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RESEARCH ARTICLE

The investigation of neuroprotective effects of pomegranate juice against low level lead induced oxidative stress in rats brain

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Düşük seviyede kurşunun ratların beyin dokusunda oluşturduğu oksidatif strese karşı nar suyunun nöroprotektif etkilerinin araştırılması

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Öz

Amaç: Bu çalışmada, düşük düzeyde kurşun (Pb) maruziyetinin sıçanlarda beyin dokusu ve antioksidan sistem üzerinde oluşturduğu hasara karşı nar suyunun (NS) nöroprotektif potansiyelinin araştırılması amaçlandı.

Gereç ve Yöntem: Araştırmada kullanılan toplam 40 adet rat, her birinde 10'ar rat bulunan 4 gruba ayrıldı. Kontrol grubu (1), standart rat yemi ve içme suyuyla beslendi. Pozitif kontrol grubuna içme suyuyla günlük 2000 ppm kurşun (kurşun asetat); düşük tedavi grubuna (3), içme suyuyla günlük 2000 ppm kurşun ve gavajla 30 µL NS; yüksek tedavi grubuna (4) ise içme suyuyla günlük 2000 ppm kurşun ve gavajla 60 µL NS verildi. Araştırma 5 hafta sürdürüldü. Sıçan beyinlerinde malondialdehide (MDA) ve glutatyon (GSH) seviyeleri ile süperoksit dismutaz (SOD) ve katalaz (CAT) aktiviteleri belirlendi. Ayrıca beyin dokusunda histopatolojik incelemeler yapıldı.

Bulgular: Nar suyu kurşunun sebep olduğu lipid peroksidasyonu azaltarak (düşük MDA düzeyi) antioksidan enzim (SOD ve CAT) aktivitelerini ve GSH düzeyini artırarak oksidatif stresi hafifletti. Sadece kurşun alan grupta şiddetli nörodejeneratif değişiklikler görüldü. Nar suyunun, kurşunun beyin dokusunda oluşturduğu hücresel hasarı kısmen önlediği belirlendi.

Öneri: Düzenli nar suyu tüketimi artan yoğun sanayileşmeden kaynaklanan kronik kurşun maruziyetine karşı faydalı olabilir.

Anahtar kelimeler: Kurşun, nar suyu, fenolik bileşenler, oksidatif stres, antioksidan

Abstract

Aim: The aim of the study was to investigate the neuroprotective potential of pomegranate juice (PJ) against the damage of brain tissue and antioxidant system induced low level lead (Pb) exposure in rats.

Materials and Methods: A total of 40 rats were divided into four groups containing 10 rats in each. The control group (1) was fed standart rat feed and daily water. A positive control group (2) received a daily dose of 2000 ppm lead (lead acetate) in drinking water; a low treatment group (3) that received a daily dose of 2000 ppm lead together with 30 µL PJ by oral gavage; and a high treatment group (4) that received 2000 ppm lead and 60 µL PJ by oral gavage daily. The experiment was lasted for 5 weeks. Levels of malondialdehyde (MDA) and glutathione (GSH) were determined as well as the activities of superoxide dismutase (SOD) and catalase (CAT). Morever, histopathological examination was also performed in the brain of the rats.

Results: Pomagranete juice alleviated oxidative stress by decreasing lipid peroxidation (low MDA level) and increasing the activities of antioxidant enzymes (SOD and CAT) and GSH level in the rats exposed to lead. Severe neurodejenerative changes were observed in only groups received lead. Cellular damage of the brain was partially prevented by PJ.

Conclusion: Regular consumption of pomegranate juice may provide significant benefits against the threat of chronic heavy metal exposure due to increasing intensive industrialization.

Keywords: Lead, pomegranate juice, phenolic compounds, oxidative stress, antioxidant.

Introduction

Lead (Pb) is one of the most important environmental pollutants and low-dose exposure to lead in daily life is an important public health due to the increasing industrialization. Red blood cells, liver kidneys and brain have been considered as the main target organs of lead exposure (Flora et al 2004, Arslan et al 2011, Aksu et al 2012, Radad et al 2014). Lead has direct neurotoxic effects including apoptosis, excitotoxicity, neu-rotransmitter alterations and damage to neuronal cells in the brain (Lidsky and Schneider 2003). It was reported that long time low dose of lead exposure is considered as a preparative cause for neurodegenerative diseases like, Alzheimer's and Parkinson's diseases (Coon et al 2006, Bakulski et al 2012). One possible molecular mechanism of the Pb neurotoxicity is the imbalance of the prooxidant/antioxidant ratio and generation of reactive oxygen species (ROS) (Adonaylo and Oteiza 1999) which can cause to brain damage via oxidative activity to critical biomolecules such as lipids, proteins and DNA. Naturally antioxidants such as herbal antioxidants, essential oil derived from plants, vitamin-C and E, selenium have been reported to prevent and treat lead-induced toxicity (Reckziegel et al 2011, Aksu et al 2012).

Pomegranate juice (PJ) contains relevant amounts of phenolic compounds and the their major components are; organic acids (gallic acid, caffeic acid, ellagic acid etc), flavanoids (anthocyanins) and tannins (punicalagin and punicalin) (Espin et al 2007). Phenolic compounds are natural substances found in plants, fruits and vegetables. Polyphenols show their antioxidant effects through various actions like inducing expression of protective genes aganist oxidative stress, regulation of reactive oxygen species and scavenging metal ions (Kelsey et al 2010). Among the different compounds that could serve as unequivical makers in a fruit juice products, organic acids and total phenolic compounds are potentially the most useful because of their ubiquity, specifity and multiplicity (Poyrazoglu et al 2002). The molecular interactions of phenolic compounds with biological systems remain mostly speculative. The free-radical scavenging capability of polyphenols has been primarily tested with in vitro studies. However, phenolic compounds are structurally altered in vivo. The current study was designed to investigate the possible neuroprotective potential of PJ against low level Pb exposure on the brain damage and antioxidant system in rats.

Materials and Methods

Fresh pomegranate fruit (Punica granatum) was purchased from a local retailer (Antakya, Hatay, Turkey). Total phenolic content in pomegranate juice extract was determined as described by Ough and Amerine (1988) and measured at 765 nm using a spectrophotometer.

Totally, forty adult male Sprague Dawley rats, each weighing about 300 g were randomly assigned to treatment groups (4 treatments and ten rats for each treatment). Treatments groups were designed as a control group that reared with normal food and water; a positive control group that applied daily dose of 2.000 ppm lead (lead acetate) with drinking water; a low treatment group that applied a daily dose of 2.000 ppm lead plus 30 µL pomegranate juice (PJ; equivalent to 1.050 µmoL total polyphenols) by oral gavage; and a high treatment group that applied 2.000 ppm lead plus 60 µL PJ (equivalent to 2.100 µmoL total polyphenols) daily by oral gavage. The experiment lasted five weeks. These dosage of PJ was used based on results of previus study (Aviram et al 2000, Kaplan et al 2001). At the end of the study, the rats were sacrificed under ether anesthesia following overnight fasting. The brain tissue was excised and washed in cold ice saline (0.9%) to measure of parameters.

For histopathological examination, tissue sections were taken from tissues samples and fixed 10% neutral buffered formalin, embedded in paraffin wax, sectioned at 5 µm thicks, and stained with haematoxylin and eosin (HE) (Presnell and Schreibman 1997). After, sections were examined under a light microscope. The study protocol was approved by the Ethical Committee of the Mustafa Kemal University (B.30.2.M.K.U.0.00/05).

Malondialdehyde (MDA) levels in brain homogenate was determined using the method described by Yoshoiko et al (1979). The optical density was measured at 535 nm by spectrophotometer. Tissue glutathione (GSH) concentrations were determined using the method described by Sedlak and Lindsay (1967). These procedure is based on the method of Ellman. All of the nonprotein sulfhydryl groups of cells are in the form of reduced GSH. Catalase (CAT) activity in tissues homogenate was assayed by the decrease in absorbance of hydrogen peroxide at 240 nm as per the method of Aebi (1984). Superoxide dismutase (SOD) activity in tissues homogenate was assayed spectrophotometrically as described by Sun et al (1988). Brain protein levels were determined by Bradford reagent.

Data were performed by using one-way ANOVA (the general linear models procedure of SAS). Differences between means were determined by Duncan's multiple range test at a significance level of $P<0.05$.

Results

Total phenolic content of pomegranate juice was estimated as 6645 mg/L. MDA formation increased in brain tissues of the rats exposed the lead ($P<0.001$) while PJ administration significantly decresed the level of MDA (Table 1). When the group received to lead alone compared with the control, GSH level significantly decreased ($P<0.001$) while SOD and CAT activities significantly increased ($P<0.001$). GSH levels

in both of the groups received PJ were significantly lower (P<0.001) than the control. GSH level tend to increase in the group that received at 30 µL PJ whilst this level significantly increased in the group that received at 60 µL PJ (P<0.01). When examined CAT and SOD activities of the groups received both levels of pomegranate juice, CAT and SOD activities was lower than the group received to lead alone, whilst it was higher than the control's level (P<0.001). SOD activities in the brain tissue of the group received to 60 µL PJ were close to that of the control group (P<0.001).

Histopathological exemination of the brain tissue of the experimental groups are shown Figure 1. The tissues of control groups showed a normal histological structure (Figure 1-A). The finding results from light microscope examined preparations of brain of rats showed some differences between experimental groups (Figure 1-B, C and D).

Discussion

Lead is known as environmental and industrial pollutants which induces physiological, biochemical and behavioral dysfunction. Well documents are available about this pollutant causes neurological deterioration like is brain injury, mental breakdown and many behavioural problems (Radad 2014). Numerous studies showed that phenolic compounds could decrease tissues loading of heavy metal (Xia et al 2010, Liu et al 2011, Aksu et al 2012, Radad et al 2014). In this study, the neuroprotective potential of polyphenolics enriched PJ on the brain damage and antioxidant system were investigated in rats against low level Pb exposure. Although the exact mechanism of Pb toxicity is still not very clear, previoulsy studies showed that Pb exposure stimulates production of ROS and thus reduces the cellular antioxidant capacity (Adonaylo and Oteiza 1999). Lead enhances lipid peroxidation by inhibiting of SOD and other related enzymes (Villeda-Hernandez et al 2001). One of the main reasons to cause damage in membranes, DNA, or proteins and subsequent destroy of tissues or systems is an imbalance of prooxidant/antioxidant ratio in tissue and cellular components (Hsu and Guo 2002). Therefore, regular comsumption of antioxidant rich ingredients like pomegranate juice would have an advantageous role on the cell's antioxidant defences to counteract heavy-metal intoxication.

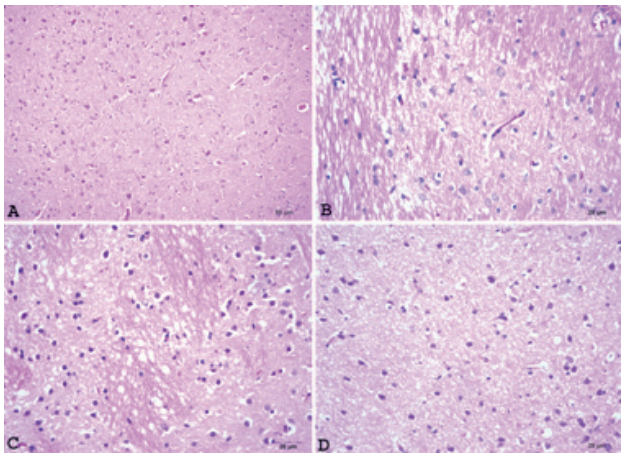

Figure 1. Photomicrographs of brain tissue from rats that control (A), lead treated (B), lead plus 30 µL PJ (C), and lead plus 60 µL PJ (D). A. Normal histological appearance in the brain of control group Bar: 50 nm. B. The presence of hyperemia in brain vessels, perivascular cell infiltration, edema, vacuol formation and satellitosis in the brain of the group received to lead alone. Bar: 20 nm. C. The presence of neuronal degeneration, necrosis, vacuolation and satellite cell in the brain of the groups received to 30 µL PJ together with lead. Bar: 20 nm. D. The presence of edema, vacuolation, neuronal degeneration and the glial cells in the brain of the groups received to 60 µL PJ together with lead. Bar: 20 nm

In this study, as expected, the lead exposure enhanced the generation of ROS and lipid peroxidation and caused cell damage. Lipid peroxidation is a well-known mechanism of oxidative damage caused by ROS and it has been used a potential marker of oxidative stress. In this study the increases in MDA level, a lipid peroxidation marker, in the brain of the lead-exposed rats were accompanied by alterations in the animals' antioxidant defence systems including decreased GSH levels in brian tissues examined and increased SOD and CAT activity (Table 1). Lead-acetate not only induces the oxidative stress but also stimulates antioxidant enzymes activity as a defence mechanism. Lead causes an elongation in fatty acids by increasing the number of double bonds, thereby increasing lipid peroxidation in cell membranes. Additionally, the affinity of lead for sulfhydryl groups (-SH) adversely affects the integrity of cell membranes. When peroxidation occured in the phospholipid structure of cells, membrane integrity is disrupted and ultimately to cell death (Sharma et al 2010). GSH is the most important non-enzymatic antioxidant and contains reactive sulfhydryl groups (-SH) which protects the cells membrane against ROS aggression. However, GSH is rapidly oxidized by oxidants (Sanfeliu et al 2001). An decreasing of intracellular GSH refers to increased cytotoxicity of lead in endotelial cells of the organs. In addition to high MDA and low GSH levels observed in the tissues of the lead alone received groups, some histopathological findings was determined in tissue sections such as brain edema, neuronal degeneration and encephalomalacy, and in some cases vacuol formatin and satellitosis, which were supported this information. Antioxidant enzymes such as SOD and CAT provides vital contrubitions to the cellular defence mechanism against oxidative damage (Gurer and Ercal 2000). In this study, the increase in SOD and CAT activities in brain tissues of the rats exposed to lead may be a compensation mechanism to counteract the decreasing level of GSH. Studies have suggested that lead-induced oxidative stress and auto-oxidation of excessively accumulated aminolevulinic acid dehydratase seemed to result in the formation of superoxide and hydrogen peroxide (Sharma et al 2010, Dixit et al 2012). SOD and CAT enzyme activities may have increased due to their protective effects against the generation of highly reactive species such as hydroxyl radicals (OH•), hydrogen peroxide (H_2O_2) and superoxide anions (O_2•). The defence activity of cellular SOD

Table 1. Brain lipid peroxide levels and antioxidant status in experimental groups.

	Groups					
	Control	Pb	Pb + PJ (30 µL)	Pb + PJ (60 µL)	SEM	P
Brain MDA (µmol/L)	38.23^{c}	92.75^{a}	62.75^{b}	50.91^{bc}	3.894	0.001
GSH (µmoL/mg protein)	5.234^{a}	2.020^{c}	2.505b^{c}	3.034^{b}	0.226	0.001
CAT (U/mg protein)	0.224^{c}	0.749^{a}	0.372^{b}	0.384^{b}	0.033	0.001
SOD (U/mg protein)	2.521^{c}	7.537^{a}	4.301^{b}	3.442^{bc}	0.344	0.001

MDA: Malondialdehyde, GSH: Glutathione, CAT: Catalase, SOD: Superoxide dismutase, a, b, c: Different letters in the same line are statistically significant (P<0.001).

and CAT against the oxidative tissue damage is focused on the breakdown of superoxide radicals to hydrogen peroxide and oxygen. Thus, hydroxyl radical formation is reduced, and hydrogen peroxide is removed (Sharma et al 2010).

In this study, PJ could alleviate oxidative stress by decreasing lipid peroxidation (indicated by low MDA level), healing the activities of antioxidant enzymes (SOD and CAT) and increasing GSH level (Table 1). This contribution could be attributed to the presence of phenolic compounds and flavonoids in PJ. These compounds have ability in scavenging of O_2 and H_2O_2 through a flavonoids and phenolic redox cycle. In this system some radicals such as phenoxy or flavonoxy are formed during the peroxidase process (Yamasaki et al 1997). They snatch electrons from other radicals which avoide the polymers formation (Perez et al 2002). The findings from the current study of oxidant/antioxidant status are not fully supported with the current histopathologic findings. The histological examination of the brain tissues of rats exposude to lead showed severe histopathological changes as compared to normal rats (Figure 1A and B). Application of PJ could not exactly repair the tissue compared to the normal cell structure. Oxidative damage formed by lead in brain tissue were not significantly reduced by polyphenolic compounds even normal cell structures were observed in the group received to 2100 µmoL total phenolic compounds according to microscopic examinations. This situation may be due to the excess easy oxidable components such as polyunsaturated fatty acids or neurotransmitters and the sensitivity of the brain to oxidative stress caused by lower total antioxidant capacity in brain tissue.

Conclusion

The data of this study suggest that PJ and their components are not completely prevent lead caused oxidative damage in rat brain. Polyphenols in PJ restricted the lead-induced lipid peroxidation and enhanced the antioxidant defence system but not restricted fully histopathological changes in brain tissues. It was concluded that the supplementation with antioxidants enriched PJ may be a potential therapy in the prevention of long time low dose Pb intoxication. In particular, in terms of public health, regular consumption of pomegranate juice can be considered to provide significant benefits against the threat of chronic heavy metal exposure due to increasing intensive industrialization.

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