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Aerobic exercise and unripe grape extract as antioxidant do not protect the kidney against cisplatin induced nephrotoxicity

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ABSTRACT

Background: Nephrotoxicity is the cisplatin therapy side effect due to stress oxidative formation and lack of antioxidants.

Objectives: The role of aerobic exercise and three different doses of unripe grape extract as an antioxidant on cisplatin-induced nephrotoxicity were studied

Materials and Method: Seventy male Wistar rats were randomly divided into groups of control, cisplatin (2.5 mg/kg/d for 7 days), aerobic exercise + cisplatin and unripe grape extract and also aerobic exercise + unripe grape extract. Aerobic exercise was administered in period of 7 weeks (5 days/week, 1 h/d), and unripe grape extract was used in three different doses of 20, 100, 200 mg/kg/d for period of 7 weeks.

Results: Cisplatin elevated the serum levels of blood urea nitrogen (BUN), creatinine and malondialdehyde (MDA), and also kidney weight and kidney tissue damage score and decreased body weight and the kidney tissue levels of nitrite significantly ($P<0.05$), but aerobic exercise or unripe grape extract did not alter the parameters toward normal levels.

Conclusions: Based on these findings, aerobic exercise and antioxidant such as unripe grape extract may not protect the kidney against cisplatin-induced nephrotoxicity during anti-cancer therapy.

Original Article

Implication for health policy/practice/research/medical education:
To avoid using aerobatic exercise and unripe grape as antioxidants to expect the prevention of cisplain induced nephrotoxicity.

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1. Background

Chemotherapy is recommended for most of cancer to limit the mortality rate. Cisplatin is a drug which is used for chemotherapy (1). However it is accompanied by side effects of ototoxicity, gastrointestinal disturbance, bone marrow suppression, allergic reactions, or nephrotoxicity (2,3). Cisplatin increases serum level of creatinine and blood urea nitrogen (BUN) and decreases serum level of magnesium (4-6). It also causes inflammation, apoptosis and cell death (2). To reduce the side effect of cisplatin during chemotherapy, administration of antioxidants is suggested. Antioxidant agents such as l-arginine (7) and vitamin E (8) decrease cisplatin-induced nephrotoxicity. As a medical plant agent, pomegranate extract reduces cisplatin-induced nephrotoxicity in animal models (9). Unripe grape juice has a protectieve role on lipid profile (10), and its juice has a sour taste and texture similar to grape and is rich in polyphenol compound

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as an antioxidant mixture (11). Aerobic exercise is also considered as an antioxidant, and plays an important role in lifestyle. Aerobic exercise improves muscle strength and function of the heart in cancer patients who were subjected to chemotherapy (12). Aerobic exercise also increases superoxide dismutase (SOD) activity (13) while it did not protect the kidney against cisplatin induced nephrotoxicity in female animal (14).

2. Objectives

In this study the effects of aerobic exercise and administration of unripe grape extract on cisplatin induced nephrotoxicity were investigated.

3. Material and Methods

Seventy adult male (203 ± 3 g) Wistar rats (Animal Centre, Isfahan University of Medical Sciences, Isfahan, Iran) were used.

3.1. Aerobic exercise protocol

The animals were placed on the rodent's treadmill for a week of compatibility, and the animals forced to run at the speed of 16 m/min/d for 15 minutes. The aerobic exercise protocol was designed for seven weeks (5 days a week, 1 h/d). In first two weeks, the treadmill velocity was adjusted to 20 m/min, and it was increased to 23 m/min during weeks 3 and 4, 25 m/min during week 6, and 28 m/min during last week (14).

3.2. Study design

The rats were divided into nine groups as follows:

Group 1 (named control); the rats placed on a treadmill without running and they received vehicle (Tween 80, Sigma, Missouri USA) by orally gavage during the study, and also received saline during the last week.

Group 2 (named cisplatin); the rat received similar regimen as group 1 except cisplatin (2.5 mg/kg/d, IP; intraperitoneal) during the last week instead of saline.

Group 3 (named aerobic exercise + cisplatin): the animals run on a treadmill for period of 7 weeks (5 days/week, 1 h/d) and received cisplatin (2.5 mg/kg/d, IP) during the last week.

Groups 4, 5 and 6 (named U20, U100, and U200); the animals received unripe grape extract orally gavage (20, 100, 200 mg/kg/d, for period of 7 weeks), respectively, and received cisplatin (2.5 mg/kg/d, IP) during the last week. unripe grape extract was prepared as described before (15). Briefly, fresh unripe grapes (*Vitis vinifera* L. CV Shiraz) were dried, powdered and then extracted based on percolation method using 70% ethanol. The extract was subjected to concentrate and dry powder was obtained. Finally, based on Folin-Ciocalteu method, total polyphenolic compounds were evaluated.

Groups 7, 8 and 9 (named aerobic exercise +U20, aerobic exercise +U100, aerobic exercise + U200); the rats run on a treadmill for period of 7 weeks (5 days/week, 1 h/d) accompanied with orally gavage of unripe grape extract (20, 100, 200 mg/kg/d) and received cisplatin (2.5 mg/kg, i.p, daily) during the last week.

3.3. Measurements

At the end, the rats were anesthetized with chloral hydrate (450 mg/kg; IP). Blood samples were obtained by heart puncture. The kidneys were excised and weighed immediately. The left kidney fixed in 10% formalin solution for the staining (H&E) and pathology investigation. The injury severity named kidney tissue damage score (KTDS), and it was graded by a pathologist (who was blinded to the study) from 1 to 4 while zero score was assigned to normal tissue. The right kidney was homogenized and centrifuged. The serum levels of creatinine and BUN were determined using quantitative diagnostic (Pars Azmoon, Iran) by Technicon, RA1000 Model. The serum and kidney levels of nitrite (stable NO metabolite) were measured using a colorimetric assay that involves the Griess reaction. The serum and renal levels of malondialdehyde (MDA) were measured manually as described before (14).

3.4. Ethical issues

This project was approved by Ethics Committee of Isfahan University of Medical. Prior to the experiment, the protocols were confirmed to be in accordance with the guidelines of Animal Ethics Committee of Isfahan University of Medical Sciences (#ir.mui.rec.1394.2.241)

3.5. Statistical analysis

Data were expressed as mean ± SEM. One-way ANOVA followed by Fisher's least significant difference (LSD) was applied to compare the BUN, creatinine, nitrite, and MDA plasma levels, and kidney weight (KW) and body weight (BW) between the groups. To compare the KTDS between the groups, Kruskal-Wallis and Mann-Whitney U tests were applied. Additionally, $P \leq 0.05$ was considered statistically significant.

4. Results

Cisplatin administration increased the serum concentrations of BUN and creatinine, and KTDS and KW and decreased the body weight significantly when compared with control group ($P<0.05$) (Figure 1). However, aerobic exercise, different doses of unripe grape

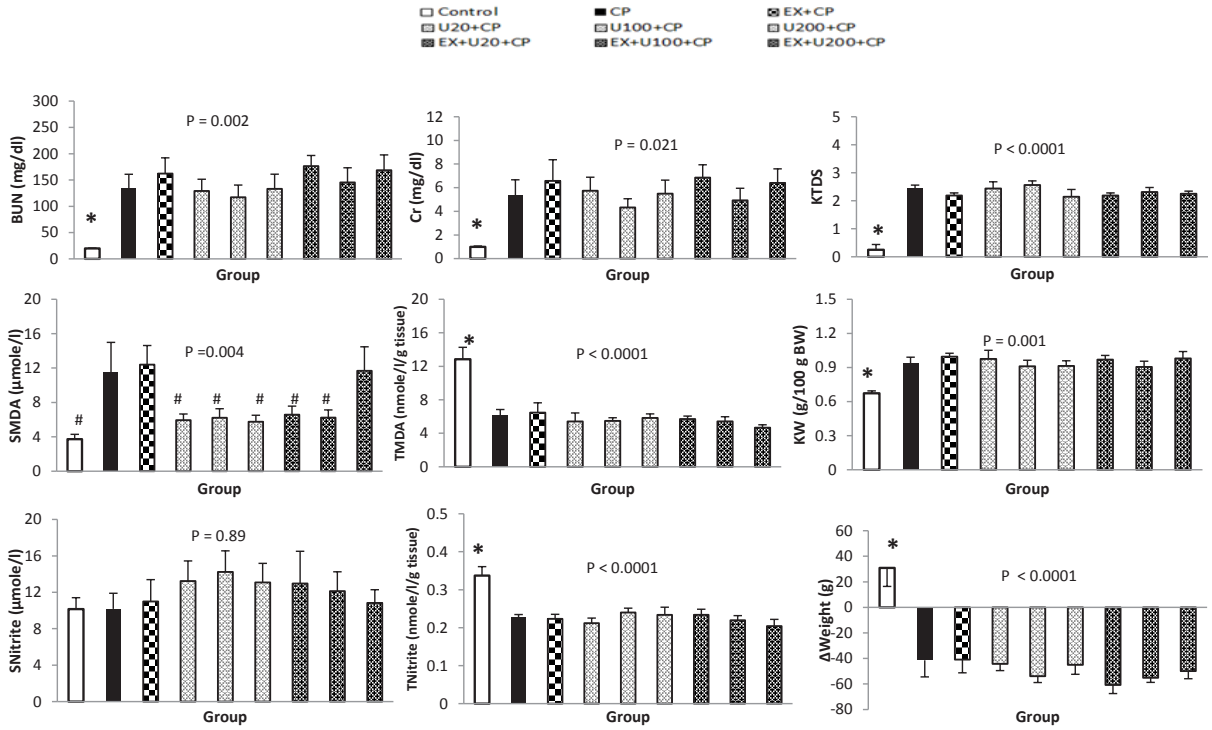

Figure 1. The serum levels of BUN, Cr, MDA and nitrite, and KTDS, KW, kidney tissue levels of nitrite and MDA, and body weight changes in 9 experimental groups. CP: cisplatin; EX: aerobic exercise; U20, U100,U200; unripe grape extract 20, 100, 200 mg/kg. * indicates significant differences from other groups ($P<0.05$), and # stands for significant differences from groups CP, EX+CP or EX+U200+CP ($P<0.05$). n= 6 in control group and n=8 in each others groups. See the text for group information.

extract alone or unripe grape extract combined with EX did not alter these parameters toward normal level (Figure 1). Cisplatin alone or combined with aerobic exercise, also increased the serum concentration of MDA significantly ($P<0.05$), however, the increased serum MDA concentration was reduced toward normal level by unripe grape extract alone. The kidney tissue levels of MDA and nitrite in the control group were significantly greater than other groups ($P<0.05$). No significant differences were observed in serum nitrite levels between the groups. Based on tubule dilatation, debris or inflammation, the histopathology data demonstrated the higher injury severity in all cisplatin treated groups compared to the control group ($P<0.05$). The sample tissue staining images are shown in Figure 2.

5. Discussion

Anti-cancer drugs usually accompanied with stress oxidative formation and serious side effects that need to be controlled with special cares. Physical movement and some herbal drugs as antioxidant agents against stress oxidative formation are candidate for such purpose. Cisplatin is a famous anti-cancer drug which is accompanied with nephrotoxicity. The aim of this study was to investigate the effect of aerobic exercise and unripe grape extract on cisplatin-induced nephrotoxicity. Our results showed that administration of cisplatin increased the BUN, creatinine and MDA concentration in serum as well as KTDS and KW as expected (7,16). However, aerobic exercise and unripe grape extract either alone or combined did not attenuate cisplatin-induced nephrotoxicity. The effects of eight weeks aerobic exercise on cisplatin-induced nephrotoxicity has been reported (17,18). Eight weeks of aerobic exercise did not attenuate vanadyl sulphate induced nephrotoxicity (18). However, it may have a protective effect against cisplatin-induced nephrotoxicity (18). It is reported that aerobic exercise elevates glutathione erythrocyte peroxidase enzyme (GSH) activity and SOD activity (13). In addition, aerobic exercise reduces fatigue, anxiety, and depression (19). In agreement with this study, it is revealed that aerobic exercise training could not affect kidney function against chronic renal failure (20), and aerobic exercise exacerbated the overall damage in animals with chronic nitric oxide synthase (NOS) inhibition (21). Sport also has a negative effect on the immune complex mediated glomerulonephritis (22).

Previous studies have reported that administration of unripe grape extract had a protective effect on blood pressure (15,23), and polyphenolic compounds in

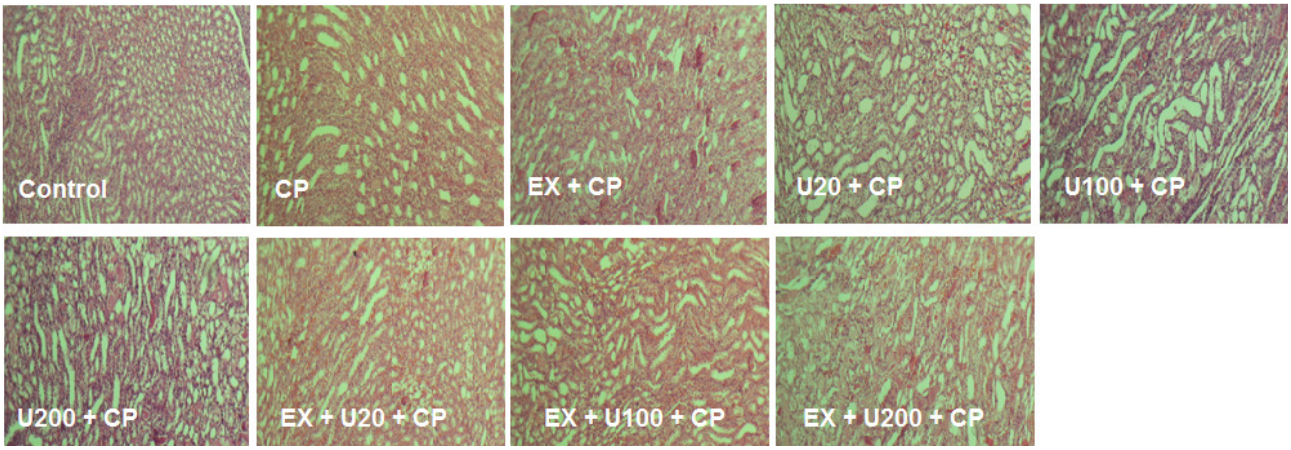

Figure 2. The sample images (×100) from all the experimental groups. The staining was performed by H&E method. The group name is shown on each picture (see the text for group information). CP: cisplatin; EX: aerobic exercise; U20, U100, U200; unripe grape extract 20, 100, 200 mg/kg.

unripe grape extract increased its antioxidants effect. It is also reported that exercise activity changes antioxidant content (24,25) and decreased MDA level (26).

6. Conclusions

It is concluded that aerobic exercise or unripe grape extract as antioxidants are expected to protect the kidney against cisplatin-induced stress oxidative and nephrotoxicity. However this expectation was failed in this study. It seems that special attention should be made to choose the right aerobic exercise protocol and antioxidant agents to obtain protective effect against, cisplatin side effect of nephrotoxicity.

Authors' contribution

SK and BM conducted the experimental procedures and prepared the first draft of the manuscript. SK helped in study design and supervised. BZ helped in study design and in preparation of herbal extract. AT analyzed the pathology data, and MN designed, supervised and analyzed the data research and completed the manuscript. All authors read and signed the paper.

Conflicts of interest

The authors declare no competing interests.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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