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BENEFICIAL EFFECTS OF GRAPE SEED EXTRACT AGAINST CISPLATIN-INDUCED TESTICULER DAMAGE IN RABBITS

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Biological compounds with antioxidant properties may contribute to the protection of cells and tissues against deleterious effects of reactive oxygen species (ROS) and other free radicals induced by cisplatin. This study was performed to investigate the possible protective role of antioxidant treatment with grape seed extract (GSE) on cisplatin-induced testes oxidant injury using biochemical approaches. The degree of protection produced by GSE was evaluated by determining the level of malondialdehyde (MDA) and glutathione (GSH), the activity of catalase (CAT), glutathione peroxidase (GSH-Px), were estimated from testes of rabbits. Male New Zealand white rabbits were divided into 3 groups (n=6):1-Control group(1 ml saline. i.p) 2-Cisplatin group (a single dose of cisplatin (5 mg/kg, i.p) 3- A single dose of cisplatin (5 mg/kg, i.p) + GSE (250 mg/kg/day, i.p) for 6 days. MDA level were significantly higher when compared to Control (p<0.05). In Cisplatin+grapes groups MDA levels were found significantly lower than cisplatin group (p<0.05). GSH levels were decreased with cisplatin compared to control but in case of cisplatin+ GSE it increased (p<0.05). In treated rabbits, the activity of CAT and GSH-Px was decreased in cisplatin and cisplatin+ GSE groups compared to control (p<0.001). These results indicate that the antioxidant GSE might have a protective effect against cisplatin-induced testiculer damage and oxidative stress in rabbit.

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1. Introduction

Oxidative stress may result in overproduction of oxygen free-radical precursors and/or decreased efficiency of the antioxidant system [1]. The oxygen free-radical generation is associated with auto-oxidation of glucose, impaired glutathione metabolism, alterations in the antioxidant enzymes and formation of lipid peroxides [2,3,4]. MDA is used as marker of oxidation of membrane phospholipids through lipid peroxidation [5]. Antioxidants are our first line of defense against free radical damage, and are critical for maintaining optimum health and well-being. The need for antioxidants becomes even more critical with increased exposure to free radicals [6]. There are various endogenous defence mechanisms against free radicals, such as the enzymes SOD, GSH-Px and CAT, whose activities eliminate superoxide, hydrogen peroxide and hydroxyl radicals [7]. Cisplatin is a highly effective antineoplastic drug commonly used for treatment of wide variety of solid tumors [8].

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Flavonoids, which are polyphenolic antioxidants, occur naturally in vegetables and fruits [9]. Fruits and vegetables contain a vast array of antioxidant components, mainly polyphenols and flavonoids [10,11]. Flavonoids possess several physiological properties: antioxidant, antibacterial, antiviral, antiinflammatory, antimutagenic and antitumoral activity, as well as the activation or inactivation of certain enzymes [12].

The grape is becoming increasingly popular as a fruit and is a significant source of nutritional antioxidants, such as polyphenols, anthocyanins as well as biologically active dietary components [13]. Grape seed extract is a naturel extract from the seed of *Vitis vinifera*. It is a rich source of one of the most benefical groups of plant flavonoids, proanthocyanidins oligomers. These flavonids exerts many-health-promotings effects including the ability to increase intracelular vitamin C levels, decrease capillar permability and fragility and scavenge oxidants and free radicals [14]. There are many references in the literature to the composition and antioxidant properties of grape polyphenols [15,16].

In the present experiment, we studied the influence of GSE on cisplatin-induced testiculer damage in rabbits.

2. Experimental

2.1. Chemicals

Cisplatin (50 mg/100 ml, Code 1876A) was purchased from Faulding Pharmaceuticals Pic (Warwickshire, UK). GSE was kindly provided by Mikrogen Pharmaceuticals in Istanbul, Turkey.

2.2 Animals and experimental procedure

Rabbits were obtained from the Veterinary Control and Research Institute, Elazig, Turkey. Eighteen healthy male New Zealand white rabbits (weighing 2.5- 3 kg) were housed individually under diurnal lighting conditions (12–12 h) with free access to drinking water and standard commercial rabbit chow (pellet form, in the sack, Elazig Food Company). The environment in the animal rooms was maintained at a temperature of 24 ± 3 °C. The protocol of this study was approved by the Veterinary Control and Research Institute Ethics Committee.

The animals were divided into three groups, each consisting of six animals. Control group: a single dose of 0.9% saline was administered. Cisplatin group: a single dose of cisplatin 5 mg/kg body weight was administered. Cisplatin+GSE group: was treated with GSE (GSE were dissolved in water and administered to animals by gavage at the dose of 250 mg/kg body weight) for 6 consecutive days before and 6 consecutive days after a single intraperitoneal cisplatin injection.

2.3. Biochemical Assays

At the end of the experiment, the rabbits were decapitated under slight ether anaesthesia and the testes were removed immediately. The testicular tissue was homogenized in glass-glass homogenizer with a buffer containing 1.5% potassium chloride to obtain 1:10 (w/v) whole homogenate. Homogenates were centrifuged 10 min at 700 g at $+4^0$C to determine of GSH, MDA levels and CAT activity, but in GSH-P_X activity, we centrifuged the homogenates in (105.000 *g*, 15 min, 4 °C) and the supernatant was subjected to enzyme assays immediately.

The levels of lipid peroxidation (as MDA) in testes tissues were measured with the thiobarbituric acid reaction using methods described by Placer et al. [17]. The testes tissue CAT activity was determined according to the method of Aebi [18]. Testes GSH-Px activity was determined according to the method of Lawrence and Burk [19]. The GSH content of the testes homogenate was measured at 412 nm using the method of Sedlak and Lindsay [20]. The protein content in the testes was measured by method of Lowry et al. [21].

2.4. Statistical analysis

All values are presented as mean ± S.E.M. All groups were compared by one-way analyses of variance (ANOVA) and post hoc multiple comparasions were done with Duncan test in SPSS/PC software program (version 12.0; SPSS Inc., Chicago, IL, USA) to determine the differences in all parameters.

3. Results

The testes MDA levels were significantly increased in Cisplatin treated group when compared to control. In Cisplatin+GSE group, MDA levels were increased when compared to control but this increase was not significant statically. Administriation cisplatin and grape decreased the MDA levels of testes when compared to cisplatin group (Table 1).

CAT and GSH-P_X activity were decreased depending on Cisplatin and Cisplatin+GSE administriation compared to control. In Cisplatin+GSE group CAT and GSH-P_X activity were increased when compared to cisplatin group (Table 1).

Table 1. Effects of GSE Aganist Cisplatin-Induced Oxidative Stress in Testes Tissue of Rabbits

Groups	MDA nmol/g tissue	CAT k/g protein	GSH nmol/g tissue	GSH-Px IU/g protein
Control	4.23 ± 0.47^{b}	134.00 ± 12.37^{a}	1.98 ± 0.02^{ab}	34.00 ± 1.15^{a}
Cisplatin	13.33 ± 2.94^{a}	68.25 ± 0.94^{c}	1.92 ± 0.01^{b}	20.66 ± 2.02^{b}
Cisplatin+GSE	8.13 ± 0.28^{ab}	103.50 ± 7.30^{b}	2.04 ± 0.23^{a}	25.26 ± 2.41^{b}

Different letters in same column indicate statistically importance according to Duncan's Multiple Range test ($p<0.05$)

In Cisplatin group, GSH levels were decreased but in Cisplatin+GSE group were increased compared to control. GSH levels were increased with administriation of Cisplatin + GSE compared to Cisplatin group (Table 1).

4. Discussion

In this study magnitude of cisplatin-induced testicular injury in the rabbits was assessed with the estimation of testicular MDA, GSH levels and CAT, GSH-P_X activity.

As a result of our study, cisplatin caused to decrease GSH levels but in Cisplatin+GSE group GSH levels increased again. The depleted level of GSH in cisplatin toxicity may due to scavenging of toxic radicals and inhibition of the synthesis and increased rates of turnover [22]. Depletion of cellular glutathione (GSH), which may act as a radical scavenger [23], potentiates the cisplatin-induced cytotoxicity [24,25]. Although cisplatin has been demonstrated to deplete cellular GSH, to alter intra cellular thiols, and to induce lipid peroxidation [26,27].

The beneficial effects of GSEs are well documented in earlier studies. Fujii et al. [28] suggested that GSPs (grape seed polyphenols) have protective effects against high glucose-induced cytotoxicity. Chis et al. [29] found that long-term daily administration of grape seed extract offers enhanced antioxidant potential and protection against tissue lipid peroxidation and protein oxidation. Pari et al. [30] suggested that grape leaf extract exerts its protective effect by decreased the lipid peroxidation and improving antioxidants status, thus proving itself as an effective antioxidant in alcohol induced oxidative damage in rats. Suwannaphet et al. [31] showed that intake of grape seed extract may be a feasible therapeutic strategy for prevention of a high-fructose diet-induced insulin resistance and oxidative stress. Dulundu et al. [32] found that GSE reduces oxidative stress and fibrosis in experimental biliary obstruction. Safa et al. [33] demostrated that pretreatment with red grape seed extract protects against gentamicin-induced acute kidney injury as evident on tissue histology. Atessahin et al. [34] demostrated that lycopene have a possible protective effect against cisplatin-induced spermiotoxicity, effect of giving lycopene after cisplatin being superior to the giving it before cisplatin. Yuce et al. [35] showed

that experimental cisplatin administration increased lipid peroxidation in rats. Ellagic acid protects against cisplatin-induced toxicity by inhibition of the inactivation of glutathione and antioxidant system by cisplatin, up-regulation of GSH-Px and CAT levels in the liver and heart. Thus, the moderate ellagic acid supplementation may play a protective role against cisplatin-induced oxidative stress. Saalu et al. [36] demonstrated that epidoxorubicin treatment resulted in testicular oxidative stress and morphological impairment. They also show that pretreatment with GSE might attenuate this injury in rats.

In our study, decreased activities of GSH-Px, catalase and increased MDA levels were also found in cisplatin group. It was observed that cisplatin induced negative effects in antioxidant enzymes and MDA levels were prevented by grape compared to the cisplatin alone group. Oxidative damage to polyunsaturated fatty acids of cell membranes has long been considered to result in the impairment of membrane fluidity and permeability. The sperm cell membrane contains high levels of polyunsaturated fatty acids resulting in damage of germ cells and mature sperm [14]. Free radical scavenging enzymes such as SOD, CAT, GSH-Px and GST are the first line of defense against oxidative injury. The inhibition of antioxidant system may cause the accumulation of H_2O_2 or products of its decomposition [37]. Our data indicate that GSE (250 mg/kg body weight) has a protective action against cisplatin-induced testiculer toxicity as evidenced by the lowered tissue lipid peroxidation and elevated levels of the enzymic and non-enzymatic antioxidants in testes. Polyphenolic compounds are present in grape, which are powerful antioxidant properties, i.e free radical scavenging activity [38]. We supposed GSE may inhibit lipid peroxidation by scavenging free radicals and increasing intracellular concentration of glutathione.

5. Conclusion

Our study suggests that GSE play a beneficial role in the treatment of cisplatin induced testiculer tissue damage, which could be one of its therapeutic values. These results are correlated with literature but we need further studies to understand compensating effects of GSE in cisplatin-induced testicular injury rabbit model.

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