The protective effect of pomegranate juice in paracetamol-induced acute hepatotoxicity in rats

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Abstract

Aim: Being the most commonly used antipyretic and analgesic, paracetamol is one of the most common causes of childhood poisoning in the world and maintains its importance also in our country. Paracetamol poisoning is one of the most common causes of liver failure. This study aimed to investigate if pomegranate juice had protective effect in acute liver toxicity related with paracetamol.

Material and Methods: A total of 36 Wistar-Albino rats were divided into four groups as the paracetamol group (3 000 mg/kg paracetamol), the pomegranate juice + paracetamol group (1.5 mL pomegranate juice plus 3 000 mg/kg paracetamol), the pomegranate juice group (1.5 mL pomegranate juice) and the control group (1.5 mL distilled water). Pomegranate juice and distilled water were administered for eight days. Paracetamol was administered on day 8. The level of thiobarbituric acid reactive substances, as an oxidative marker, was measured in the blood and liver tissue on day 9. In addition, liver tissues were evaluated histologically (in terms of increased connective tissue, granular degeneration, mononuclear cell infiltration, necrotic cells and vascular congestion).

Results: The liver tissue and blood thiobarbituric acid reactive substances levels were found to be significantly lower in the pomegranate juice + paracetamol group compared to the paracetamol group ($p < 0.05$). Histologically, structural changes related with damage were observed in both the paracetamol group and pomegranate juice + paracetamol group. The extent of damage was statistically significantly lower in the pomegranate juice + paracetamol group ($p < 0.001$).

Conclusions: Our results related with oxidative and histologic evaluation showed that pomegranate juice might have a preventive effect in paracetamol-induced acute liver damage. (Türk Pediatri Ars 2016; 51: 72-8)

Keywords: Hepatotoxicity, paracetamol, pomegranate juice

Introduction

Paracetamol (PCT) is one of the leading pharmacological causes of acute hepatic failure. It is the leading cause of drug poisonings in USA and Europe (1-3). However, it is the most commonly used antipyretic and analgesic drug in children. It can be obtained over-the-counter and is an essential member of the medicine cabinets in houses. It is considered safe as long as it is used at the recommended doses (10-15 mg/kg) with the recommended frequency (≤ 5 dose/day) and with a maximum daily total dose of 75 mg/kg.

Following intake of high dose paracetamol the enzyme systems which allow its metabolism by transforming

this substance to inactive compounds by way of conjugation with sulphate and glucuronide are saturated and formation of the toxic intermediate metabolite named 'N-acetyl-p-benzoquinone-imine' (NAPQI) increases. 'N-acetyl-p-benzoquinone-imine' causes rapid depletion of glutathione stores which are found scarcely in the liver, binds irreversibly with the hepatocytes and leads to hepatic necrosis (4). Treatment targets excretion of PCT from the body and restoring glutathione stores. Activated charcoal decreases absorption of PCT, whereas acetylcysteine acts as a precursor molecule for synthesis of glutathione in the liver. If hepatic damage is serious, liver transplantation may be needed. However, these therapies are not sufficiently efficient and there is no ideal treatment for PCT poisoning (5).

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Medicinal herbs which have been used for centuries in some cultures have also a wide coverage in current modern medicine. The effects of various plants on paracetamol toxicity have been investigated in many studies. In experimental liver damage induced by paracetamol, beneficial effects of the plants including Lantadine A, Clitoria ternatea, Taraxacum officinale, Clausena dentata, Phyllanthus acidus and Telfairia occidentalis have been demonstrated in various studies (6-11).

Pomegranate (*Punica granatum*) which grows in the Mediterranean Region and is usually consumed as fresh fruit or beverage has drawn interest for long years because of its beneficial effects. Pomegranate juice and shell have marked antioxidant capacity. Pomegranate juice is composed of water (85%), sugar (10%), pectin, ascorbic acid and polyphenols (1.5%) (12). It is thought that the beneficial effects of pomegranate juice on human health arise from the strong antioxidants in its content (13, 14). Pomegranate juice contains anthocyanins (delphinidin-3-glucosidase, delphinidin-3.5 glucosidase, cyanidin and pelargonidin) and ellagitannins (for example, pomegranate juice contains 2g/L polyphenol) which reduce the effects of free radicals. Punicalagin is the main ellagitannin which is responsible of the antioxidant efficiency of pomegranate. It is rich in flavonoids (for example: quercetin, kaemferol and luteolin glycosides) and polyphenolic acids (for example: elaic and galic acid) (15-17). The extracellular antioxidant efficiency of pomegranate juice has been shown to be three-fold higher compared to red wine and green tea and 2-6-fold stronger compared to other natural beverages (16, 18, 19). In a recent study, it was shown that pomegranate juice increased antioxidant defense mechanisms and had protective effect in the testicular tissue against acute toxicity originating from CCl₄ in rats (20). In another study, Matthaïou et al. (21) showed that consumption of pomegranate juice increased the levels of glutathione and decreased lipid and protein oxidation. Since antioxidant mechanisms and glutathione level have a significant role in hepatic damage related with PCT, these studies suggest that pomegranate juice may have preventive or therapeutic effects in terms of hepatic toxicity.

As far as we know, the effects of pomegranate juice on PCT toxicity have not been investigated yet. In this study, we aimed to investigate if pomegranate juice has a protective effect on PCT-induced hepatotoxicity by measuring the level of tiobarbituric acid reactive substance (TBARS) in serum and hepatic tissue and examining the histopathological changes in the liver.

Material and Methods

Experimental animals: 36 female Wistar-Albino rats which were just weaned with a weight of 150-200 g (4-5 weeks of age) were used in the study.

The rats were kept in the light/dark and at room temperature (22-24°C) for 12 hours. Adequate amounts of drinking water and standard rat food were given.

The study was conducted in the Experimental Research Center, Biochemistry and Histology Laboratories in accordance with the ethics committee rules for animal experiments after obtaining approval from the Experimental Animals Ethics Committee (24.04.2012-06).

Pomegranate juice: The pomegranates were washed and peeled and squeezed by hand each morning.

Organization of the experiment groups and conduction of the experiment: Thirty six animals were randomly assigned to the following four groups:

Paracetamol group (n=10): 1.5 mL/day distilled water was given by gavage for eight days, paracetamol was given orally at a dose of 3 000 mg/kg on the 8th day,

Pomegranate juice + Paracetamol (n=10): 1.5 mL/day pomegranate juice was given by gavage for eight days, paracetamol was given orally at a dose of 3 000 mg/kg on the 8th day.

Pomegranate juice group (n=8): 1.5 mL/day pomegranate juice was given orally for eight days,

Control group (n=8): 1.5 mL/day distilled water was given orally for eight days.

Anesthesia, obtaining tissue and blood samples: On the 9th day, anesthesia was achieved by intraperitoneal ketamine (80 mg/kg) and xylazine (10 mg/kg) before sacrifice and a 4-6 mL blood sample was obtained from the aorta with sterile injector. The blood samples were centrifuged at 5 000 RPM for 5 minutes and their sera were separated. At the end of the study, the abdomen was cut in the midline and the right lobe of the liver was removed for histological examination.

Measurement of lipid peroxidation (TBARS): Lipid peroxidation was studied in serum and hepatic tissue by

way of colorimetric measurement method. Malondialdehyde and tiobarbituric acid (TBA) complex is formed with interaction of MDA and TBA at high temperature and colorimetric measurement is performed in an acidic environment. TBARS concentrations of the samples were calculated by way of standard-absorbance graphic. The results were given as $\mu\text{M}/\text{mL}$ in serum and as $\mu\text{M}/\text{g}$ protein in hepatic tissue.

Histopathological examination: The tissues were fixed in 10% neutral formaldehyde solution and paraffin blocks were prepared. Five-micron-thick sections were obtained from the paraffin blocks prepared. The samples were stained with hematoxylin-eosine for histological evaluation.

The samples which were stained were examined with Olympus BX50 binocular microscope. The hepatic sections were examined by a single histologist who was blind to the study groups and scoring was performed. The hepatic tissues were examined in terms of increase in connective tissue, granular degeneration, mononuclear cell infiltration, necrotic cells and vascular congestion. The severity of each finding was scored as 0 (normal), +1 (mild), 2++ (moderate) +++ 3 (severe) according to the study of Yahya et al. (23). Afterwards, the total scores of the groups were compared.

Statistical analysis

Kruskal–Wallis variance analysis was used to evaluate the differences between the study groups in terms of biochemical data and histopathological scores. A p value of <0.05 was considered significant. If the Kruskal–Wallis test showed a significant difference, Bonferroni-corrected Mann-Whitney U test was used to investigate which group was different from the others. According to the Bonferroni correction ($0.05/6$), a p value of <0.01 was considered significant. The significance level was accepted as a p value of <0.05 . The analysis of the study was performed in computer environment using SPSS 15.0 statistics program (LEAD Technologies Inc.; Chicago, IL, USA).

Table 1. Mean values of the histopathologic scores of the groups

	PST (n=10)	NS+PST (n=10)	NS (n=8)	C (n=8)
Mean value of the histopathologic scores	$11.8 \pm 0.44^*$	$4.90 \pm 0.43^{**}$	1.37 ± 0.32	0.00 ± 0

C: control; NS: pomegranate juice; PST: paracetamol

According to Bonferroni correction ($0.05/6$) a p value of <0.01 was considered significant.

*significantly high when compared with all groups ($p < 0.001$).

**significantly lower compared to PST group ($p < 0.001$).

Results

The results showing lipid peroxidation (TBARS): The TBARS levels of the groups are shown in Figure 1. The tissue and hepatic TBARS levels were markedly higher in the PST group compared to the other groups ($p < 0.05$). The tissue TBARS levels were found to be higher in the Paracetamol and pomegranate juice group compared to the pomegranate juice and control groups, but no statistically significant difference was found.

Histopathological results: The histopathological findings of the study groups are shown in Table 1. The pathological change scores were found to be statistically significantly lower in the Pomegranate juice and PST group compared to the PST group ($p = 0.000$). When the hepatic tissues of the rats which were given paracetamol were examined, a moderate increase in the connective tissue, granular degeneration, necrotic cells, necrosis in the lobular center, early fibrosis in the perivenous region and portal vein congestion with periportal area infiltration characterized with increased inflammatory cells at moderate and severe levels were observed (Figure 2).

When the hepatic tissues of the animals which were given pomegranate juice and PST were examined, slight portal vein congestion, granular degeneration, increased connective tissue and leukocyte accumulation were observed

Figure 1. Liver and serum TBARS levels

(Figure 3). On histological examination of the groups which were given distilled water and pomegranate juice, normal hepatic tissue without significant pathological finding was observed. The hepatic cells were localized radially and intact large spheric nuclei and granular cytoplasm were observed (Figure 4).

Discussion

This study shows that pomegranate juice may prevent acute hepatic damage related with PCT considering antioxidant and histopathological changes. In this study, experimental hepatotoxicity which was induced with PCT was investigated with the TBARS method which is based on measurement of oxidative state lipid peroxidation. In the paracetamol group, the TBARS levels in the hepatic tissue and blood were found to be significantly higher compared to the other groups. The TBARS levels in the pomegranate juice and PCT group were found to be significantly lower compared to the PCT group. There was no significant difference between the pomegranate juice and PCT group and the pomegranate juice and control groups. These results show that pomegranate juice can protect the liver against oxidative stress.

Similar to our study, Piriñcioglu et al. (24) showed that pomegranate juice decreased oxidative stress in experimental hepatic damage induced by CCl₄ using the TBARS method. In addition, Rosenblat et al. (13) showed that lipid peroxidation levels were significantly high in diabetic patients according to the TBARS method and the TBARS levels decreased following consumption of 50 mL pomegranate juice daily for three months. In an experimental study conducted by Faria et al. (25), it was found that pomegranate juice provided protection against hepatic oxidative stress in mice.

On histological examination in the PCT group, increased connective tissue, hemorrhagia, mononuclear cell infiltration, necrotic cells and granular degeneration were present in the hepatocytes as expected according to the histopathological findings. These degenerative changes were also found in the pomegranate juice and PCT group, but they were found to be milder compared to the PCT group. In many studies conducted with various poisonous substances, it was shown that the histologic findings showing hepatic damage in the pomegranate juice groups were close to the control groups (24, 26-28).

Picture 1. Hepatic tissue samples belonging to the paracetamol group
a1; increased connective tissue (thin arrow), infiltration (thick arrow), H-E, x20.
a2; granular degeneration (thin black arrows), vascular congestion (thick black arrow), necrotic cell (white arrow), H-E, x40.
a3; infiltration (black arrow), increased connective tissue (white arrow), H-E, x20.
a4; increased connective tissue (white arrow), infiltration (black arrow), H-E, x20.

Picture 2. Hepatic tissue samples belonging to the paracetamol group and pomegranate juice group
b1;infiltration (white arrows), H-E, x10.
b2;granular degeneration (white arrows), infiltration (black arrow), H-E, x20.
b3; granular degeneration (white arrow), vascular congestion (black arrow), H-E, x40.
b4; vascular congestion (white arrow), infiltration (black arrow), H-E, x40.

Picture 3. Hepatic tissue samples belonging to the pomegranate juice group (c) and control group which shows normal histologic findings (d), H-E, x20

The histopathological findings in our study supported the hypothesis that pomegranate juice was protective against PCT toxicity in the liver.

Fever is one of the most common complaints in children and PCT is the first line drug among the drugs used as antipyretics. Doses above 10 grams cause to hepatotoxicity in adults, whereas doses above 300 mg/kg may lead to severe poisoning and hepatic necrosis in children. Paracetamol poisoning is observed commonly in children because of

factors including widespread use, the fact that syrup forms are sweet, increased activity because of the nature of the age group and curiosity and learning instinct.

Although the efficiency of various herbal agents in prevention of paracetamol-related hepatic damage has been proven in many studies, all herbal substances used in these studies have material and method difficulties and their use is not practical. Pomegranate juice is obtained from an easily accessible fruit. In addition, it can be easily consumed by children in terms of color and taste.

Young rats which were just weaned and could represent the pediatric patient group were used in our study. In addition, effect of pomegranate juice against acute hepatic toxicity caused by PCT could not be shown in previous studies. These two properties provide new contributions to the literature.

In conclusion, the results of this study related with antioxidant properties and histopathological examination showed that pomegranate juice might be efficient in preventing PCT-induced acute hepatic damage in young rats. We think that this study might contribute to the literature as the first experimental study conducted in this area.

Ethics Committee Approval: Ethics committee approval was received for this study from Süleyman Demirel University School of Medicine (24.04.2012-06).

Informed Consent: The informed consent was not required because the study was performed on animals.

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