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Original Article

Protective effects of hesperidin on ionizing radiation-induced liver damage

Hesperidinin iyonlaştırıcı radyasyonun neden olduğu karaciğer hasarı üzerine koruyucu etkileri

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Abstract

Aim: Liver is mostly exposed to radiation during radiotherapy to the upper abdomen, the right lobe of the lung, distal esophagus tumors or total body irradiation. Radiation may lead to cellular damage, and clinical and laboratory findings of liver dysfunction. This study aimed to investigate the protective effect of peroral hesperidin on reducing oxidative stress in liver tissue caused by ionizing radiation

Material and Methods: 24 adult male rats were randomly divide into four groups. Group control was given only physiological saline, Group HES was given hesperidin at 50 mg/kg body weight (BW) for 15 days, Group RAD was given only irradiation, and Group HES+RAD was given hesperidin at 50 mg/kg BW daily and then irradiated. At the end of 15 hesperidinin days, the animals in Groups RAD and HES+RAD were exposed to a dose of 10 Gy to the abdominopelvic region. Liver and blood samples were used for determination of total antioxidant status (TAS) and malondialdehyde (MDA) and also histopathological examination was performed.

Results: Compared with the Group RAD, the plasma and tissue MAD level was significantly decreased in Group HES+RAD (p=0.002). Both plasma and tissue levels of TAS was found significantly higher in HES+RAD group (respectively, p=0.002,p=0.004). Histological examination of Group RAD, portal edema, significant intra-cytoplasmic vacuolization, swelling in the hepatocytes, necrosis, significant sinusoidal and central vein dilation and congestion were observed . In group HES+RAD periportal edema, central vein dilation and congestion were not histologically evident when compared with Group RAD.

Conclusion: Radiotherapy was found to lead to an increase in lipid peroxidation and a reduction in anti-oxidant capacity; 50 mg/kg/day hesperidin administration for 15 consecutive days was seen to reduce the histological changes of liver damage and oxidative stress in rats

Key words: Hesperidin; liver damage; oxidative stress; radiation

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Öz

Amaç: Karaciğer çoğunlukla üst batın, sağ alt akciğer, distal özafagus tümörleri için veya tüm vücut radyoterapi (RT) uygulamasında radyasyona maruz kalmaktadır. Bu çalışmada iyonizan radyasyonun karaciğer dokusunda neden olduğu oksidatif stresi üzerine hesperidinin koruyucu etkisinin araştırılması amaçlanmıştır.

Gereç ve Yöntemler: 24 yetişkin erkek rat rastgele 4 gruba ayrıldı. Kontrol grubuna sadece fizyolojik salin, Grup HES'e 15 gün 50 mg/kg hesperidin, Grup RAD'a sadece irradyasyon yapıldı ve Grup HES + RAD'a 15 gün boyunca 50 mg/kg hesperidin verildi, 15. gün sonunda abdominopelvik bölgeye 10 Gy dozunda radyasyon uygulandı. Radyasyon uygulandıktan 24 saat sonra Total antioksidan kapasite (TAK) ve malondialdehid (MDA) tayini için karaciğer ve kan örnekleri alındı ve ayrıca histopatolojik inceleme yapıldı.

Bulgular: Grup RAD ile karşılaştırıldığında, plazma ve doku MAD düzeyi Grup HES + RAD'da anlamlı olarak azaldı (p=0.002). Hem plazma hem de dokudaki TAK, HES + RAD grubunda anlamlı olarak daha yüksek bulundu (sırasıyla, p = 0.002, p = 0.004).Grup RAD'da , histolojik olarak portal alanda ödem, sinüzoitlerde dilatasyon, hepatositlerde belirgin olarak şime, intrasitoplazmik vakuolizasyon ,arada nekroz, belirgin sinüzoidal dilatasyon, santral ven dilatasyonu ve konjesyon izlendi. Nükleer hipertrofi belirgindi. Grup HES+RAD, Grup RAD ile karşılaştırıldığında periportal ödem, santral ven dilatasyonu ve konjesyon histolojik olarak belirgin değildi

Sonuç: Radyoterapinin lipit peroksidasyonunda artışa ve antioksidan kapasitede azalmaya neden olduğu; Ratlarda 15 gün boyunca 50 mg/kg/gün hesperidin uygulamasının, radyasyonun neden olduğu karaciğer hasarında görülen histolojik değişiklikleri ve oksidatif stresi azalttığı görülmüştür.

Anahtar kelimeler: Hesperidin, Karaciğer hasarı, Oksidatif stres, Radyasyon

Introduction

Excessive reactive oxygen species (ROS) formation can induce oxidative stress, leading to cell damage that can culminate in cell death. ROS are produced by living organisms as a result of normal cellular metabolism and environmental factors, such as air pollutants or ionizing radiation [1]. Cellular exposure to ionizing radiation leads to oxidizing events that alter atomic structure through direct interactions of radiation with target macromolecules . Further, the oxidative damage may spread from the targeted to neighboring [2]. The destructive effect of ionizing radiation results from reactive oxygen species including hydrogen peroxide (H2O2), superoxide anion (O-2) hydroxyl radicals that develop from dissolution of water [3]. Another mechanism of action of RT is alteration of the cell homeostasis, modifiying the signal conduction, increasing DNA damage and consequently making the cell proper for apoptosis [4].

Radiotherapy (RT) is among the most common and most important techniques used for cancer treatment [5].

Liver is mostly exposed to radiation during RT to the upper abdomen, the right lobe of the lung, distal esophagus tumors or total body irradiation. Radiation may lead to cellular damage, and clinical and laboratory findings of liver dysfunction [6,7].

The use of antioxidants either in the diet or as therapeutic agents might offer protection against radiation induced damage [8].

Phenolic compounds are mainly divided into phenolic acids and flavonoids. The structure of phenolic compounds plays an important role in the radical scavenger effect and metal chelating property. Flavonoids constitute a significant group of phenolic compounds, and more than 4000 flavonoids have been detected and they have been classified according to molecular structures [9]. Flavonoids have drawn attention of the researchers due to their properties of being radical scavengers, regulators of enzymatic activity, acting as antibiotic, anti-allergenic, anti-diarrhetic, anti-ulcer and anti-inflammatory drugs [10].The best defined characteristic of flavonoids is their acting as anti-oxidants, which removes free radicals and reactive oxygen species [11,12].

Hesperidin (3,5,7-trihydroxy flavanone-7-rhamnoglucoside) is a pharmacologically active bioflavonoid that is abundant in citrus fruit [13].The hydrogen radical scavenger effect, iron chelating activity and the reduction potency of hesperidin are greater compared to synthetic anti-oxidants such as alpha tocopherol, ascorbic acid, butylated hydroxytoluene, and butylated hydroxyanisole [14].

The present study was aimed to investigate the protective effect of hesperidin administered via the peroral route in rats with radiation-induced liver damage.

Material and Methods

The study was approved by the Institutional Animal Experiments Local Ethical Committee (2017-18-05/10) and performed with international guidelines on the care and use of laboratory animals. The 24 adult male Wistar-Albino rats, weighing approximately 300–350g were supplied by Bulent Ecevit University (BEUN) Experimental Animals Research Unit, Zonguldak,Turkey. The rats were acclimatized and maintained under controlled conditions of temperature (23±1 °C), humidity (55±5%) and light (12h of light and dark cycle), at the BEUN Animals Research Unit. All were fed with standard pellets and water ad libitum.

Male rats randomly were randomly divided into four groups .Group control was given only physiological saline (PS) (no RT; n = 6), Group HES was treated with hesperidin at 50 mg/kg body weight (BW) daily in distilled water as defined in the study of Cetin et al. (12) and 0.25 mL of PS for 15 days (no RT; n = 6), Group RAD was given only irradiation (RT; n = 6), and Group HES+RAD was pre-administrated with hesperidin at 50 mg/kg BW as a single daily dose by oral gavage and 0.25 mL of PS for 15 days and then irradiated (RT+HES; n = 6). At the end of 15 days, the animals in Groups RAD and HES+RAD were exposed to 10 Gy of to the abdominopelvic region (APR). All rats were decapitated at 24 h after exposure to radiation. Hesperidin was purchased from Sigma Chemicals, St. Louis, MO, (USA).

Irradiation of animals

The experimental model of anaesthetized rats for irradiation was used, as described by Parihar et al. (13). The animals in Groups RAD and HES+RAD were anaesthetized with an intraperitoneal injection of 100 mg/kg ketamine, then four rats in the prone position were administered a single non-lethal dose of 10 Gy using a 6-MV linear accelerator at a dose rate of ~1 Gy/min with the source axis distance (SAD) technique and a 1.0-cm bolus material on the surface. A computed tomography simulation of a rat was performed with 1-mm slices, and a dose calculation was performed with the Eclipse treatment planning system (ver. 8.9; Varian Medical Systems, Palo Alto, CA, USA). The animals were returned to their home cages following irradiation. Control animals were anaesthetized but not exposed to radiation. All irradiations were performed between 08:00 and 09:30.

Chemical analysis

Tissue samples were cut into small pieces and then homogenized in phosphate-buffered saline (pH 7.4) using a glass-Teflon homogenizer (Ultra Turrax IKA T18 Basic) for 2 min at 5,000 rpm. The homogenate was then centrifuged (5,000 g, 15 min). The supernatant was used for the analysis. Serum and tissue levels of total antioxidant status (TAS) and malondialdehyde (MDA:the end product of lipid peroxidation) were measured using a colorimetric method with a TAS and MDA kit (Oxford Biomedical Research, Oxford, USA) in accordance with the manufacturer's protocol.

Histopathology of the Liver

For the examination of the liver in the four groups, were graded with a modified version of the technique described by Howarth et al. (14) and the severity of the damage was determined using the damage severity score (DSS).The four groups were examined in terms of 8 parameters, defined as İntracytoplasmic edema, nuclear hypertrophy, dilatation of vena centralis, dilatation of portal triad, sinusoidal dilatation, inflammation of portal triad, hepatocellular necrosis, congestion of vena centralis and sinusoides. Each criterion was scored from 0 to 3 (0 = normal, 1 = mild damage, 2 = moderate damage, 3 = severe damage) in each area for a maximum of 24 points. An experienced pathologist examined the parenchyma and stroma at 20 representative sites.

Statistical analysis

All analyses were performed with the 'R' software (ver. 3.3.2). Descriptive statistics are stated as mean ± standard deviation (SD), median, minimum, and maximum values for continuous variables. Conformity to a normal distribution was assessed with the Shapiro-Wilk test. Differences between the four groups were evaluated with ANOVA and the Kruskal-Wallis test. Tukey and Bonferroni-corrected Mann-Whitney U-tests were used as post hoc tests for ANOVA and the Kruskal-Wallis test, respectively. For all statistical comparisons, a value of $p < 0.05$ was considered to indicate statistical significance.

Results

The results of the biochemical assessments of peroxidation and antioxidant capacity parameters are shown in Tables 1 and 2.

Biochemical parameters

MDA is associated with lipid peroxidation. Compared with the control group, the serum MDA level was significantly higher in Group RAD ($p = 0.002$) and was significantly decreased in Group HES+RAD ($p = 0.002$), (Table 1). The MDA levels in the liver tissue was significantly higher in Group RAD ($p = 0.002$), (Table 1), while treatment with hesperidin significantly reduced lipid peroxidation in liver tissue in Group HES+RAD ($p = 0.002$), (Table 1).

Table 1. MDA levels in plasma and tissue

	Liver (nmol/g wet tissue)	Plasma (µmol/L)
Group Control	1.78(1.25-2.01)[a]	3.40(2.60-4.23)[a]
Group HES	1.96(1.56-2.40)[b]	3.90(3.10-4.81)[b]
Group RAD	3.52(2.60-3.90)[a, b, c]	7.05(6.01-8.80)[a, b, c]
Group HES+RAD	1.83(1.69-2.00)[c]	4.89(3.47-6.00)[c]
p value	0.003	0.001

Abbreviations: HES=Hesperidin; RAD= Radiation
Values are reported as median (minimum and maximum value), (n:6) a,b,c,d,e: statistically significant with Bonferonni corrected Mann Whitney U test

TAS activity indicates anti-oxidant capacity. Compared with the control group, the serum TAS level was significantly lower in Group RAD (p = 0.002) and was significantly increased in Group HES+RAD (p = 0.004), (Table 2). TAS levels in the liver tissue was significantly lower in Group RAD (p = 0.002), (Table 2), and was significantly higher in liver tissue in Group HES+RAD (p = 0.002), (Table 2).

Table 2. TAS levels in plasma and tissue

	Liver (µmol Trolox equivalents/g)	Plasma (mmol/l Trolox equivalent)
Group Control	4.84(4.55-5.11)[a, b]	0.391(0.374-0.411)[a]
Group HES	4.50(4.21-4.87) [c, d]	0.375(0.354-0.411)[c]
Group RAD	1.41(1.21-1.62)[a, c,e]	0.331(0.308-0.360)[a, c, e]
Group HES+RAD	3.15(2.81-3.42) [b, d, e]	0.361(0.345-0.390)[e]
p value	<0.001	0.002

Abbreviations: HES=Hesperidin; RAD= Radiation
Values are reported as median (minimum and maximum value), (n:6) a,b,c,d,e: statistically significant with Bonferonni corrected Mann Whitney U test

Histopathological analysis

The liver tissue was normal in Group control and damaged, to varying degrees, in the other groups. Liver damage was scored from a maximum of 24 points on eight criteria. The liver damage scores were calculated as 0.95±094 in the control group, 13.4±1.84 in the RAD group, 2.05±1.70 in the HES group, and 6.25±2.26 in the HES+RAD group. A statistically significant difference was found between the Control group and the RAD group (p < 0.001) and between the RAD group and the HES+RAD group (p < 0.001), (Table 3).

Table 3: Damage severity scores of liver tissue

	Liver
Group Control	1 (0-3) [a, b]
Group HES	2 (0-5) [c, e]
Group RAD	13,5 (9-16) [a, c, d]
Group HES+RAD	6 (2-11) [b, d, e]
p value	<0.001

Abbreviations: HES=Hesperidin; RAD= Radiation
Values are reported as mean±SD, (n:20)
a,b,c,d,e,f: statistically significant with Bonferonni corrected Mann Whitney U test

Histopathological analysis

All figures demonstrate the histopathological changes in the liver tissues of rats in each group. Cord alignment was regular in the liver parenchyma. The portal area and central vein structures were normal (Fig 1A, 1B).

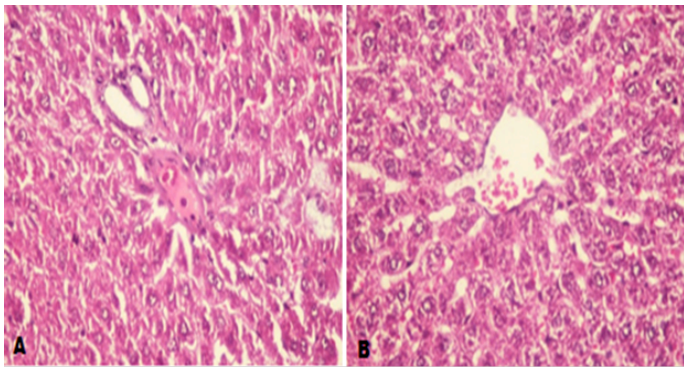

Figure 1: Group Control **A)** portal area and periportal parenchyma **B)** Central venous and peripheral parenchyma (H&E, X40)

In Group RAD, portal edema, sinusoidal dilation, mild mononuclear cell reaction, significant intra-cytoplasmic vacuolization, swelling in the hepatocytes, necrosis, significant sinusoidal dilation, central vein dilation and congestion were observed. Nuclear hypertrophy was evident (Fig 2A, 2B).

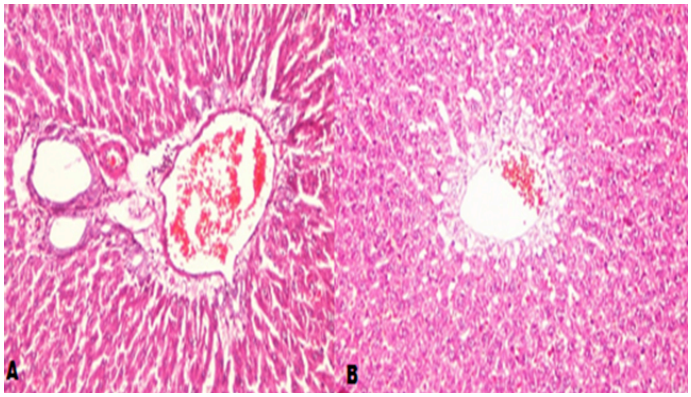

Figure 2: Group RAD. **A)** Periportal prominent edema, mild disorganization and congestion of hepatocyte cordons . Focal necrosis in hepatocytes and reactive nuclear changes **B)** Congestion and dilation of central vein and swelling and edema is prominent in pericentral hepatocytes (H&E, X20).

Histological examination of Group HES revealed focal mononuclear cell reaction in portal regions, mild sinusoidal dilation and congestion (Figure 3A, 3B).

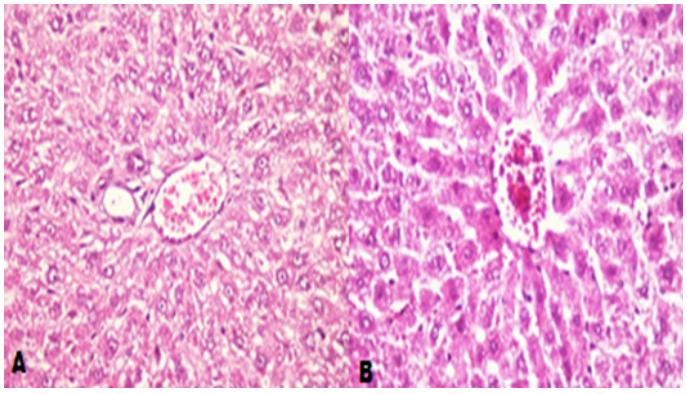

Figure 3: Group HES. A,B) Mild dilation and congestion in portal area, liver parenchyma around the central vein ,sinusoid and significant dilation and congestion in venous structures(H&E, X40).

Mild mono-nuclear cell reaction, sinusoidal dilation, significant sinusoidal dilation, central vein dilation and congestion were observed in the histological examination of Group HES+RAD. Periportal edema, central vein dilation and congestion were not histologically evident when compared with Group RAD (Figure 4A, 4B).

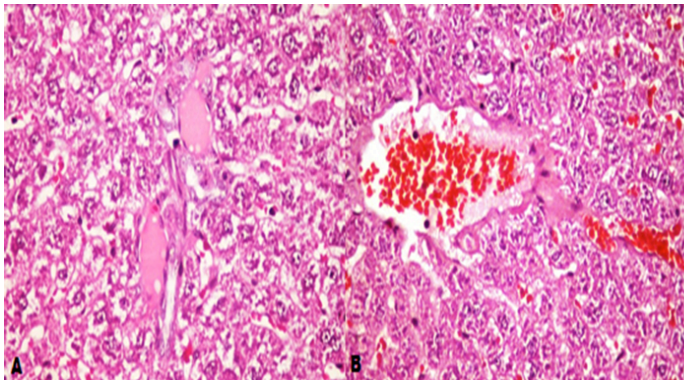

Figure 4: Group HES+RAD A) Cordon arrangement is regular in portal area and surrounding parenchyma. Focal necrosis and hypertrophy in hepatocytes , mild dilation in sinusoids **B)** Mild dilation and significant congestion in central vein and sinusoids

Discussion

This study was conducted using a rat model for assessment of the anti-oxidant effect of hesperidin on oxidative stress in the acute period of 10 Gy radiation exposure. The anti-oxidant effect was determined to increase through observation of a reduction in the oxidative stress parameter (MDA) and an elevation in the anti-oxidant parameter (TAS) in the group in which hesperidin was administered as pre-treatment.

RT is an important therapeutic agent for cancer treatment. The main purpose of RT is applying a maximum dose of ionizing radiation to tumor tissue while causing minimum damage [5].

Radiation changes the cell structure, causing ionization and activation of atoms, damages the basic compounds and consequently leads to a visible biological lesion when absorbed by a viable cell and interacts with biological systems to produce excess ROS. ROS also negatively affect intracellular concentration of antioxidants [15].

As radiation is known to induce lipid peroxidation, supplementation of antioxidants either in the diet or as therapeutic agents are believed to play major role in reducing toxicity.

Srinivasan et al. [16] showed that pretreatment with ferulic acid, a dietary phenolic acid, significantly decreased the levels of thiobarbituric acid reactive substances (TBARS) and protected the hepatocytes against with increasing dose (1, 2 and 4 Gy) of γ-radiation induced cellular damage.

Chitra et al. [17] indicated that tocopherol used as an antioxidant relieved radiotherapy-induced oxidative damage.

Hesperidin is also a flavonoid found in orange and lemon, which has anti-allergic, anti-oxidant and anti-inflammatory properties [18-20].

In rats with pretreated hesperidin (100 mg/kg/d, b.w, orally for 7 days) compared to the control group, superoxide dismutase (SOD) activity, glutathione (GSH) and MDA concentration in the lungs significantly decreased 24 hours after exposed to γ-radiation 18 Gy [21].

Shaban et al. [22] showed that 8 Gy and 10 Gy caused significant increases in DNA- fragmentation and protection is more effective when 200 mg/kg hesperidin is given before rather than after exposure of rats testis to radiation.

It was also reported that SOD, catalase and glutathione peroxide enzyme activity decreased in rats which were exposed to 1,3,5 Gy of whole body irradiation, and these anti-oxidant enzyme activites significantly increased in the group in which hesperidin was administered (50mg/kg and 100 mg/kg) for 7 days after irradiation, and the decrease in AST, ALT, ALP, LDH and gamma-GT levels was maximum in rats exposed to 5 Gy radiation [23].

In our study, MDA, which indicates lipid peroxidation, was seen to significantly decrease and TAS, which reflects an anti-oxidant status, was seen to significantly increase in rats pretreated with hesperidin for 15 days (50 mg/kg) exposed to 10 Gy radiation

On pathology examination in radiation-induced liver disease (RILD), obliteration and occlusion, retrograde congestion and secondary hepatic necrosis are observed in the central veins of the hepatic lobules [24].

Kalpana et al. [25] results revealed that in 4 Gy irradiated group there was anisocytosis of hepatocytes and some of the hepatocytes showed blast transformation, nuclear disintegration and some had pyknotic nuclei, in the 25mg/kg hesperidin pre-administered group most of the hepatocytes was within normal limits with occasional occurrence of macrovesicular type of fatty change

In our study, portal edema, sinusoidal dilation, mild mono-mononuclear cell infiltration in some areas, significant intra-cytoplasmic vacuolization, necrosis, significant sinusoidal dilation, central vein dilation and congestion were observed in the single 10 Gy irradiated group. İn HES (50mg/kg) +RAD group, cordon arrangement is regular in portal area and surrounding parenchyma. Focal necrosis and hypertrophy in hepatocytes, mild dilation in sinusoids. And also liver damage was scored from a maximum of 24 points on eight criteria and the severity of the damage was determined using DSS. In RAD group had a significantly higher score than HES+RAD group ($p < 0.001$).This result supported that hesperidin alleviated the liver damage .

Radiation was found to be associated with severe chronic side effects such as late fibrosis, which could limit the prognosis besides leading to oxidative stress. Hesperidin (100 mg/kg or 200 mg/kg) was shown to have an anti-fibrotic property through preventing the increase in serum and hepatic parameters, caspase 3 gene expression, inducible nitric oxide synthase and alpha smooth muscle actin [26,27]. Since our study included the acute period after radiation, there was no fibrosis in the radiation group.

The limitation of this study was that liver function tests were not examined and the protective effect of hesperidin was not observed at different doses.

In conclusion, radiotherapy was found to lead to an increase in lipid peroxidation and a reduction in antioxidant capacity; 50 mg/kg/day hesperidin administration for 15 consecutive days was seen to reduce the histological changes of liver damage and oxidative stress in rats

The treatment options are limited in RILD, and the disease may result in hepatic failure and death. Further human studies are required for investigating whether hesperidin administration before RT is useful or not insusceptible to RT-induced liver damage.

Declaration of conflict of interest

The authors received no financial support for the research and/or authorship of this article. There is no conflict of interest.

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