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Effect of Pomegranate Seed Extract on Free Radical Damage and Antioxidant Activity Under Cisplatin-Induced Oxidative Stress Conditions in Rabbit Testes

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The aim of this study is to evaluate the protective effect of pomegranate seed extract on cisplatin-induced oxidative stress in rabbits testes. Rabbits were divided into three groups; (i) being control group, (ii) cisplatin group and (iii) cisplatin + pomegranate seed extract group. The level of malondialdehyde and glutathione, the activity of catalase, glutathione peroxidase, were measured in testes tissue of rabbits. Malondialdehyde levels were increased depending on cisplatin. In the case of cisplatin + pomegranate seed extract, malondialdehyde levels were found to be lower than cisplatin group ($p < 0.05$). In cisplatin group, catalase, glutathione peroxidase activity and glutathione levels were decreased when compared to control but in cisplatin + pomegranate seed extract group, catalase, glutathione peroxidase activity and glutathione levels were found higher than cisplatin group ($p < 0.05$). The pomegranate seed extract supplementation may play a protective role or decrease the side effects of cisplatin-induced testes toxicity from cancer medicines.

Key Words: Pomegranate, Cisplatin, Testes, Rabbit, Antioxidants.

INTRODUCTION

Cisplatin, cisplatinum or *cis*-diamminedichloroplatinum(II) (CDDP) is a potent anticancer agent against solid tumors of the testes, ovaries, breasts, lungs, bladder, *etc*. However, in practice, the use of cisplatin is limited by its marked renal toxicity[1-4]. It has been suggested that the generation of reactive oxygen species and lipid peroxidation is responsible for the cisplatin-induced renal tubular injury[5-7].

Antioxidants, in general, are compounds which dispose, scavenge and suppress the formation of ROS and lipid peroxidation. Among the well known biological antioxidants, glutathione, glutathione peroxidase, catalase, superoxide-dismutase have a significant role as a suppressor or scavenger of free radicals[8,9]. When antioxidant defences are impaired or overcome, oxidative stress may produce DNA damage, enzymatic inactivation and peroxidation of cell constituents, especially lipid peroxidation[10]. Toxicity biomarkers, such as malondialdehyde, have been also proposed to reflect the oxidative status of exposed species[11]. Malondialdehyde is used as marker of oxidation of membrane phospholipids through lipid peroxidation[12]. Antioxidants and radical scavengers prevent the cisplatin-induced lipid peroxidation and nephrotoxicity[13].

Various agents have been suggested to protect and/or prevent the side effects of many chemotherapeutics. Such chemopreventive agents are flavonoids, which are found in almost all food categories, but primarily in fruits and vegetables. Flavonoids have many functions such as phenolic antioxidants, scavengers of free radicals, chelating agents and modifiers of various enzymatic and biological reactions[14].

In the past few years there has been an increasing interest in determining relevant dietary sources of antioxidant phenolics. Thus, red fruit juices such as grape and different berry juices have received attention due to their antioxidant activity. Pomegranate juice has become more popular because of the attribution of important biological actions[15]. Thus, the antioxidant and antitumoral activity of pomegranate bark tannins (punicacortein)[16,17] and the antioxidant activity of the fermented pomegranate juice[18] have been reported. Pomegranate juice, peel, seeds-all have a potent antioxidant activity. Kaur *et al.*[19] suggested that pomegranate flowers too boast an enormous antioxidant activity. Antioxidant potential of pomegranate juice and extracts are attributed to their high polyphenolics content including ellagic acid and ellagitannins[20].

In the present study, we have extensively studied the antioxidant effects of pomegranate seed extract against cisplatin-induced testes oxidant injury using rabbit models.

EXPERIMENTAL

Pomegranate seed extract and chemicals: Cisplatin (50 mg/100 mL, Code 1876A) was purchased from Faulding Pharmaceuticals Pic (Warwickshire, UK). Pomegranate seed extract was kindly provided by Ari Muhendislik Co., Ankara, Turkey.

Treatment of animals: Eighteen healthy male New Zealand white rabbits, weighing 2.5-3.0 kg, were used throughout this study. The animals were obtained from the Veterinary Control and Research Institute, Elazig, Turkey. The animals were kept under standard laboratory conditions (12 h light: 12 h dark and 24 ± 3 ºC). All experimental procedures were conducted in accordance with the guide to the care and use of laboratory animals. The rabbits were fed with standard commercial rabbit chow (pellet form, in the sack, Elazig Food Company). Feed and water were provided *ad libitum.*

The rabbits were randomly assigned to three groups. The first (control) group (n = 6) and second group (n = 6) received daily i.p. normal saline and i.p. cisplatin (5 mg/kg), respectively, for six consecutive days. The third group (n = 6) of rabbits was treated with pomegranate seed extract (pomegranate seed extract 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 dose of 5 mg/kg body weight cisplatin injection.

Sampling and biochemical analyses: The rabbits were sacrificed under slight ether anaesthesia at the end of the six day period. Testes were removed immediately and stored at 20 ºC until analyzed. 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. Concentrations of malondialdehyde, as proceeding lipid peroxidation, were measured in the homogenate. Homogenates were centrifuged at 5000 rpm, 20 min, at + 4 ºC to determine of glutathione level, catalase and glutathione peroxidase activity and the supernatant was subjected to enzyme assays immediately.

Lipid peroxidation (as malondialdehyde) levels in testes homogenate were measured with the thiobarbituric-acid reaction by the method of Placer *et al.*[21]. The values of malondialdehyde were expressed as nmol/g tissue. The glutathione contents in testes were measured at 412 nm using the method of Sedlak and Lindsay[22]. The levels of glutathione were expressed as nmol/g tissue for testicular tissue.

The glutathione peroxidase activity was determined according to the method of Lawrence and Burk[23]. The protein concentration was also measured by the method of Lowry *et al.*[24].

The glutathione peroxidase activity was expressed as IU/g protein for testicular tissue. The testicular tissue catalase activity was determined by measuring the decomposition of hydrogen peroxide at 240 nm, according to the method of Aebi[25] and was expressed as k/g protein, where k is the first-order rate constant.

Statistical analysis: Data are presented as mean ± standard error of means (SEM). One-way analysis of variance and post hoc Duncan's test was used to determine the differences between the groups in terms of all studied parameters using the SPSS/PC computer program (version 12.0; SPSS, Chicago, IL, USA).

RESULTS AND DISCUSSION

The malondialdehyde levels of testes were significantly increased in cisplatin treated group when compared to control. In cisplatin + pomegranate seed extract group, malondialdehyde levels were increased when compared to control but this increase was not significant statically. Administration cisplatin + pomegranate seed extract decreased the malondialdehyde levels of testes when compared to cisplatin group (Fig. 1).

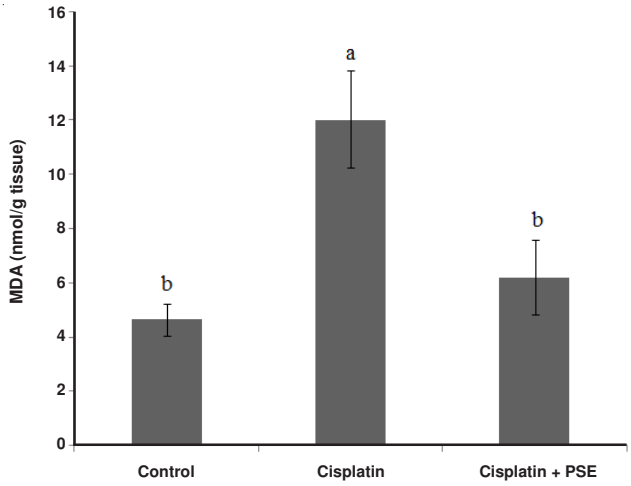

Fig. 1. Effects of pomegranate on malondialdehyde (MDA) levels under cisplatin-induced oxidative stress conditions in rabbits testes. Different letters at the top of bars denote statistical significant difference ($p < 0.05$)

Catalase and glutathione peroxidase activity were decreased depending on cisplatin and cisplatin + pomegranate seed extract administration compared to control. In cisplatin + pomegranate seed extract group catalase and glutathione peroxidase activity were increased when compared to cisplatin group (Figs. 2 and 3).

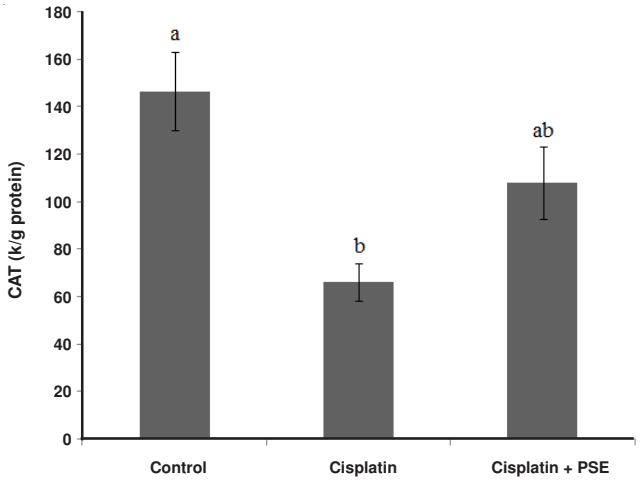

Fig. 2. Effects of pomegranate on catalase activity under cisplatin-induced oxidative stress conditions in rabbits testes. Different letters at the top of bars denote statistical significant difference ($p<0.05$).

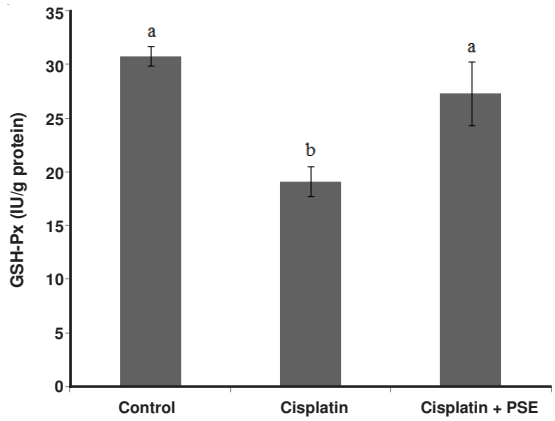

Fig. 3. Effects of pomegranate on glutathione peroxidase activity under cisplatin-induced oxidative stress conditions in rabbits testes. Different letters at the top of bars denote statistical significant difference ($p < 0.05$)

In cisplatin group, glutathione levels were decreased ($p < 0.05$) but in cisplatin + pomegranate seed extract group were increased compared to control ($p < 0.05$). Glutathione levels were increased with administration of cisplatin + pomegranate seed extract compared to cisplatin group (Fig. 4).

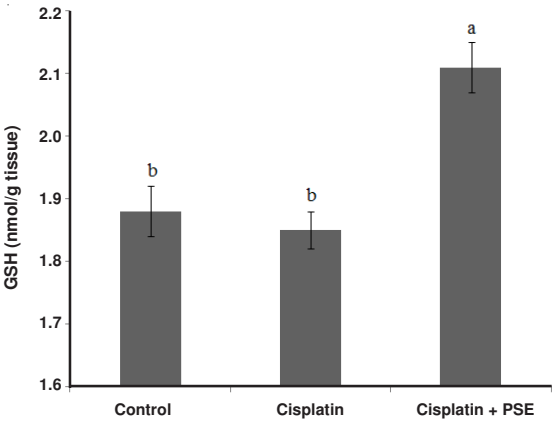

Fig. 4. Effects of pomegranate on glutathione levels under cisplatin-induced oxidative stress conditions in rabbits testes. Different letters at the top of bars denote statistical significant difference ($p < 0.05$)

The mechanisms by which cisplatin causes renal damage is not clear. However, it has been presumed that oxidative stress is involved in this procedure[6,26,27]. In the same way, many antioxidants have been shown to be protective against cisplatin-induced nephrotoxicity. In addition, various free radical scavengers have been shown to be effective in protection against cisplatin-induced nephrotoxicity and treatment with such agents provides significant protection against cisplatin-induced acute renal failure[28-30].

Pomegranate fruit (*Punica granatum*) has taken great attention for its health benefits in the last years. In the past decade, numerous studies on the antioxidant activity have shown that pomegranate juice contains high levels of antioxidants - higher than most other fruit juices and beverages[31,32]. Pomegranate juice is an important source of anthocyanins, hydrolyzable tannins punicalagin and punicalin, ellagic and gallic acids and also contains vitamin C. The antioxidant and free radical scavenging activity of phenolic compounds derived from pomegranates and vitamin C have been reported[33].

Kaur *et al.*[19] demonstrates that the alcoholic extract of pomegranate flowers possess a potent free radical scavenging, antioxidant and hepatoprotective activities. The extract is capable of protecting against oxidative damage to lipids and proteins and also of increasing/maintaining the levels of antioxidant molecules and enzymes *in vivo*. Turk *et al.*[33] investigated effects of pomegranate juice consumption on sperm quality, spermatogenic cell density, antioxidant activity and testosterone level in male rats. A significant decrease in malondialdehyde level and marked increases in glutathione, glutathione peroxidase and catalase activities and vitamin C level were observed in rats treated with different doses of pomegranate juice.

Lipid peroxidation increases in kidney tissues following cisplatin treatment *in vivo*[5,34]. Depletion of cellular glutathione, which may act as a radical scavenger[35], potentiates the cisplatin-induced cytotoxicity[36,37]. Tian *et al.*[38] and Yilmaz *et al.*[39] suggested that under oxidative stress conditions, there may be positive regulation in the glutathione biosynthesis, resulting in the increased level of glutathione contents. Faria *et al.*[40] investigated effect of pomegranate (*Punica ranatum*) juice intake on hepatic oxidative stress. Their results are compatible with a protective effect of pomegranate juice against systemic oxidative stress in mice.

In this study, decreased activities of peroxidase and catalase were found in the testes of rabbits treated with cisplatin. Glutathione peroxidase enzyme catalyses the reduction of hydroperoxides, at the expense of glutathione. Catalase is also a peroxidase and at the same time the most important enzyme involved in H_2O_2 degradation. Along with glutathione peroxidase inhibition, there was also a decrease in catalase activity in cisplatin group. As they degrade the same kind of substrates, glutathione peroxidase and catalase activities are often related. It was observed that cisplatin induced negative effects in antioxidant enzymes including glutathione peroxidase and catalase activities were prevented by pomegranate seed extract compared to the cisplatin alone group. Supplementation of pomegranate seed extract to cisplatin treated rabbits restored the non-enzymatic antioxidants levels in testes. The biologically active antioxidants found in pomegranate sparing the antioxidant activity and reduced the consumption of endogenous antioxidants, which could be responsible for the reduction of oxidative stress during cisplatin toxicity.

In conclusion, the present study demonstrates that pomegranate possess a potent free radical scavenging, antioxidant. The pomegranate is capable of protecting against oxidative damage to lipids and also of increasing/ maintaining the levels of antioxidant molecules and enzymes *in vivo*. The results of the present study also indicate that pomegranate can be used as easily accessible source of natural antioxidants against cisplatin-induced testes oxidant.

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