EFFECT OF GRAPE SEED EXTRACT ON LIPID PEROXIDATION, ANTIOXIDANT ACTIVITY AND PERIPHERAL BLOOD LYMPHOCYTES IN RATS EXPOSED TO RADIATION

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ABSTRACT

The radioprotective efficacy of grape seed extract (GSE) and vitamin E against whole body X-radiation was studied in Albino Wistar rats. Three groups of rats were investigated: control group (CG) intraperitoneal (i.p.) physiological serum 1 ml/day (n = 10), i.p., vitamin E group (VG) 50 mg/kg/day (n = 10), and i.p. grape seed extract group 50mg/kg/day (n=10). Four weeks later, 6 Gy radiation dose was given to rats. The blood samples were taken 24 hours later after irradiation and Lymphocyte, malondialdehyde (MDA), reduced glutathione (GSH), nitrate, nitrite, reduced ascorbic acid, retinol, β-caratone and ceruloplasmin concentrations were analyzed. The levels of GSH ($p < 0.05$), retinol ($p < 0.001$), β-caratone ($p < 0.05$) and ceruloplasmin concentration ($p < 0.001$) in GSE group were found to be higher than control group but the level of MDA ($p < 0.001$) and nitrite concentration ($p < 0.05$) in rats supplemented with GSE group were found to be lower than the CG.

The results show that GSE has enhanced the antioxidant status and have decreased the incidence of free radical-induced lipid peroxidation in blood sample of rats exposed radiation.

Keywords: Grape seeds; Lipid peroxidation; Peripheral blood lymphocytes; X-rays; Rat

1. INTRODUCTION

The most important action of ionizing radiation on ions, exited molecules and free radicals had a variety effects, including peroxidation of lipids in membranes, inactivation of enzymes, dipolimerization of polysaccharides, and disruption of nucleic acids[1)]. Each lipid peroxide molecule that forms is also a free radical. Thus, on it starts, the process of peroxidation can become autocatalytic, with each lipid peroxide attacking a neighboring fatty acid to yield additional lipid peroxide product[2)]. Reactive free radical formed within cells can oxidize biomolecules and lead to cell death[3)]. X-rays are ionizing radiation which carries enough energy to ionize an atom or molecule. These ionizations can be very destructive to living tissue therefore genetic materials of the cell are destroyed, thereby halting growth.

An antioxidant has been described as any substance that interferes with the reaction of any substance with oxygen or hindered a free radical reaction. In metabolism, the existences of antioxidant systems protect the cell membrane against damage caused by free radicals. Such antioxidants involves enzymatic (superoxide dismutase, glutathione peroxidase and catalase, among others) and no enzymatic (glutathione, ceruloplasmin, vitamins) systems. General supplementation with antioxidants, such as vitamins A, C, and E, and with the sulfur-amino acids cysteine and glutathione is known to reduce free-radical damage caused by radiation therapy[4)]. While protection of healthy tissue would best be accomplished if these nutrients were administered prior to radiation therapy, some scientists think that these nutrients might help protect the normal cells against the radiation-generated free radicals needed to kill all of the cancer cells[5)].

The seed extracts of *Vitis vinifera* are mentioned to have antimicrobial and free radical scavenging properties[6)] reduce the risk of chronic disease by protecting against free radical mediated damage[7)] and prevent incidence of stroke due to the radical scavenging action and inhibition of lipid peroxidation[8)].

Vitamin E (α-tocopherol) is a natural component of cell membranes and is the strongest antioxidant of the tocopherols; it reacts quickly with peroxyl radicals and forms a α-tocopheroxyl radical, which interrupts the free radical chain reaction[9)]. A wide range of radioprotective effects associated with vitamin E have been demonstrated, such as preservation of the small bowel crypt, increase in the rate of the DNA repair process[10)] and reduction in the number of micronuclei in human lymphocytes in vitro[11)].

The cytochemical demonstration of alpha- naphthyl acetate esterase (ANAE) activity provides a method for identifying human T lymphocytes[12], and animals[13,14]. Alpha-naphthyl acetate esterase is a non-specific esterase. The pattern of esterase activity revealed by this method provides a discriminating marker for mature T-lymphocytes showing dense, localized, dot-like positive responses.

The purpose of the study was to investigate the effect of supplemental GSE and vitamin E on lipid peroxidation and on the antioxidant systems in rats exposed to X-radiation. These studies demonstrate that antioxidant effect of GSE given animal was more effective than that of vitamin E administered rats exposed radiation.

2. MATERIALS AND METHODS

PREPARATION OF GRAPE SEED EXTRACT (GSE)

Grape was cultivated in the Cal region of Denizli on October. Grape seeds were separated from the pulp and peel. The seeds were dried in drying oven at 50°C for 72 hrs. Then, dried seeds were ground to fine powder by grinder. About 50 g of each powder were extracted with 500 ml of 100% ethanol at 40 °C for 4 hours and stood for 1 day at room temperature. The extract was then filtered, and the ethanol was removed by using rotary evaporator under vacuum at 40°C. The residue obtained was dissolved in water and kept in freezer for further experiment.

ANIMAL AND PROTOCOL DESIGN

Before commencing the work, permission from Institutional Animal Ethics Committee of Ege University was obtained. All animals were habituated to the laboratory conditions at least 2 weeks prior to training. Thirty male Albino Wistar rats weighing approximately 150-200 g were housed five animals per cage and were fed a standard rodent chow diet and water ad libitum. They were kept at a constant temperature (22 ^{0}C) on 12 h cycles of light and dark. The rats were randomly allocated to three groups:

Controls Group: (n = 10): For the 4 weeks prior to irradiation, each rat received a daily intraperitoneal (i.p.) injection of 1 ml physiological serum.

Vitamin Group: (n = 10): For the 4 weeks prior to irradiation, each animal received a daily i.p. injection of 50 mg/kg vitamin E (Sigma, St. Louis, USA).

GSE Group: (n = 10): For the 4 weeks prior to irradiation, each rat received a daily i.p. injection of 50 mg/kg GSE.

Four weeks later, 6 Gy radiation dose was given to rats. The rats were sacrificed under intense ether atmosphere 24 hours later after radiation. Blood samples were taken. The serum was prepared by centrifugation (3000×*g*, 10 min, +4 °C).

IRRADIATION

The rats were placed in plastic cages for exposure, and were irradiated using a Indico 100 Rab X-ray instrument (CPI, Ontario, Canada). Whole-body of each rats was exposed to X-radiation at the distance of 100 cm from the source to deliver the dose-rate of 3 Gy/min.

BIOCHEMICAL ANALYSES

Whole blood malondialdehyde MDA levels were measured according to a method of Jain et al.[15]. Whole blood reduced glutathione GSH concentration also was measured by spectrophotometric method[16]. The concentrations of nitric oxide (nitrate and nitrite) were detected by the methods of Miranda et al.[17]. Serum vitamin C (ascorbic acid) level was determined after derivatization with 2,4-dinitrophenylhydrazine[18]. The levels of β-carotene at 425 nm and vitamin A (retinol) at 325 nm were detected after the reaction of serum: ethanol: hexane at the ratio of 1: 1: 3, respectively[19] Ceruloplasmin (CLP) level was studied by the spectrophotometric method[20].

HISTOLOGICAL ANALYSES

Heparinized blood samples were taken from rats for ANAE staining for the purpose of this study. For ANAE demonstration[21], air-dried blood smears were fixed in glutaraldehyde-acetone mixture for 3 minutes at -10 °C.

Following fixation, the smears were rinsed in distilled water and then allowed to dry at room temperature for 30 minutes. Blood smears were incubated (pH 7.2) for 4 hours, and after incubation the preparations were washed in distilled water and counterstained with 1% methyl green for 30 minutes. Following dehydration in increasing concentrations of ethanol, the preparations were cleaned in xylene and mounted in DPX. After application of ANAE enzyme stain, they were examined under a light microscope. The proportions of ANAE (+) and ANAE (-) lymphocytes were determined for each blood smear by counting 500 lymphocytes in a total of five areas (100 each one).

STATISTICAL ANALYSES

Statistical analyses of data were analyzed with SPSS statistical software (SPSS for Windows; Release 10.0.1 Standard Version). Comparisons between different groups were performed by one-way ANOVA; if ANOVA revealed significant differences, the post-hoc comparisons were performed by Tukey's multiple range tests. Differences between means of $P < 0.05$ were considered significant. The results are expressed as means ± standard deviation.

3. RESULTS

The results of all determination for the two experimentals (GSE and vitamin E) and the control group are given in figure 1 and figure 2. The MDA concentration of GSE group was found significantly lower than the control group ($p < 0.001$). When MDA concentration of control group compared with VG, it has diminished in VG ($p < 0.01$) but this decrease is smaller than the GSE group with respect to control group. Both nitrate and nitrite concentrations in the GSE group were lower than in the VG and in the CG. The decreasing of nitrate concentration ($p < 0.01$) in GSE group is slightly larger than in VG ($p < 0.01$) compare to the control group and the same changing is observed in nitrite concentration between the groups but the p values ($p < 0.05$) are found differently. The results of the present study demonstrate that whole-body irradiation in rats causes damage in serum, as assessed by increased lipid peroxidation and decreased GSH levels. GSH concentrations have increased in GSE and VG group but its increases in the GSE and VG weren't identical. The GSH level in GSE group was found to be significantly higher in both VG ($p < 0.05$) and control group ($p < 0.001$) respectively and there was significant difference between VG ($p < 0.05$) and control group. There were significant differences serum ascorbic acid concentrations between VG ($p < 0.01$) with GSE and control groups. Although serum ascorbic acid concentrations appeared to be higher in the GSE group, the differences between GSE and control groups were not statistically significant. Retinol levels in all of the studied in serum were found to be significantly higher in both in the GSE group ($p < 0.001$) and VG ($p < 0.05$), as compared to control group. There were no significant differences in β-carotene concentration was noted between VG and CG but this concentration was found to be a important difference between in GSE ($p < 0.05$) with control groups.

The results of ANAE staining are shown in figure 3. In a light microscope examination, ANAE (+) lymphocytes had 1–3 distinct, red-brownish granules representing the reaction product by which they recognized T-lymphocytes. No reaction product was present in B-lymphocytes. The numbers of ANAE (+) lymphocytes in the GSE was higher than that in other experimental group ($P<0.05$). The size of ANAE (+) lymphocytes was similar in all groups. Our study demonstrated that vitamine E groups ANAE (+) T lymphocytes counts significantly fewer compared with control group ($P < 0.05$).

4. DISCUSSION

The presence of MDA is taken as an indicator of free radical damage through membrane lipid peroxidation[22]. The increase in MDA and hydroperoxide levels in serum suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms. When MDA concentration of control group compared with GSE group and VG group, its concentration has decreased in GSE group and VG with respect to control group. These decreased values have been found 32% and 22% in GSE group and VG, respectively. This result shows that GSE is effective than vitamin E for radical capture through membrane lipid peroxidation. Decrease of vitamin E concentration is consistent with previous report about the protective effects of vitamin E against lipid peroxidation, [4,5,23] activity. Falling of serum MDA concentration values in supplemented GSE group coherent with Ahn et al. reported that dietary GSE has strong radical scavenging[24].

The ubiquitous presence of nitric oxide (NO) in the living body suggests that NO plays an important role in the maintenance of health. Being a free radical with vasodilator properties NO exerts dual effects on tissues and cells in various biological systems. At low concentrations NO can dilate the blood vessels and improve the circulation, but at high concentrations it can cause circulatory shock and induce cell death[25]. Thus, diseases can arise for extreme physiological NO concentrations. When examination of serum nitrite and nitrate (such as nitric oxide markers) concentrations, its values in control group are greater than the examined groups and changing of two parameters concentration in nitrate are found (36 %) and nitrite (22 %). That's why GSE and vitamin E have effected identically over serum nitrite and nitrate concentrations. Because of the decreasing of serum nitrite and nitrate concentrations in rats receiving the GSE and vitamin E, this substances have antioxidant properties.

GSH provides major protection in oxidative injury by participating in the cellular system of defense against oxidative damage[26]. GSH scavenges $O_2^{\cdot}$ and protects protein thiol groups from oxidation. GSH also has a major role in restoring other free radical scavengers and antioxidants, such as vitamin E and ascorbic acid to their reduced state. It was reported that tissue GSH levels and the activities of glutathione reductase and glutathione peroxidase, which are critical constituents of GSH-redox cycle, were significantly reduced due to oxidative stress and the authors propose that impairment of antioxidant defense mechanisms could permit enhanced free radical induced tissue damage[27, 28]. It has been reported that GSH has shown anticarcinogenic and antimutagenic effects also it has got an important protective factor against the damaging effect of free radicals induced by radiation in blood serum, in lung and in brain[29]. It was stated that GSH levels are affected during radiation therapy, thus increasing the risk of DNA and other cellular damage[30]. In this study, GSH values have increased in GSE and vitamin E group but its increases in the GSE and vitamin concentration weren't identical. GSE treatment significantly increased the level of GSH group rats. GSH and vitamin E treatment depresses lipid peroxidation and replenishes GSH content in these tissues, which verifies the protective effect of GSH against the oxidative injury. This could be explained by the expression of γ-glutamyl cysteine synthetase, the rate- limiting enzyme for GSH synthesis in flavonoids[31] present in grape seed extract.

Ascorbic acid has been shown to directly regenerate the α-tocopherol from α-tocopherol radicals, and is thus an integral part of the redox antioxidant-recycling network[32]. Ascorbic acid being a free radical scavenger[33], it is more likely utilized in scavenging the free radicals involved in stress or radiation. After the Chernobyl nuclear accident, individuals exposed to intense ionization radiation indicated severe vitamin C deficiency[34]. GSE group rats showed increasing tendency in ascorbic acid concentration levels in serum when compared to control rats but there were no significant differences. Meanwhile, ascorbic acid concentrations in VG rats were significantly higher than those of control and GSE group rats, 39 % times and 34 % times, respectively ($p < 0.01$ with respect to control and GSE). The level of ascorbic acid in GSE and vitamin E group is higher than the control group show that these substances strengthen the antioxidant system to radiation.

Radioprotective effect of vitamin A (retinol) and β-carotene has been reported[35] in mice exposed to partial-body irradiation or total-body irradiation. Vitamin A and related carotenoid compounds have a considerable role in cancer prevention[36] and are of crucial importance to health, in their capacities as potent antioxidants and immune modulators. Carotenoids and vitamin A are of considerable importance to the prevention and treatment of many diverse cancers in a variety of ways: attack and destroy cancer cells; prevent the appearance or proliferation of tumors, and actually reverse precancerous lesions[37]. Serum retinol concentration of GSE and vitamin E group rats was significantly higher than that of the control group rats ($p < 0.001$ and $p < 0.05$ with respect to control, respectively). β-Carotene concentrations in the GSE and vitamin E group have been found to be higher than the control group but its concentration of GSE ($p < 0.05$ with respect to control) was significantly higher than that of vitamin E group. Vitamin C may support the effects of other vitamin such as thiamin, pantotenic acid, folic acid and vitamin A[38]. In the present study, GSE enhanced the levels of vitamin A in rats that is why we think that GSE can act as vitamin C.

Ceruloplasmin is an important extracellular antioxidant and free radical scavenger. In order to overcome the potential harmful effect of free radicals and to reduce the damage by oxidants, a variety of pharmacological antioxidants such as GSH, ceruloplasmin and transferrin have been examined[39]. In the present study, serum ceruloplasmin concentration in dietary GSE ($p < 0.001$ with respect to VG and control group) rats was more intense than in dietary VG ($p < 0.05$ with respect to control) rats compared with control group rats.

Hemopoietic tissue and blood cells are highly radiosensitive. The most marked effects are on the parent (stem) cells of the leukocytes, lymphocytes and platelets. As can be seen in Table 2, T lymphocytes counts were significantly protected with 50mg/kg/day dose of GSE against irradiation. ANAE staining results revealed that

positive ANAE staining could be used as a marker for the T-lymphocytes of rats. In this study the similar ANAE (+) staining patterns were found with previous authors[13]. ANAE (+) lymphocyte proportions were 79.2%, 75.3% and 63.5% in control, GSE and vitamin E groups, respectively. These proportions are close to the peripheral blood T lymphocyte ratio of dogs (Aştı et al., 1993) and and humans[40], but more than that of chicken[23,24,31].
The results indicate that GSE and vitamin E have enhanced the antioxidant status and have decreased the incidence of free radical-induced lipid peroxidation in blood sample of rats exposed X-radiation. However, antioxidant effect of GSE given animal was more effective than that of vitamin E administered whole-body irradiation rats.

5. ACKNOWLEDGEMENTS

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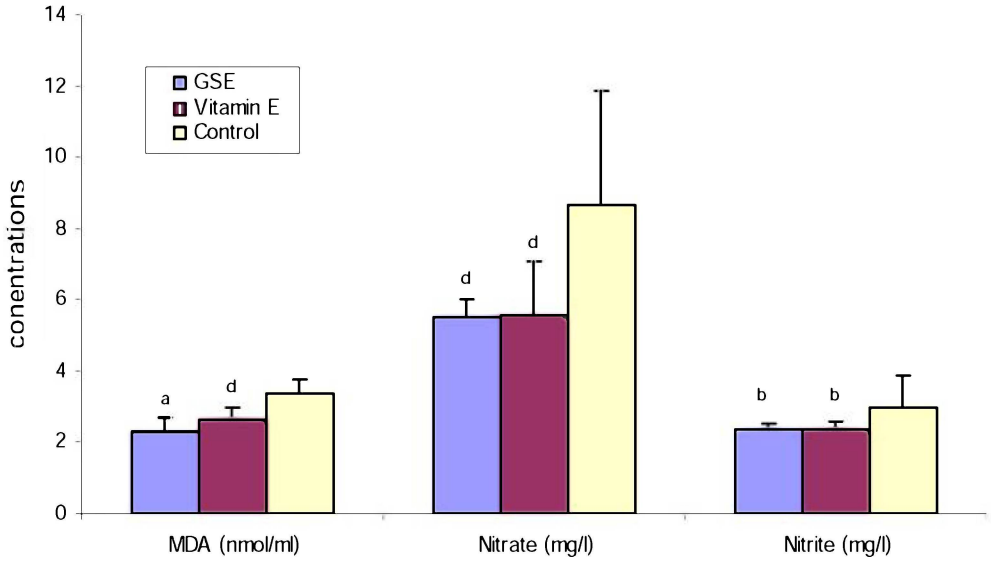


Figure 1. Effects of grape seed extract (GSE) and vitam E on blood sample of rats exposed to X-radiation. [a]: $p < 0.001$ with respect to control [b]: $p < 0.05$ with respect to control,[d]: $p < 0.01$ with respect to control.

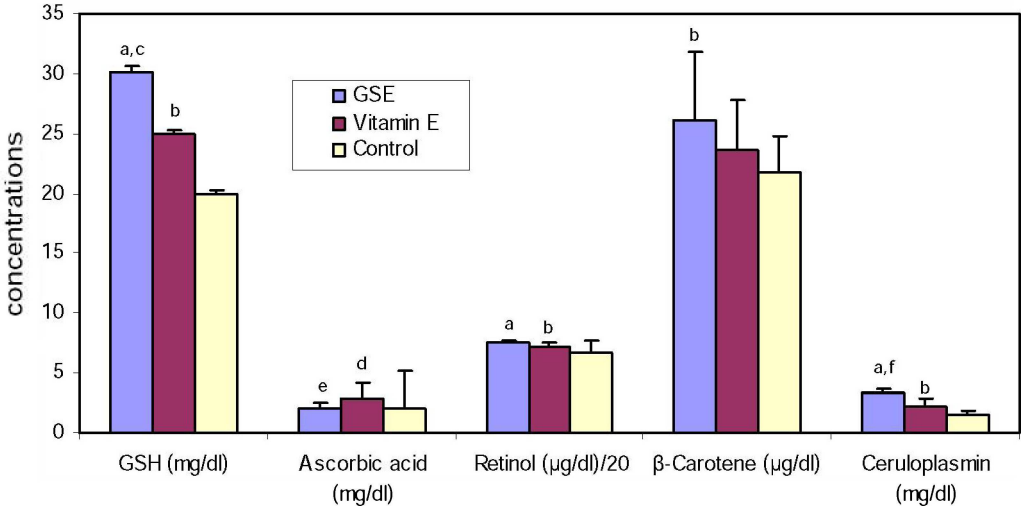


Figure 2. Effects of grape seed extract (GSE) and vitam E on blood sample of rats exposed to X-radiation. [a]: $p < 0.001$ with respect to control, [b]: $p < 0.05$ with respect to control, [c]: $p < 0.05$ with respect to vitamin E, [d]: $p < 0.01$ with respect to control, [e]: $p < 0.01$ with respect to vitamin E [f]: $p < 0.001$ with respect to vitamin E

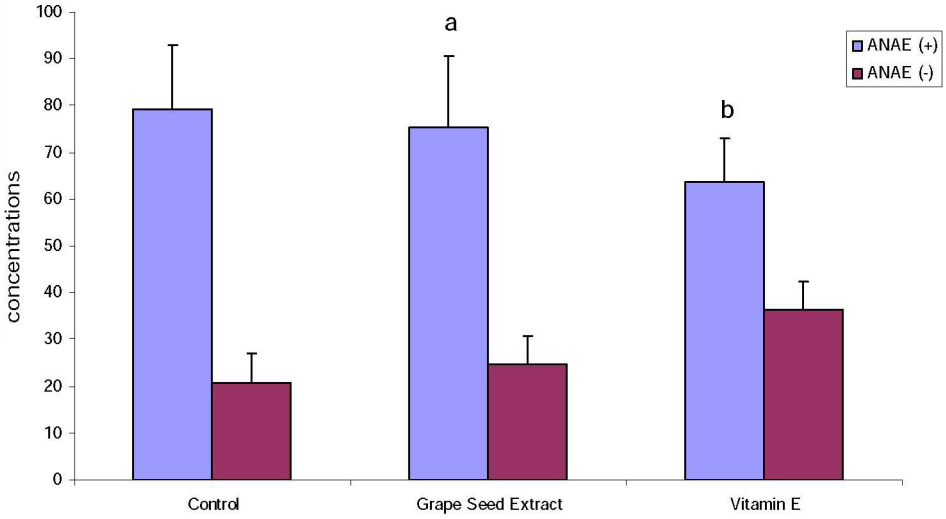


Figure 3. Anae staining in peripheral blood lymphocytes in rats. [a]: $p < 0.05$ with respect to vitamin E, [b]: $p < 0.05$ with respect to control group.

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