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Antioxidant Effects of Hydro-Alcoholic Extract of Pomegranate (*Punica granatum* L.) on Some of Tissues

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Pomegranate (*Punica granatum* L.) is rich in polyphenolic antioxidant, which includes tannins and anthocynins and flavonoids. Researchers reported that pomegranate exhibits antivirus, anti-proliferative, anti-cancer, antidiarrheal and anti-diabetic activities. The aim of this study was to investigate the antioxidant properties of pomegranate on kidney, liver and testis tissues of male rats. Eighteen rats were divided into three groups; Group 1, received distilled water; animals in the Groups 2 and 3 received hydro-alcoholic extract of pomegranate in doses of 250 and 500 mg kg^{-1} respectively. The extract was administrated for 8 weeks by gavage, then oxidative stress and antioxidant enzymes were studied in kidney, liver and testis tissues of all groups. The hydro-alcoholic extract of pomegranate significantly decreased ($p<0.05$) malondialdehyde (MDA) level, but increased ($p<0.05$) the superoxide-dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) activity of kidney, liver and testis when compared to control group. In conclusion, hydro-alcoholic extract of pomegranate possesses a powerful antioxidant activity and can increase levels of antioxidant enzymes in some tissues.

Key words: *Punica granatum*, Antioxidant enzymes, Oxidative stress.

Oxidative stress represents an imbalance between the production of free radicals and reactive oxygen species (ROS) and biological systems ability to repair their damaging effects[1]. Reactive oxygen species (ROS) are strongly reactive oxidizing molecules belonging to the class of free radicals. Formation of ROS in different organs is a natural process but excessive in their production promote the oxidation and DNA injury of Cells[2]. There are numerous documents that show oxidative stress has key role in the pathophysiology of the aging process and likely to be involved in the pathogenesis of atherosclerosis, neurodegenerative diseases, cancer and diabetes[3] .Antioxidants, are compounds that scavenge, and suppress the production of ROS and lipid peroxidation. The well known biological antioxidants include; glutathione (GSH), glutathione peroxidase (GPx), catalase (CAT), superoxide-dismutase (SOD) which play a important role in suppressor or scavenger of free radicals. Thus, the use of ROS suppressors may be useful for the prevention of some diseases[4].

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The application of synthetic antioxidants in food is decreasing due to their suspected function as compounds of carcinogenesis, also due to lack of use of synthetic food additives by the consumer. Several studies demonstrated that the use of synthetic antioxidants is limited due to their health risks and toxicity[5]. Thus, replacement of synthetic antioxidants with plant compounds has greatly increased. The pomegranate is one of the major plant sources of antioxidant[6]. Pomegranate (*Punica granatum* L.) was used in ancient medicine of many civilizations particularly in the Middle East. Pomegranate is rich source of polyphenolic antioxidant which includes tannins and anthocynins and flavonoids[7,8]. The amount of soluble polyphenols in pomegranate juice varies in the range of 0.2 to 1.0% which mainly consists of tannins, ellagic tannins, catechins, anthocyanins, gallic and ellagic acids[9]. These antioxidants are more powerful than other antioxidants such as; coenzyme Q 10, Vitamins C and E and α lipoic acid[10]. Also, it has been proven that pomegranate has higher levels of antioxidants than to green tea and red wine[11]. It is extensively reported which pomegranate displays antivirus, anti-proliferative, anti-cancer, antidiarrheal, and anti-diabetic activities[12,13]. The current study aims to evaluate the antioxidant effects of pomegranate on testis, liver and kidney that may makes it one of the most important foods or herbal medicine for the future.

MATRIALS AND METHODS

Plant material

The fresh pomegranate fruits purchased in October 2013 were dried and powdered before extraction

Hydro-alcoholic extract

The powder (500 gr) was extracted with 70% ethanol for 72 hr using macerated method. The mixer was filtered with through a funnel blocked with cotton wool. The solvent of the filtrate was evaporated at ambient temperature. The powder obtained was further extracted with 600 ml of hot water then evaporated to dryness[14].

Experimental animals

Eighteen male Wistar rats (180-200 gr) were prepared from animal house central of Ahvaz Jundishapur University of medical science. Animals were kept in wire bottomed cages in a room under standard condition of illumination with a 12-hours light-dark cycle at 25±1°C. They were provided with water and balanced diet *ad libitum*. The experiments were approved by Ahvaz Jundishapur University Ethical Committee (AJUEC). Rats were divided into three Groups of 6 rats each.

1. Group 1 served as control and received distilled water by oral gavages (1ml) for 8 weeks.
2. Group 2 received oral administration of extract at a dose of 250 mg/kg for 8 weeks.
3. Group 3 rats were given extract orally by gavages at a dose of 500 mg/kg for 8 weeks.

At the end of the experiment, rats were sacrificed under ether anesthesia, the kidney, testis and liver were separated, then a part of these organs were used for further enzymatic analysis, whereas the other part was stored in 10% formalin for histopathological examination.

Homogenate preparation

The organs washed in normal saline and homogenates 10% prepared in 5% (w/v) of potassium chloride by using a homogenizer (Heidolph Silentcrosher M, Germany). The homogenates were centrifuged in 8000×g for 10 minutes at 4°C and supernatants were used for measurement of oxidative stress by determination of lipid peroxidation as well as antioxidant enzymes (AOEs) such as SOD, GPx and CAT separately in each organs.

Lipid peroxidation

Tissue levels of lipid peroxidation were determined as TBARS (thiobarbituric acid reactive substance) calculated as MDA (15)**.** The absorbance was measured at 532. The concentrations were expressed as nm MDA/mg protein.

SOD activity

The SOD activity was measured according to the method of Suttle[16] by Ransod kit (Randox, lab. Crumlin, UK). This method is based on the formation of red formazan from the reaction of 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) and superoxide radical. The absorbance was measured at 505 nm. The inhibition of the produced chromogen is proportional to the activity of the SOD present in the sample. A 50% inhibition is defined as 1 unit of SOD, and specific activity is expressed as U SOD/mg protein.

GPx activity

GPx activity was estimated by using the Ransel kit (Randox, lab. Crumlin, UK) based on Paglia and Valentine method (17). GPx catalyses the oxidation of Glutathione (GSH) by Cumene Hydroperoxide. In the presence of Glutathione Reductase (GR) and NADPH the oxidized Glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP+. The decrease in absorbance was measured at 340 nm. Specific activity is expressed as U/mg protein.

CAT activity

CAT activities were assayed by Claiborne method [18]. H2O2 (19 mM) was prepared in 50 mM potassium phosphate (pH 7.0) added to 50 ml sample. The reaction was initiated by the addition of H_2O_2, and absorbance changes were measured at 240 nm (25°C) for 30 s. The molar extinction coefficient for H2O2 is 43.6 M^{-1} cm^{-1} . One unit is deûned as one µmol of hydrogen peroxide consumed per minute, and the speciûc activity is reported as U/mg protein. Protein was measured by the method of Bradford.

Histopathological Examination

The fixed specimens were then trimmed, washed, and dehydrated in ascending grades of alcohol, then cleared in xylene, embedded in paraffin, sectioned at 5ìm and stained with Hematoxylin and Eosin (H&E) and examined microscopically.

Statistical analysis

All data are expressed as the mean ± SE. Statistical significance of differences was assessed with one-way ANOVA by SPSS for Windows (version 15) followed by Tukey´s-test. P<0.05 was assumed as statistically significant

RESULTS

Body weight

Data in Figure I demonstrate that none of pomegranate extract doses had statistically significant (p > 0.05) effect on body weights of the rats when compared to the control group.

Lipid peroxidation

Figure 2 shows the level of MDA in various groups. The levels of MDA were significantly (p<0.05) decreased in kidney, liver and testis tissue of rats that received pomegranate extract when compared to control rats. However, there was no difference between the groups that received pomegranate extract (p > 0.05).

Antioxidant enzymes

The SOD, GPx and CAT activity levels in pomegranate extract (250 mg kg^{-1} and 500 mg kg^{-1}) were significantly (P<0.05) more than control rats in all of organs studied. (Figure 2, 3 and 4). But we did not detect differences between the groups receiving the extract.

Histopathological Examination

Microscopic evaluations showed that the kidney and liver tissues in different groups were

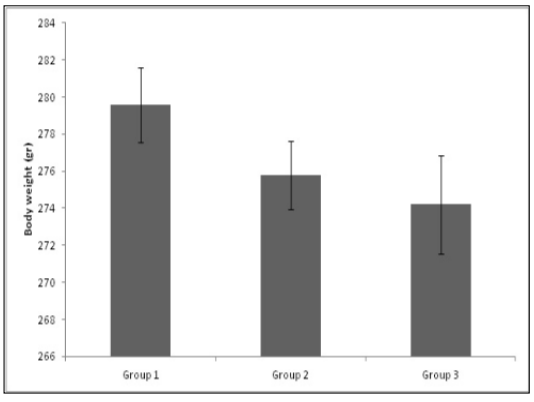

Fig. 1. Body weight changes between groups, group 1 (control), group 2 (250 mg kg^{-1} pomegranate extract), group 3 (500 mg kg pomegranate extract). Data are expressed as the mean ± SE. There was not different between groups.

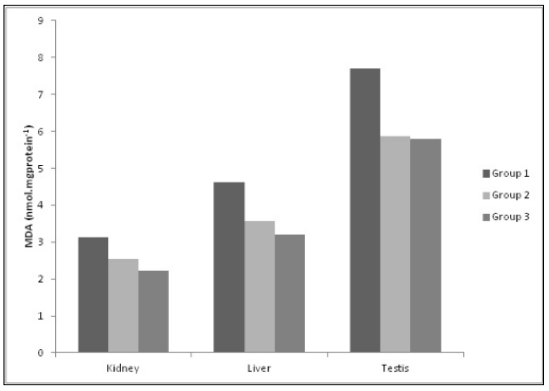

Fig. 2. Changes of lipid peroxidation level in different organs and groups. Data are expressed as the mean ± SE. * P<0.05 as compared with control, ** P<0.05 as compared with control.

natural and we did not observe any changes in these tissues between different groups but testes showed an increase in thickness of germinal epithelium as well as decrease in lumen diameter of seminiferous tubules in groups that received pomegranate extract when compared to control group (Figure 6).

DISSCUSSION

Pomegranate is an rich source of punicalin, tannins punicalagin , anthocyanins, gallic and ellagic acids and also contains vitamin C. The antioxidant and free radical scavenging activity of pomegranate phenolic compounds and vitamin C have been reported[2,19,20]. In this study we didn't find any different in body weight of animals between groups. These results are similar to previous study[2]. Also in this study, it was observed that pomegranate extract decreased significantly levels of MDA in kidney, liver and testis tissues when compared to control. Lipid peroxidation is defined as the oxidative deterioration of polyunsaturated lipids[21]. Beginning of a peroxidation process in a membrane or polyunsaturated fatty acid is because removing a hydrogen atom from the double bond in the fatty acid. The free radical tends to be stable by a molecular rearrangement to formation a conjugated diene, that typically react rapidly with oxygen to yield a peroxy radical. Peroxy radicals can remove a hydrogen atom from another lipid molecule to give a lipid hydroperoxide, R-OOH. May be locator fate of peroxy radicals are to transformed into cyclic peroxides or cyclic endoperoxides that break into aldehydes such as MDA and polymerization

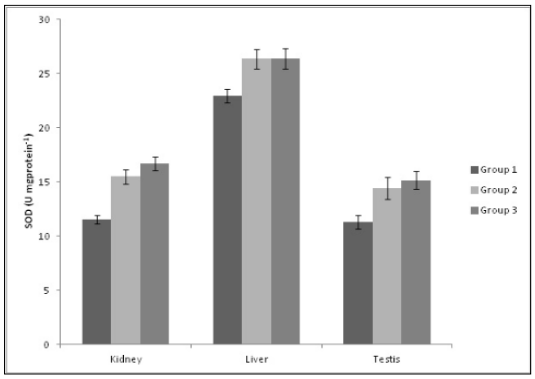

Fig. 3. Changes of SOD activity in different organs and groups. Data are expressed as the mean ± SE. * P<0.05 as compared with control, ** P<0.05 as compared with control

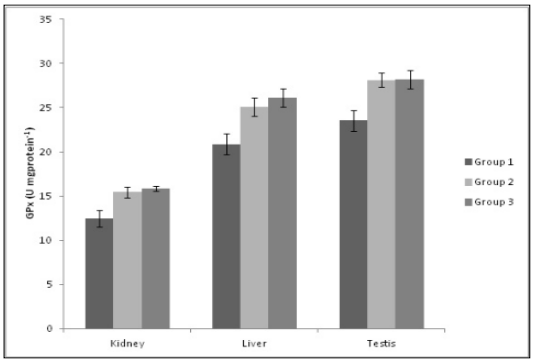

Fig. 4. Changes of GPx activity in different organs and groups. Data are expressed as the mean ± SE. * P<0.05 as compared with control, ** P<0.05 as compared with control

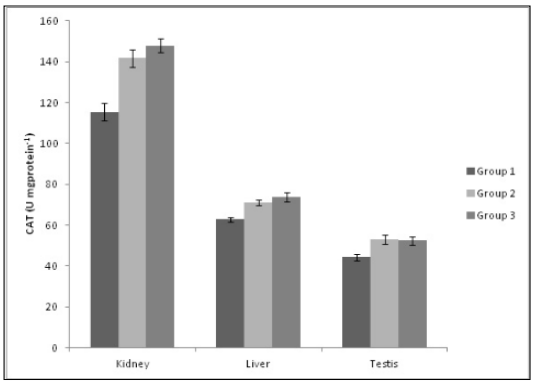

Fig. 5. Changes of CAT activity in different organs and groups. Data are expressed as the mean ± SE. * P<0.05 as compared with control, ** P<0.05 as compared with control

products[22]. The function of the pomegranate extract to suppress hydroxyl radicals is probably related to the inhibition of progress of the sequence of lipid peroxidation and due to oxygen species scavenging activity of pomegranate, thus reducing the rate of chain reaction[23]. Our observations are in agreement with previous study[24]. The protective antioxidants include the metal chelating proteins and the endogenous antioxidant enzymes include; CAT, GPx and SOD. The condensations and sites of these antioxidants are highly regulated because their major operation is to arrest the production of free radicals to prevent cellular injure or elevate cell survival[25]. These three enzymes are also the first class of defenses for the body against oxidant-induced cytotoxic challenges[24]. CAT

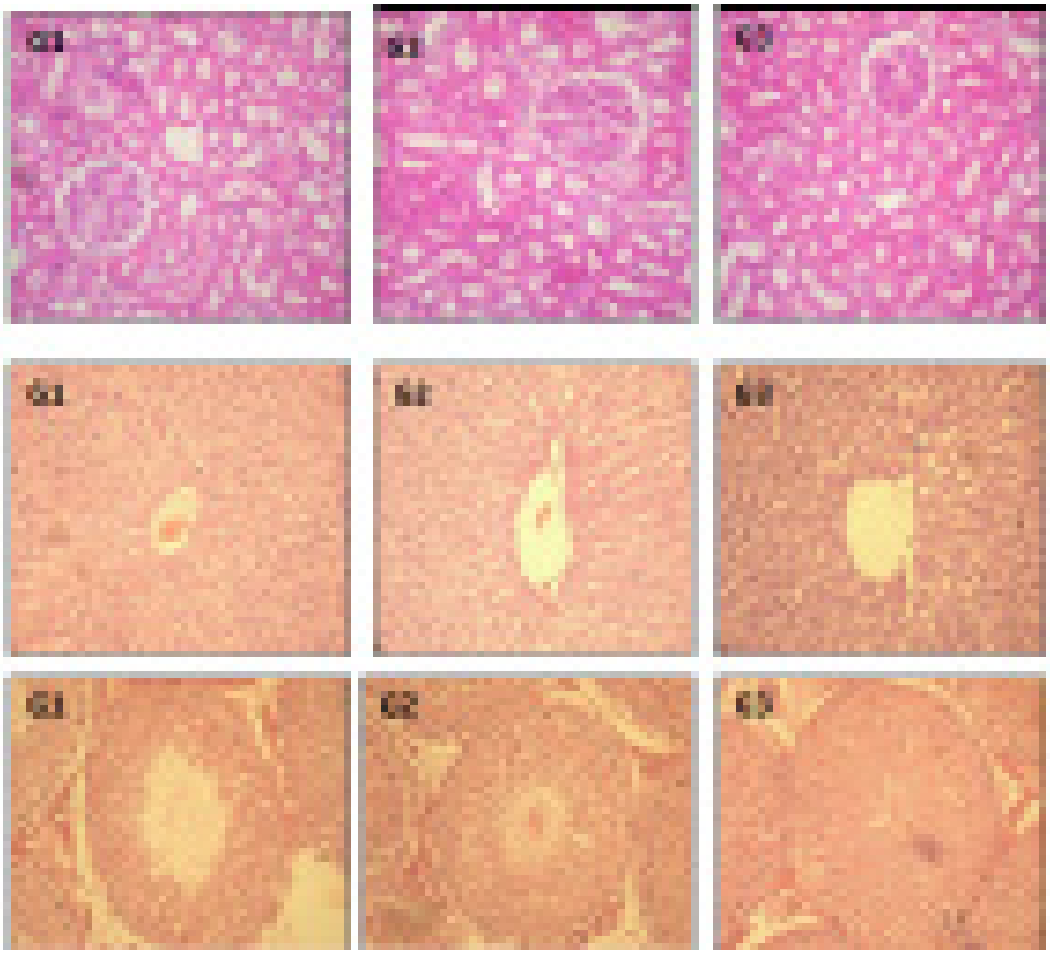

Fig. 6. Microscopic images of kidney, liver and testis in different groups. G1 (received distilled water), G2 (received 250 mg.kg^{-1} extract), G3 (received 500 mg.kg^{-1} extract), (H&E ×300)

operates to detoxify hydrogen peroxide (H2O2) by converting it to oxygen and water. Decreased CAT is accompanied with an enhanced risk of chronic diseases that induced by oxidative stress include: neurodegenerative diseases, diabetes, atherosclerosis, and postmenopausal osteoporosis[26]. GPx is found in the plasma and the cytosolic or membrane portion of tissues that catalyzes the conversion of H2O2 to water and oxygen, by reduced glutathione (GSH) as a substrate that is oxidized to glutathione disulfide (GSSG) in the procedure[27]. SOD catalyzes the dismutation of $O2^{o-}$ to oxygen and H2O2 that is subsequently converted to water and oxygen by CAT or GPx. SOD centralized in the intracellular area of tissues and is filtered by the kidney before being reabsorbed and catabolized in the proximal tubules[27]. Free radicals which were not properly restrained by protective antioxidants they start peroxidative chain reactions that must be stopped by the chain-breaking, radical-scavenging antioxidants[25]. Our study also indicated a significant increase of SOD, GPx and CAT in kidney, liver and testis of groups that received pomegranate extract. Antioxidant activity in different parts of the pomegranate has been examined in many studies[28]. All these activities probably are related to the various phenolic compounds present in pomegranate, such as punicalagin isomers and anthocyanins. These compositions are well-known for their properties to scavenge free radicals and to suppress lipid oxidation[29]. It also reported that Pomegranate polyphenolic molecules undergo redox reactions

because phenolic hydroxyl groups easily present hydrogen for reducing agents[30]. Our findings are similar to previous study[31]. Histopathological results didn't show structural changes in kidney and liver between groups but we observed an increase in germinal epithelium in seminiferous tubules in groups that received pomegranate extract. It may be attributed to inhibition of extreme production of free radicals, produced by spermatozoa themselves, by means of their antioxidant activity of pomegranate. These results are agreement with previous studies[20, 32]. It could be concluded that pomegranate extract is more effective in improving antioxidant enzymes level and reducing oxidative stress in some of organs and this is due to antioxidant properties of pomegranate.

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