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Increase of free radical levels in the periodontal tissues of therapeutic dose radiation applied rats and potential protective effects of bioflavonoids and polyphenols (ENOANT®)

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ABSTRACT

The aim of this study is to determine the effects of the therapeutic dose radiation application on the periodontal tissues of rats and to examine the protective effects of antioxidant application against free radicals prior to radiotherapy. 40 Wistar albino rats were divided into groups of ten animals each: radiotheraphy group; 20 days ENOANT® group; 1 day ENOANT® group, and control group. Then, 3 Gray (Gy) therapeutic dose radiation were applied to head and neck area of the rats outside the control group. After the application, Malondialdehyde (MDA) levels in serum and periodontal tissues were measured by Thiobarbituric acid (TBA) method in the radiotherapy group, long and short term ENOANT® used groups and the control group. The obtained values were statistically compared, the statistical analysis of obtained results showed that according to tissue MDA level, a significant degree of decrease was found in long term ENOANT® used group compared to the radiotherapy group ($p<0,05$). According to these the long-term use of ENOANT® is more effective than its short-term use.

Key words: Antioxidants, Bioflavonoids, Free radicals, Polyphenols, Radiotheraphy.

INTRODUCTION

Radiotherapy, which is a method of high-energy ionising beams, is used for the treatment of malign tumours. It is used as primary therapy method for the treatment of cancer patients and with other therapy methods such as surgery (Baskar *et al.*, 2012).

The biological effects of radiation on the tissues occur with direct or indirect mechanisms. The direct mechanism affects directly the DNA molecule and cause cell destructions or DNA breakings. However the indirect effect decomposes water molecules and causes release of free radicals, which then lead to damage on the cell nucleus. (Karbownik and Reiter, 2000; Stanton and Stinson, 1992; White and Pharoah, 2014).

The free radicals increase with radiation, cardiac problems, liver diseases, infections, chronic alcoholism and smoking. They have destructive effects on proteins, neurotransmitters, nucleic acids (DNA) and cell membrane lipids. (Gulyasar, 2003; Wells and Oddle, 2005).

Antioxidants are a large group of compound which is formed of enzymes, vitamins, some metals and they may prevent oxidative damage in the cell due to free radicals. (Maturu *et al.*, 2018). Many authors showed, anti-inflammatory, antiallergenic, antioxidant and anti-carcinogenic effects of bioflavonoids and polyphenols in their studies. (Babukumar *et al*., 2017; Singh *et al*., 2017; Hritcu *et al.*, 2017; El Shahat *et al.*, 2017; Fan *et al*., 2018; El Shahat *et al.*, 2018) Polyphenols exist in many vegetables and fruits, like grapes. Especially grape peel and seeds contain a great amount of polyphenols. Since peel and seed of grape are used during its production red wine has a strong antioxidant effect. Research on red wine has shown it prevents cardiovascular diseases and also it has radioprotective effect. (Ogay *et al.*, 2001; Melnichenko *et al*., 2001).

ENOANT®, is a grape concentrate which is produced in Ukraine, from the peels and seeds of Cabernet-Sauvignon grapes, similar production technology to that of red wine, but it is alcohol free. The polyphenol concentration of red wine is 0,2 g/dm³ whereas the polyphenol concentration of ENOANT® is 18-20 g/dm³ . Also the antioxidant level of ENOANT® is 3000 times more than the antioxidant level of blood. (Ogay *et al.*, 2001; Melnichenko *et al.*, 2001; Babanin *et al.*, 2001; Schmitt-Schillig *et al.*, 2005).

Senyel established polyphenol concentration of ENOANT® with HPLC (High Performance Liquid Chromatography) analysis as;
Catechin: 1,47 mg/ml
Epicatechin: 0,88 mg/ml
Resveratrol: 23 µg/ml
Quercetin: 130 µg/ml. (Senyel, 2004).

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ENOANT® has antioxidant, antibacterial and antiallergic properties, it has been successfully used in a large amount of clinical research and also with therapeutic and prophylactic purpose, such as the treatment of cardiovascular diseases, respiratory and immune system diseases, allergy and bacterial infections, and against the injurious effects of chemotherapeutic agents and ionising radiation. (Melnichenko *et al.*, 2001; Babanin *et al.*, 2001; Kara *et al.*, 2011).

MATERIALS AND METHODS

For the experiment 40 rats were separated into four groups. All groups were fed in standard conditions (room temperature (23 ± 2°C), illumination (12 hour light, 12 hour dark) and moisture (%50 ± 10) through the experiment. First group was fed *ad libitum* with standard rat food and tap water for 20 days. Second group was fed *ad libitum* with standard rat food and tap water for 20 days, but this time, in addition each rat was fed with gavage method with a 1 ml tap water and 0,5ml/kg ENOANT®. Third group was fed *ad libitum* with standard rat food and tap water for 20 days and then on the last day those rats was fed once with gavage method with a 1 ml tap water and 0,5ml/kg ENOANT®. Fourth group was a control group and this group was fed *ad libitum* with standard rat food and tap water for 20 days.

Radiotherapy application: On the twentieth day of ENOANT® application all the rats of first, second and third group were anesthesized intramusculary with 100 mg/kg Ketalar® (Pfizer) and then 3 Gy therapeutic radiation was applied with the Co-60 Cirus radiotheraphy unit (SSD=80 cm, dose speed 76,45 cGy/min) to the head and neck area of rats.

Obtaining and evaluating tissue and serum examples: On the following day of radiotheraphy application four groups of rats were sacrificed with ether anaesthesia and then blood sample were collected by the use of heart puncture. The collected blood samples were centrifuged for to obtain serum for biochemical analysis and the level of Malondialdehyde (MDA) was measured in the obtained plasma. The oral mucosa and the soft tissues of rats were removed and the tissue homogenate was prepared for the measurement of MDA levels.

The MDA levels of tissue homogenate was measured by TBARS (Thiobarbituric acid reactive substances) method. This method is based on determination of spectrofotometric absorbance, with 535 nm wavelength, of the coloured complex that is composed from one molecule of MDA and two molecules of thiobarbituric acid condensed together. (Fernandez *et al.*, 1997)

RESULTS AND DISCUSSION

The obtained results were analyzed statistically. Plasma MDA and tissue MDA variables' means were evaluated with one-way variance analysis and post-hoc paired comparisons were evaluated with Bonferroni and Dunnet methods. Statistical analysis was evaluated with the statistical software, SPSS for Windows version 7.0. The value of significance limit accepted for evaluations is 0.05.

The results of plasma MDA levels statistical analysis were given on Table 1.

The statistical analysis made according to these results showed that there was no significant difference between free radical levels of irradiated rats and control group ($p>0.05$). Also, no significant difference was found for free radical levels after long or short term Enoant® use in 95% interval of reliability ($p>0,05$).

The results of tissue MDA levels statistical analysis were given on Table 2.

The statistical analysis of obtained results showed that according to tissue MDA level, a significant degree of decrease was found in long term ENOANT® used group compared to the radiotherapy group ($p<0,05$). However, no significant difference was found between free radical levels

Table 1: Plasma MDA levels statistical analysis.

Group	Mean	Median	Variance	Standard deviation	Minimum μ mol/L	Maximum μ mol/L
Radiotherapy	6,77	6,75	0,59	0,77	5,75	8,04
20 days ENOANT®	6,38	6,36	0,48	0,69	5,36	7,31
1 day ENOANT ®	6,69	6,64	0,97	0,98	5,36	7,98
Control	7,16	7,48	0,44	0,66	6,06	7,93

The statistical analysis made according to these results showed that there was no significant difference between free radical levels of irradiated rats and control group ($p>0.05$).

Table 2: Tissue MDA Levels statistical analysis.

Group	Mean	Median	Variance	Standard deviation	Minimum μ mol/L	Maximum μ mol/L
Radiotherapy	0,67 *	0,59	0,04	0,21	0,45	0,98
20 days ENOANT®	0,42 *	0,42	0,007	0,08	0,30	0,52
1 day ENOANT ®	0,61	0,61	0,001	0,04	0,57	0,66
Control	0,53	0,55	0,003	0,06	0,42	0,57

The statistical analysis of obtained results showed that according to tissue MDA level, a significant degree of decrease was found in long term ENOANT® used group compared to the radiotherapy group (*$p<0,05$).

of short term Enoant® used group and the control or the radiotherapy group (p>0,05).

Research in oncology has shown that ionising radiation causes increase in free radicals levels in human and rat bodies. For example, Mut *et al*. applied 24 Gy radiation to brains of rats and found that free radical levels of radiotherapy group increased significantly compared to the control group (Mut *et al.*, 2004).

Similarly, Hardmeier *et al*. applied 2, 4 and 6 Gy radiation to two groups (radiosensitive and radioresistant) of rats' whole bodies. After the radiation application they have measured the formation of lipid peroxidation, enzyme activity and MDA levels in rats' livers. They stated that antioxidant enzyme activity of radioresistant rat increased significantly and although there was no significant difference between radioresistant and radiosensitive group in terms of MDA level, lipid peroxidation increased according to radiation dose in both groups (Hardmeier *et al.*, 1997).

Hamza *et al* (2018). gave rats dried oyster mushroom before whole body γ radiation (6 Gy). After the exposure of radiation, they anaesthetized the rats with diethyl ether and they analyzed the liver enzymes and antioxidant activity. The activity of liver enzymes was increased in the serum of gamma-irradiated rats in comparison to control group. The antioxidant activity of the group treated with 10% dried mushrooms was significantly increased in comparison to the irradiated group. According to these results, the authors suggest that oyster mushroom can improve the antioxidant status and minimize the radiation-induced injury (Hamza *et al.*, 2018).

There are various antioxidants which prevent free radical formation and increase. Most research on antioxidants is about removing harmful effects occur after radiotherapy applications. For example, Sener *et al*. (2003) applied 800 cGy therapeutic radiation to rats' whole bodies and they gave rats a certain amount of antioxidant before and after the radiotherapy. They found that MDA levels of the groups which used antioxidant decreased significantly compared to the control group. In this study, the reason of antioxidant application to rats before radiotherapy is to explain the protective effect against the radiation injury. (Sener *et al.*, 2003).

Jagetia *et al.* (2005). gave rats naringin before 0.5, 1, 2, 3 and 4 Gy radiation applications to their whole bodies. After that they measured lipid peroxidation rate and antioxidant enzyme levels in tissue. They found that lipid peroxidation in naringin group after 2 and 3 Gy radiation applications decreased very significantly and after 4 Gy radiation application decreased very highly significantly ($P< 0.001$) in comparison to the control group. However, the gluthatione, catalase and superoxide dismutase activity levels increased significantly ($P<0.05$) or very highly significantly ($P< 0.001$) in comparison to the control group, depending on the radiation doses. (Jagetia *et al.*, 2005)

Prasad *et al*. (2005) applied 1, 5, 10 μg/ml sesamol to in vitro lymphocyte cultures before 1, 2 and 4 Gy radiation application. Then they measured micronucleus formation in cells, dicentric aberration rates, free radical formation, and antioxidant enzyme levels. Micronucleus formation, dicentric aberration rates and free radical formation have increased in direct proportion with radiation dose. Antioxidant enzymes' levels have increased in direct proportion with sesamol concentration. During 1 Gy radiation application all sesamol concentrations prevented radiation injury. (Prasad *et al*., 2005).

Gulbahar *et al*. (2009) researched the effects of radiation on protein oxidation and lipid peroxidation in the brain tissue. Thirty-two guinea pigs were divided into groups of eight animals each: control group (group I); radiation group (group II); N-acetylcysteine (NAC) group (group III), and NAC administered before radiation exposure group; (group IV). Guinea pigs in groups II and IV were exposed to 612 cGy radiotherapy. The levels of protein carbonyl content and malondialdehyde (MDA), which is a marker for lipid peroxidation, were measured. The protein carbonyl and MDA levels of group II were significantly greater than those of group I ($P < .01$). The protein carbonyl and MDA levels of group IV were lower than those of group II. The results indicated that radiation increased protein oxidation and lipid peroxidation in the brain, and NAC administration before radiation exposure may aid in the treatment by decreasing protein and lipid oxidation. (Gulbahar *et al*., 2009).

In the present study, we preferred ENOANT® than other polyphenols because its antioxidant levels is 3000 times more than that of blood, which is higher than most other antioxidant compounds. Also it has a radioprotective effect like red wine but it is alcohol free, therefore it can be used in children and people who do not consume alcohol.

We gave 0,5 ml/kg ENOANT® to second group of rats for 20 days and to third group for one day, then we applied 3 Gy therapeutic dose radiation to rats' head and neck area.

Similar to literature findings, we found that MDA levels in periodontal tissues of radiotherapy group increases compared to the control group. Also a significant decrease was found in MDA level after long term ENOANT® use although no significant difference was found after short term ENOANT® use.

CONCLUSION

It is suggested that, long-term use of ENOANT® before radiotheraphy application can protect against harmful effects of the free radicals. However, further studies to determine the effects of use of ENOANT® in radiotheraphy patients is recommended.

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