^{a}	8.62 ± 0.40^{b}	***
GSH-Px (U/g prot)	57.76 ± 2.99^{a}	38.74 ± 0.97^{b}	41.49 ± 1.11^{b}	***
GSH (nmol/g prot)	0.82 ± 0.02^{b}	1.33 ± 0.15^{a}	0.6 ± 0.07^{b}	***
SOD (U/g prot)	3.78 ± 0.15^{a}	4.01 ± 0.24^{a}	2.28 ± 0.34^{b}	***

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, NS: Non-significant

[abc]Mean values with different superscripts within a row were significantly different.

MDA: malondialdehyde, GSH-Px: glutathione peroxidase, GSH: glutathione, SOD: superoxide dismutase

Results

A significant increase in MDA was detected in the liver ($P < 0.001$), kidney ($P < 0.001$) and brain ($P < 0.001$) tissues of the rats as a result of irradiation ($P < 0.01$). Irradiation caused a significant decrease in the GSH-Px ($P < 0.01$) and SOD ($P < 0.05$) activity of the liver. As a result of irradiation, a significant decrease in GSH-Px activity ($P < 0.01$) in all tissues (except for kidney) was determined; whereas, a significant increase was observed in GSH level in the kidney and ovary tissues ($P < 0.001$) (Table 1).

While curcumin administration returned the increased MDA levels in the kidneys and brain in result of irradiation to normal ($P < 0.01$), it was determined that it did not return the increased MDA levels in the liver tissue to normal ($P < 0.001$) despite significantly reducing them. In the heart, spleen and ovary tissues, MDA values showed a significant decrease with the addition of curcumin compared to both control and irradiation groups ($P < 0.001$) (Table 1).

While the decreased GSH-Px and SOD activity in the liver in result of irradiation increased by the addition of curcumin ($P < 0.05$), the increased GSH levels in the kidneys and ovaries returned to the control level ($P < 0.001$). Addition of curcumin significantly reduced the GSH-Px, GSH, and SOD levels in the heart and the GSH-Px, SOD levels in the spleen, brain and ovaries compared to the control group and/or irradiation group ($P < 0.01$) (Table 1).

Table 2. Immunohistochemically cleaved caspase 3 expression.

Groups	Control	Irradiation	Irradiation + Curcumin
Cleaved caspase 3	0.85 ± 0.14^{c}	2.42 ± 0.20^{a}	1.57 ± 0.20^{b}

[abc]Mean values with different superscripts within a row were significantly different ($P < 0.05$).

A significant difference was observed between the groups in terms of cleaved caspase 3 expression ($P < 0.05$, Table 2). Cleaved caspase 3 expression was at a relatively low level in the control group (group 1) than the irradiation + curcumin (group 3) and irradiation group (group 2). While the expression of cleaved caspase 3 was at a severe level in the irradiation group, it was determined that cleaved caspase 3 expression started to decrease in the curcumin and irradiation group (Plates XII–XIII, Figs 1–3).

Discussion

Being a sensitive biomarker of lipid peroxidation, MDA is considered a suitable indicator of oxidative stress status. Increased MDA levels indicate the activation of lipid peroxidation as a result of the reaction of ROS with unsaturated free fatty acids of membrane lipids. It is known that exposure to irradiation induces liver damage (El-Gazzar et al. 2016; Khattab et al. 2017; Ashry et al. 2017; Radwan and Mohamed 2018;). It is also reported that γ-radiation causes multiple organ dysfunction especially in the liver and an increase of MDA levels in various tissues depending on the time and dose (Simsek et al. 2012; Tawfik et al. 2013; Xie et al. 2014; El-Gazzar et al. 2016). In this study, γ-radiation caused a significant increase in MDA in the kidney and brain tissues together with the liver but a non-significant increase in the heart, spleen and ovary tissues. As seen, irradiation causes damage of more than one tissue. Being a radiosensitive organ, the liver has higher susceptibility against radiation damage (Radwan and Mohamed 2018). This is verified by the increase in MDA in the liver which was 3-fold compared to the kidney and brain (2-folds).

Enzymatic and non-enzymatic antioxidant systems enable cells to protect themselves against oxidative damage. SOD is the first line defense antioxidant enzyme against ROS that

catalyzes the dismutation of the highly reactive superoxide anion (O_2^-) to the less reactive hydrogen peroxide (H_2O_2). Furthermore, GSH-Px has a significant role in the protection against oxidative damage by catalyzing the reduction of H_2O_2 to H_2O and using GSH as a substrate (El-Gazzar et al. 2016). Alterations in the balance of endogenous antioxidant enzymes are related to exposure to radiation. In this study, while the MDA levels in the liver and brain increased as a result of irradiation, significant decreases were observed in the SOD and GSH-Px activities in the liver and in the GSH-Px activity in the brain. The decreased SOD activity is associated with inactivation of the enzyme, possibly due to increased superoxide radical production or inhibition of the enzyme due to H_2O_2, as a result of decreased GSH-Px activity, which degrades H_2O_2 (El-Gazzar et al. 2016; Ashry et al. 2017). Significant decreases were observed in the GSH-Px activity in the heart, spleen, and ovary tissues in which MDA showed a non-significant increase as a result of irradiation. Inactivation of GSH-Px activity through lipid peroxidation byproducts in irradiated rats may lead to a significant decrease in GSH-Px activity (Asahi et al. 1995). The decrease in the GSH-Px activity in most of the tissues studied as a result of irradiation suggests that the first affected enzyme in the irradiation is GSH-Px. In various studies, it was reported that irradiation caused a decrease at SOD, GSH, GSH-Px levels in the liver (Tawfik et al. 2013; El-Gazzar et al. 2016; Ashry et al. 2017; Khattab et al. 2017; Radwan and Mohamed 2018). The reduction in antioxidant enzymes is due to the depletion of enzymes during oxidative stress that occurred as a result of irradiation (Khattab et al. 2017). In contrast to studies indicating a decrease in GSH in the kidneys (Ekici et al. 2016; Ashry et al. 2017), a significant increase in GSH levels in the kidneys and ovaries as a result of γ-radiation was observed in this study. GSH is a major non-enzymatic antioxidant involved in the cell defense system against oxidative damage, directly as a free radical scavenger or indirectly by repairing the initial damage to macromolecules being able to maintain protein and non-protein SH group in a reduced way (Ross 1988; Scibior et al. 2008). The increased GSH levels observed in irradiated rats may be associated with the adaptive response to radiation. Simsek et al. (2012) reported that while irradiation did not cause a change in the ovarian MDA, it caused an increase in GSH-Px and CAT activities.

It is a known fact that antioxidants are important in mitigating the detrimental effects of oxidative stress on cells (Ashry et al. 2017). Curcumin is a natural phenolic compound having impressive antioxidant properties (Xie et al. 2014). In this study, the significant increase in MDA in the kidney and brain tissues due to γ-radiation was able to reach the control values with the addition of curcumin; however, despite a significant decrease in MDA levels in the liver, they could not reach the control values. This indicates that the curcumin administered was insufficient for the liver even though it protected the kidney and brain. A significant decrease in MDA was observed in heart, spleen, and ovary tissues compared to control and irradiation groups as a result of the administration of curcumin, which may be associated with strong antioxidant characteristics of curcumin. Various researchers have reported that as a result of irradiation, curcumin decreased the elevated MDA levels in the rat plasma (El-Gazzar et al. 2016), rat liver (Tawfik et al. 2013) and brain (Xie et al. 2014) and increased the decreased antioxidant levels such as SOD, GSH-Px, CAT and GSH. Similar results are also shown in the rat hepatocyte *in vitro* cultures (Srinivasan et al. 2008). When the results of these studies were examined, it was determined that despite an improvement was observed in all these values, normal levels could not be reached (Srinivasan et al. 2008; Tawfik et al. 2013; Xie et al. 2014). Interestingly, in this study the curcumin administration significantly reduced the antioxidants examined in all the tissues compared to the control and/or irradiation groups, which may be a result of reducing the need for antioxidants studied due to the strong antioxidant property of curcumin.

The enzymes controlling apoptosis are known as caspases (Metzstein et al. 1998).

Caspase 3, which is one of these protease enzymes, has an important place in the apoptosis mechanism (Korsmeyer 1999; Hugle and Fulda 2015). It has been reported in some studies that there is a correlation between irradiation and caspase 3 expression (Ozyurt et al. 2014; Li et al. 2015; Ismail et al. 2016). Li et al. (2015) applied irradiation to the brains of rats and examined the effect of N-acetylcysteine (NAC). They determined that the caspase 3 level decreased in the NAC+ irradiation group, compared to the irradiation group. Ozyurt et al. (2014) examined the protective effects of quercetin on the kidneys and bladder following irradiation in rats. They determined that the caspase 3 level in the irradiated group was higher compared to the quercetin + irradiation group. In a study examining the effect of radiation on the liver, the protective effect of grape seed oil (GSO) was investigated and it was observed that GSO decreased the caspase 3 level, compared to the irradiation group (Ismail et al. 2016). Caspase 3 levels were also examined immunohistochemically in the study. It was suggested that the strong cleaved caspase 3 expression in the irradiation group and the mild cleaved caspase 3 expression in the curcumin + irradiation group were caused by the antioxidant property of curcumin.

Consequently, irradiation caused a significant increase in MDA in the kidney and brain tissues together with the liver. Upon examination it was determined that curcumin decreased the increased MDA levels in the kidney and brain but although it decreased the MDA level in the liver, it could not return them to the control values. Considering its effect on the liver, curcumin at the dose used during radiotherapy or used for reducing the oxidative damage resulting from irradiation may remain insufficient. Therefore, further studies are needed to investigate different doses of curcumin for reducing the effect of irradiation on the liver.

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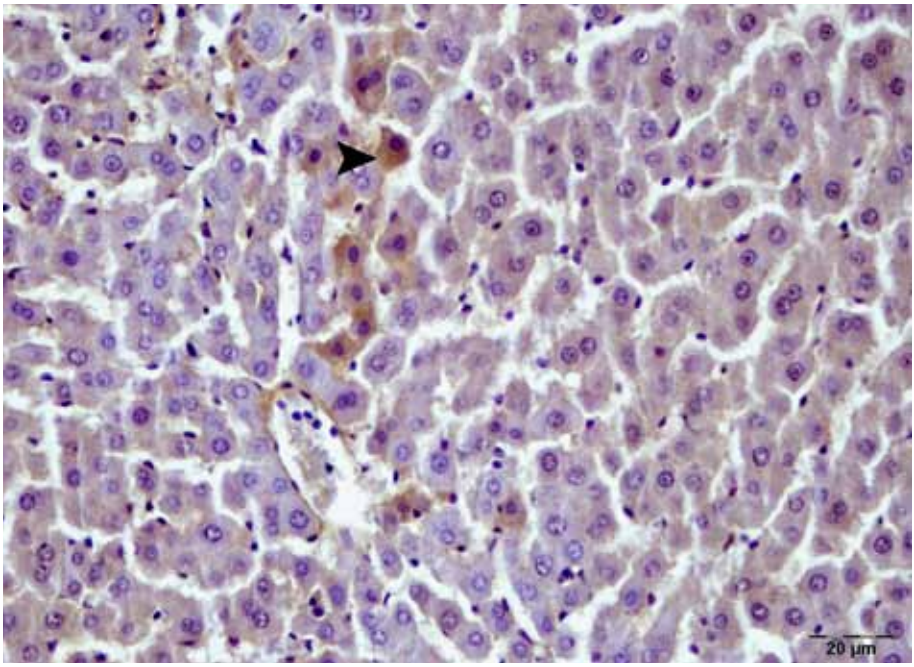

Fig. 1. Slight expression of cleaved caspase 3 in the control group

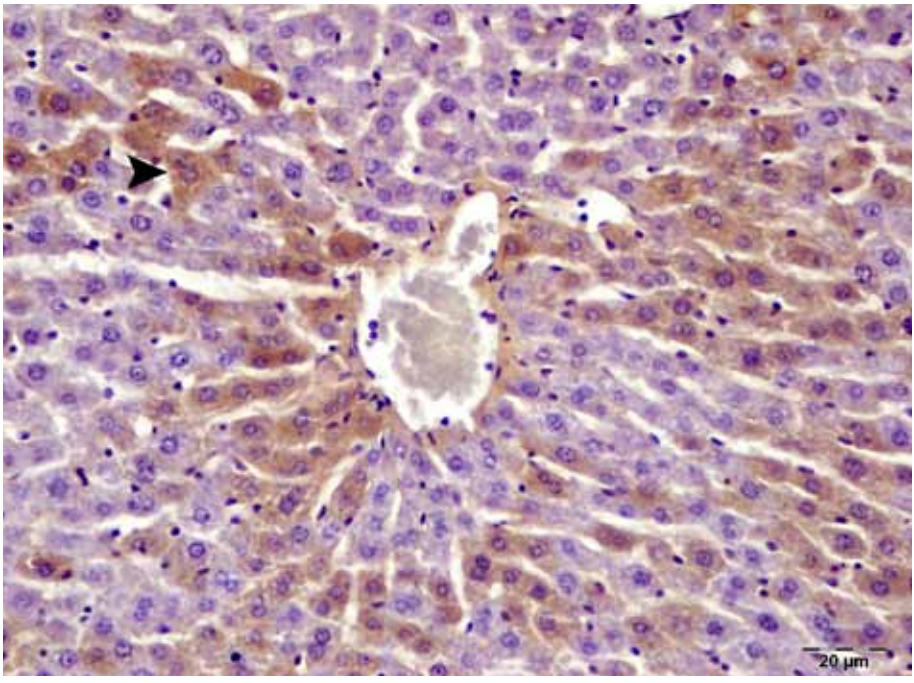

Fig. 2. Severe expression of cleaved caspase 3 in the irradiation group

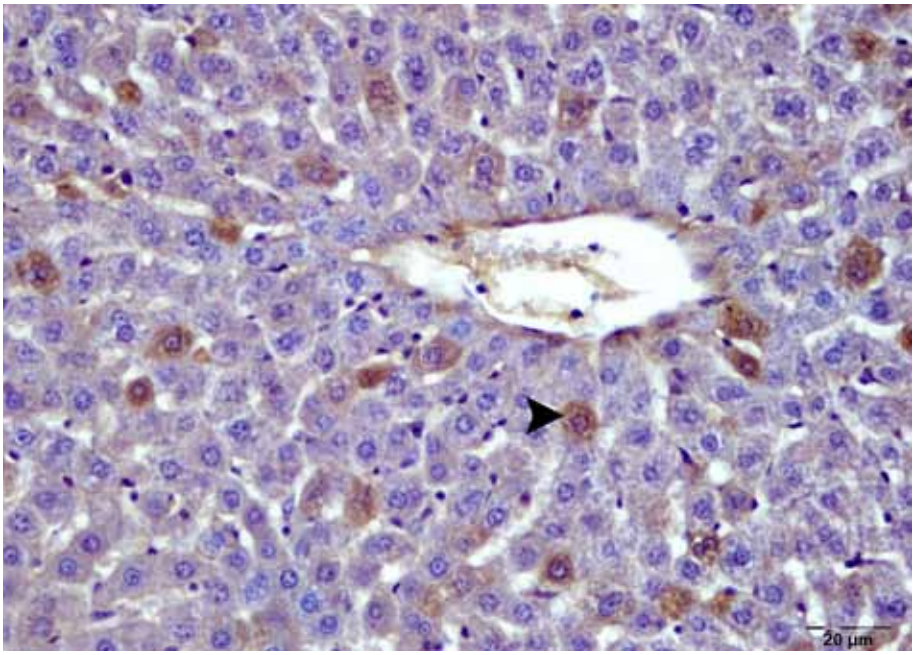

Fig. 3. Moderate expression of cleaved caspase 3 in the irradiation + curcumin group