Hepatoprotective Effect of *Punica granatum* Seed Oil Extract on Paracetamol Intoxication in Rat Via Inhibory Effect of CYP2E1 [1][2]

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Abstract

Punica granatum seed oil enriched by punikalagins has been shown to have a therapeutic effect against antidiabetic, anticancer, antiinflammatory and some organ toxicities. We aimed to reveal both protective and therapeutic effects of punica granatum seed oil, which has strong antioxidant and anti-inflammatory activity on paracetamol-induced hepatic toxicity via biochemically, molecularly and pathologically in our study. Our study, 64 albino wistar rats were fasted for 24 h and then divided into 8 equal groups. Group 1: Healthy, Group 2: 2 g/kg of paracetamol (2a: 24 h, 2b: 48 h) (orally), Group 3: 140 mg/kg of n-acetylcysteine (orally) + paracetamol, Group 4: 0.32 mg/mL *Punica granatum* (i.p) + paracetamol, Group 5: 0.64 mg/mL *Punica granatum* + paracetamol, Group 6: Paracetamol + 0.32 mg/mL *Punica granatum,* Group 7: Paracetamol + 0.64 mg/mL *Punica granatum,* Group 8: Paracetamol + 140 mg/kg n-acetylcysteine. The study was terminated at 24 and 48 h after paracetamol administration. Serum ALT and AST levels were significantly increased at 24th and 48th h of paracetamol administration according to toxicity. While malondialdehyde, CYP2E1 and TNF-α levels also increased in the liver, superoxide dismutase and glutathione peroxidase levels decreased significantly. Increased ALT, AST levels with malondialdehyde and TNF-α levels significantly decreased by punica granatum seed oil (low doses) application and antioxidant levels were also significantly improved. *Punica granatum* seed oil may be used as a potential therapeutic agent in the future by strengthening the antioxidant system and preventing inflammation, especially liver toxicity due to overdose of paracetamol in suicide- battered individuals.

Keywords: Rat, Paracetamol, Toxicity, Liver, Punica granatum

Sıçanlarda CYP2E1 İnihibisyonu İle Parasetamol İntoksikasyonunda *Punica granatum* Çekirdek Yağı Ektraktının Hepatoprotektif Etkisi

Öz

Punikalaginlerle zengin olan *Punica granatum* çekirdek yağının, antidiyabetik, antikanser, antiinflamatuar ve bazı organ toksisitelerine karşı terepötik etkileri gösterilmiştir. Çalışmamızda, parasetamol ile indüklenen hepatotoksisite üzerine güçlü antioksidan ve anti-inflamatuvar etkinliğe sahip punica granatum çekirdek yağının biyokimyasal, moleküler ve patolojik olarak koruyucu ve terapötik etkilerini ortaya koymayı amaçladık. Çalışmamızda 64 albino wistar sıçan 24 saat aç bırakıldıktan sonra 8 eşit gruba ayrıldı. Grup 1: Sağlıklı, Grup 2: Parasetamol (2a: 24 saat, 2b: 48 saat) (oral), Grup 3: 140 mg/kg n-asetilsistein (oral) + parasetamol, Grup 4: 0.32 mg/mL *Punica granatum* (i.p.) + parasetamol, Grup 5: 0.64 mg/mL *Punica granatum* + parasetamol, Grup 6: Parasetamol + 0.32 mg/mL *Punica granatum,* Grup 7: Parasetamol + 0.64 mg/mL *Punica granatum,* Grup 8: Parasetamol + 140 mg/kg n-asetilsistein. Çalışma, parasetamol uygulamasından 24 ve 48 saat sonra sonlandırıldı. Parasetamol uygulamasının 24. ve 48. saatlerinde toksisiteye göre serum ALT ve AST düzeyleri anlamlı şekilde arttı. Malondialdehit, CYP2E1 ve TNF-α düzeyleri karaciğerde de yükselirken, süperoksit dismutaz ve glutatyon peroksidaz düzeyleri anlamlı olarak azaldı. *Punica granatum* çekirdek yağı (düşük dozlarda) uygulaması ile artmış ALT, AST düzeyleri, malondialdehit ve TNF-α seviyeleri anlamlı derecede azalmış ve antioksidan düzeyleri önemli derecede düzelmiştir. *Punica granatum* çekirdek yağı, intihar girişiminde bulunan kişilerde parasetamolün aşırı dozundan dolayı oluşan karaciğer toksisitesini önleyerek ve antioksidan sistemi güçlendirerek potansiyel terapötik bir madde olarak kullanılabilir.

Anahtar sözcükler: Sıçan, Parasetamol, Toksisite, Karaciğer, Punica granatum

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INTRODUCTION

Paracetamol is a drug that has been used safely in baby, children and adults for many years as an analgesic and antipyretic [1]. Intoxication is frequently encountered because paracetamol is easily accessible. Especially in childhood, drug intoxication comes first [2,3]. Upon overdose, paracetamol is metabolized to N-acetylbenzoquinonimine (NAPQI), a intermediate reactive metabolite by the cytochrome p450 enzymes in the liver. NAPQI accumulation in the liver depletes free glutathione deposits leading to oxidative stress in the cells. It also leads to oxidation of sulfhydryl groups leading to apoptosis by increasing Ca^{++} concentration. This developing intoxication causes hepatic necrosis if not treated and liver failure in advanced cases [4-6].

Nowadays, Nacetylcysteine (NAC) is used as an antidote for hepatotoxicity due to paracetamol. NAC, a glutathione precursor, inhibits hepatic necrosis by providing NAPQI detoxification [7,8]. However, both NAC and other antioxidant acting agents can't able completely reverse the liver damage due to paracetamol toxicity. Therefore, it is important to develop drugs with stronger antioxidant and antiinflammatory activity to prevent liver damage due to paracetamol toxicity.

Punica granatum has grown up commonly and consume all around the world. It has been known strong antioxidant effects and has been used as a medication in ancient times. Also, *Punica granatum* and its compartments have benefiacial effects on diarrhea, dental problems, erectil dysfunction, protecting radiation, alzheimer, arthritis, obesity [9,10]. The *Punica granatum* fruit has three parts: the kernel, the shell and the water. The *Punica granatum* kernel is rich by its chemical content [11]. It also contains important antioxidants such as ellagic acid and gallic acid [12]. In that way there are number of different and possible biological effects. *Punica granatum* has strong antioxidant and antiinflammatory activity and has been used as an antiparasitic, anticoagulant, aphtha, diarrhea and ulcer healing agent [9,10]. The protective effects of *Punica granatum* juice and *Punica granatum* crust extract at liver toxicity depending to paracetamol has shown on that article [12]. However, *Punica granatum* seed will have different physiological effects due to the presence of many biologically active materials, unlike oil, water and crust extracts. Especially *Punica granatum* seed oil enriched by punikalagins has shown the theapeutic effects against antidiabetic, anticancer, antiinflammatory and some organ toxicities (brain, liver, kidney). However, there are no studies on the effects of *Punica granatum* seed oil or punicalagins on paracetamol toxicity. For this reason, we aimed to reveal on paracetamol-induced hepatic toxicity both protective and therapeutic effects of *Punica granatum* seed oil (PSO) with strong antioxidant and antiinflammatory activity in biochemical, molecular and pathological studies.

MATERIAL and METHODS

A total of 64 male albino wistar rat were used in the experiments. Each rat weighed 200-250 g, and all were obtained from Ataturk University's Experimental Animal Laboratory at the Medicinal and Experimental Application and Research Centre. The animal experiments and procedures were performed in accordance with national guidelines for the use and care of laboratory animals and approved by Kafkas University's Local Animal Care Committee (KAÜ-HADYEK: 2016/014). The rat was housed in standard plastic cages on sawdust bedding in an air-conditioned room at 22±1°C. Standard rat food and tap water were given *ad libitum*.

Chemicals

All of the chemicals used in our laboratory experiments were purchased from Sigma Chemical Co. (Munich, Germany). *Punica Granatum* spp., known as Hicaz in our country, was purchased from city of Mersin (obtained year: 2016) Thiopental sodium was obtained from IE Ulagay A.S. (Istanbul, Turkey).

Preparation of Punica granatum Seed Oil Extract

Essential oils of the plants were obtained on a Clevenger (Wisd-Wise Therm) device by water vapor distillation. For this purpose, the fruits were dried and then the seeds were separated. 160 g of the plant was pulverized in the shredder. The sample was placed in a glass balloon and 1600 mL of distilled water was added to it, then placed in a Clevenger apparatus and the apparatus was operated. After the evaporation started, it was left to stand for three hours. During this time, the hydrosol accumulated in the Clevenger in collection tube was taken in a sterile separate bottle. After the taken last hydrosol accumulated in the collection tube, the remaining volatile oil was stored in the dark bottles in the refrigerator at +4°C until used in the experiment.

Analysis of Ingredients in Punica granatum Seed Oil Extract by HPLC-DAD

Gallic acid, ellajic acid and punicalajin A and B were detected in extract and quantitative determinations were made on the Agilent 1200 Series High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-DAD) (Germany).

Sample Preparations

The extract was dissolved in methanol to a concentration of 1 mg/mL and a stock solution was obtained and centrifuged at 10.000 rpm for 5 min. Workup solutions were prepared by diluting the supernatant with phosphate buffer (pH=2.5, 0.025 M). The working solutions were injected into the system after passing through the injection filter. Each injection was repeated three times *(Table 1)*.

Table 1. *Amounts of substance in 1.00 g extract*

Substance	Amounts (mg/g)
Gallic acid	14.45±0.53
Punikalajin A	191.56±0.36
Punicalajin B	189.48±0.62
Ellageic Acid	68.02±0.42

Experimental Design

Animals fasted for 24 h and were divided into eight equal groups (n=8). Group 1: Control; did not receive any medication. Group 2 received only orally paracetamol 2 g/kg for 24 h (in 1% Carboxymethylcellulose (CMC) in 1X Phosphate Buffered Saline (PBS) [13-15] 2 mL). Group 2b received only orally paracetamol 2 g/kg for 48 h (in 1% CMC in 1X PBS 2 mL). Groups 3; received orally N-acetylcysteine 140 mg/kg 24 h after received paracetamol. Groups 4; received i.p. injection PSO 0.32 mg/kg 24 h after received paracetamol. Groups 5; received i.p. injection PSO 0,64 mg/kg 24 h after received paracetamol. Groups 6; received i.p. injection PSO 0,32 mg/kg before 24 h received paracetamol. Groups 7; received i.p. injection PSO 0,64 mg/kg before 24 h received paracetamol. Groups 8; received orally N-acetylcysteine 140 mg/kg before 24 h received paracetamol. The rats were allowed food post-administration of drugs for the next 24 h until they were sacrificed.

Following the experimental period, Group 1, 2, 6, 7 and 8 were killed on the 24th h after the administration of paracetamol and Group 2b, 3, 4 and 5 were killed on the 48th h after the administration of paracetamol by an overdose of a general anesthetic (thiopental sodium, 50 mg/kg). Blood samples were collected into heparinized bottles by heart puncture. The liver was removed immediately after sacrifice.

Biochemical Analyses

Serum Measurements of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), total protein serum samples were separated by centrifuging at 4.000 rpm for 10 min at 4°C within 1 h after collection, and were stored in a -86°C freezer before being used for biochemical analysis (including ALT, AST, total protein). To assess hepatic function, ALT and AST from each sample were measured in duplicate with highly sensitive Enzyme Linked Immuno-sorbent Assay (ELISA) kits specifically designed for rats, according to the manufacturer's instructions (BEN SRL-B0127E and B1253E (ITALY), respectively).

Liver Measurements of Oxidant, Antioxidants, CYP2E1 and TNF-a

Rat tissues were kept at -86°C. 100 mg of tissues from each rat was firstly perfused with PBS/heparin. All tissue samples from each rat were grinded in liquid nitrogen using a TissueLyser II grinding jar set (Qiagen, Hilden, Germany). Then they were centrifuged according to the manufacturer's instructions. Then, Superoxide Dismutase (SOD) activity, and Malondialdehyde (MDA) level, Glutathione Peroxidase (GSH) level, Cytochrome P450 2E1 (CYP2E1) and Tumor Necrosis Factor Alpha (TNF-α) levels from each supernatant were measured in duplicate with highly sensitive kits (Cayman-706002, 10009055, 703002 (USA)), and CSB-E09782r (China), specifically designed for rat tissue, according to the manufacturer's instructions. The protein concentrations were determined by the Lowry method using commercial protein standards (Sigma Aldrich, Total protein kit-TP0300-1KT-(USA)). All the data was presented as the mean±standard deviation (S.D.) results based on per mg of protein.

Real-Time PCR Analyses

The samples for molecular analyses were immediately stored at -80°C. Before storage, all of the tissue samples from each rat were first perfused with PBS/heparin and then ground in liquid nitrogen with a TissueLyser II grinding Jar Set.

RNA Extraction and cDNA Synthesis

The liver tissues (20 mg) were stabilized in an RNA stabilization reagent (RNAlater, Qiagen) and then disrupted with the TissueLyser II (Qiagen) (2x2 min). All of the RNA was purified using an RNeasy Mini Kit (Qiagen) and RNA samples were reverse-transcribed into complementary DNA with a high-capacity cDNA reverse transcription kit (Qiagen) according to our previous study [16,17].

Gene Expression

Relative rat TNF-α (PP-RA-300 taqman probe (Qiagen)) mRNA expression analyses were performed with StepOnePlus Real-Time PCR System technology (Applied Biosystems) using cDNA synthesized from RNA of ovary tissue described as previously [16].

Histopathological Procedures

The tissues reserved for histopathological examination were rapidly fixed in 10% buffered formalin for 24 h. After fixation, each tissue sample was routinely processed described as previously [16]. In haematoxylin Eosin stain (H&E) apoptotic cells are evaluated according to nucleus morphology because it stains chromatin. Observable changes include: Cell shrinkage or cytoplasmic shrinkage, condensation of chromatin and collection at the periphery of the nucleus membrane, shrinking or fragmentation of the nucleus.

Statistical Analyses

IBM SPSS statistical software Version 20.0 was used for the biochemical analysis. The results are presented as the means±standard deviation (SD). The between-group

comparisons for biochemical and molecular analyses were performed with one-way ANOVA and Duncan's multiple comparison tests. Significance was accepted at P<0.05.

RESULTS

Serum ALT and AST Levels

Primer indicators of liver toxicity are serum ALT and AST levels were measured as gold standart. Serum AST and ALT levels were significantly increased at 24th and 48th h of paracetamol according to toxicity. At our study Serum AST and ALT levels decreased in paracetamol toxicity after *Punica granatum* oil was added (P<0.05). Low dose PSO administration has lower AST and ALT levels more than high dose PSO administration (P<0.05). There was no significant difference between our study and NAC and low dose PSO administration. Finally, in our study, we found that low dose PSO administration significantly reduced AST and ALT levels compared to high dose PSO

administration in the groups in which we wanted to show a therapeutic response to toxicity (P<0.05). Again, no significant difference was found between NAC and low dose PSO in our study. Improvement of ALT and AST levels, which improved with PSO administration, suggests that PSO prevents liver toxicity *(Fig. 1)*.

Tissue Oxidative Marker

The primary mechanism of injury in paracetamol toxicity is increase of oxidative stress. The MDA levels of membrane phospholipids, the ultimate degradation product, are evaluated to show the tissue damage caused by the oxidative stress. Tissue MDA levels were significantly increased according to the duration of exposure at the 24th and 48th h of paracetamol administration exposed to the control group (P<0.05). In our study, *Punica granatum* reduced the MDA level due to the protective effect of the seed oil (P<0.05). Low dose PSO application has reduced MDA level more than high dose PSO application
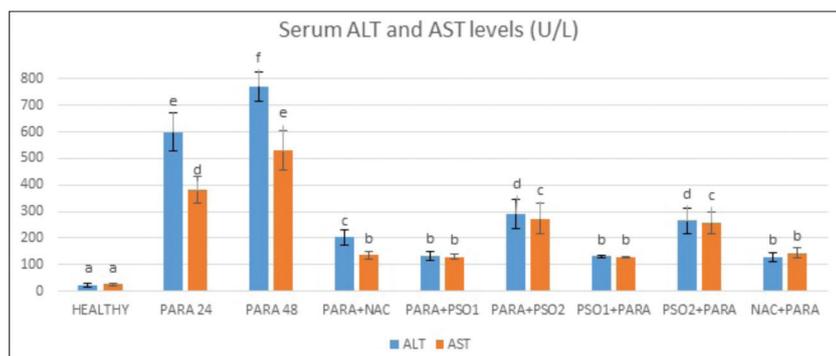
Fig 1. Effects of PSO on changes in the ALT and AST level in paracetamol toxicity. Means in the same column with the same letter are not significantly different; means in the same column with different letters indicate significant differences between the groups. P<0.05 (PSO: *Punica granatum* seed oil, PARA: Paracetamol)

Fig 2. Effects of PSO on changes in the SOD, MDA activity and GSH level in paracetamol toxicity. Means in the same column with the same letter are not significantly different; means in the same column with different letters indicate significant differences between the groups. P<0.05 (PSO: *Punica granatum* seed oil, PARA: Paracetamol)
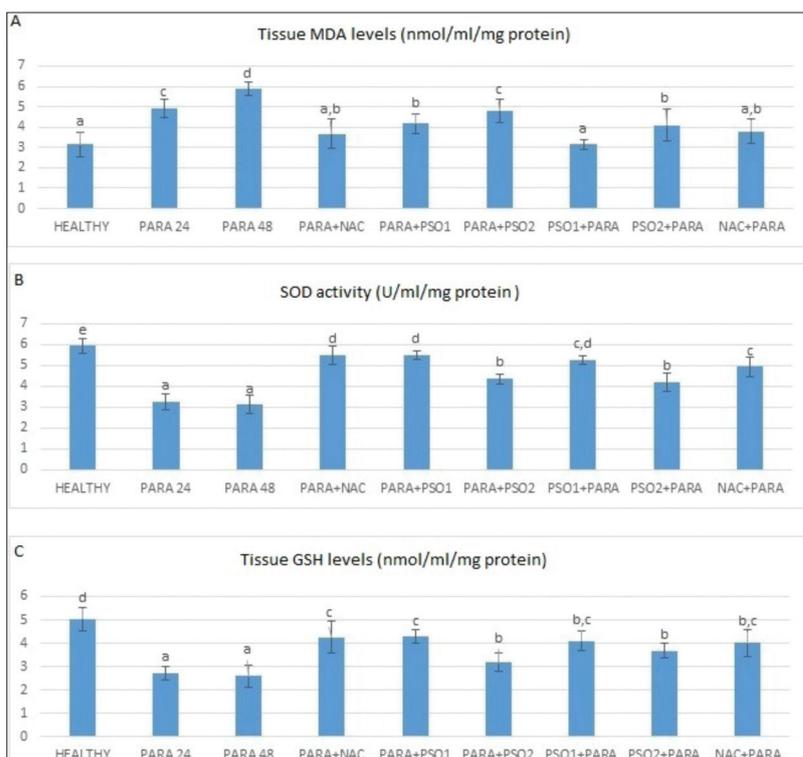
($P<0.05$). MDA level decreased with NAC application and no significant difference was observed with low dose PSO. MDA levels has decreased more at PSO threatment groups than paracetamol toxicity groups. Comparing both protective and therapeutic groups of PSO in our study, PSO has a strong protective effect and reduce damage in the treatment process *(Fig. 2)*.

Tissue CYP2E1 Levels

NAPQI which is produced from paracetamol by CYP2E1 in the liver. Active metabolite is responsible for the development of paracetamol toxicity. Increased CYP2E1 activity due to damage causes to increase of damage. Therefore, pharmacological agents with antioxidant pro-

perties can also correct the increased CYP2E1 enzyme activity at the same time, changing the style o the damage. In our study, groups with paracetamol treated showed an increase CYP2E1 activity in liver, depending on the duration of exposure ($P<0.05$). In the treated groups, However, low dose PSO application caused a further decrease in CYP2E1 activity more than high dose PSO application ($P<0.05$). CYP2E1 activities in the NAC treatment group was found lower than low dose PSO treatment group *(Fig. 3)*.

Tissue Antioxidant Markers

One of the most important defense systems of our body is antioxidants. Deficiency of antioxidants leads to increased oxidative stress and to necrosis of the liver. In
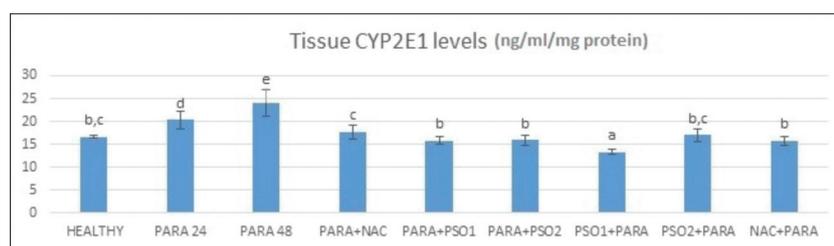

Fig 3. Effects of PSO on changes in the CYP2E1enzym levels in paracetamol toxicity. Means in the same column with the same letter are not significantly different; means in the same column with different letters indicate significant differences between the groups. $P<0.05$ (PSO: *Punica granatum* seed oil, PARA: Paracetamol)

Fig 4. Effects of PSO on changes in the TNF-α mRNA expression levels (A) and tissue TNF-α levels (B) in paracetamol toxicity. Means in the same column with the same letter are not significantly different; means in the same column with different letters indicate significant differences between the groups. $P<0.05$ (PSO: *Punica granatum* seed oil, PARA: Paracetamol)
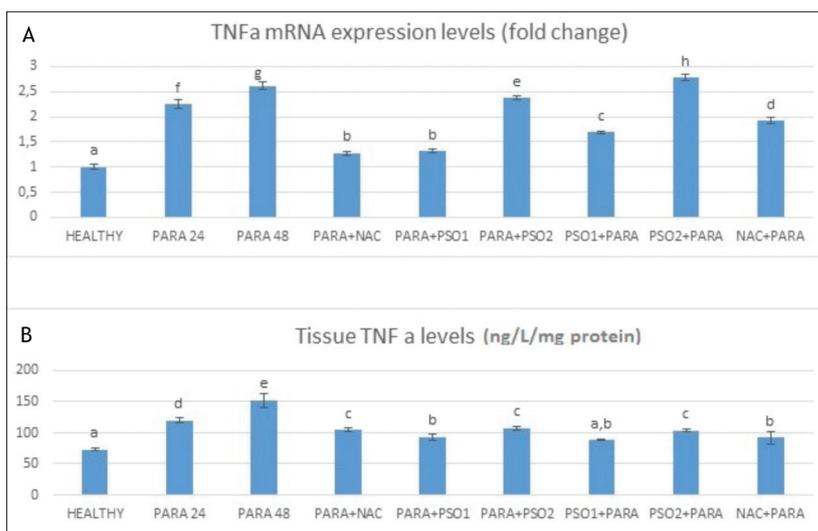
Table 2. *Pathological scoring*

Groups	Periportal Inflammation	Congestion	Cellular Damage Severity	Comman Cellular Damage	Necrosis
Healthy	minimal	++	none	none	none
PARA24	++	+	minimal	minimal	++
PARA48	++	+	minimal	minimal	none
PARA + NAC	+	+	none	none	none
PARA + PSO1	+	++	none	none	++
PARA + PSO2	+	+	none	none	none
PSO1 + PARA	+	+	none	none	none
PSO2 + PARA	+	+	none	none	none
NAC + PARA	+	+	none	none	local
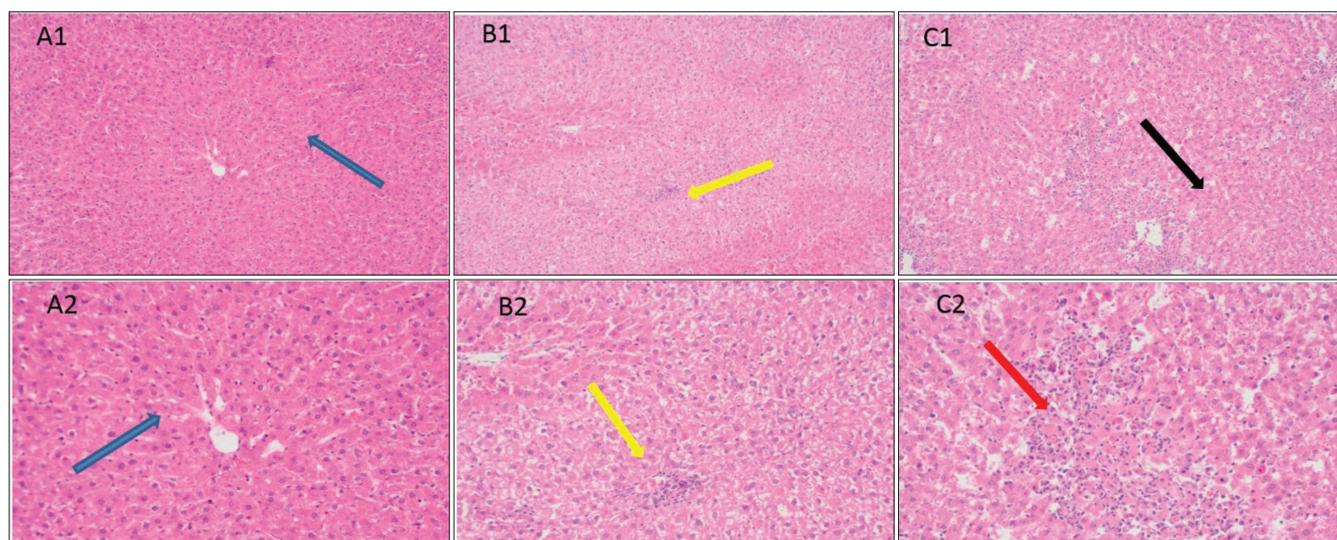
Fig 5. A) 1-40x, 2-100x, H-E staining results of healthy, B) 1-40x, 2-100x, H-E staining results of paracetamol toxicity 24 h, C) 1-40x, 2-100x, H-E staining results of paracetamol toxicity 48 h. *blue arrow:* cellular damage minimal, *yellow arrow:* periportal inflammation, *red arrow:* necrosis, *black arrow:* apoptosis
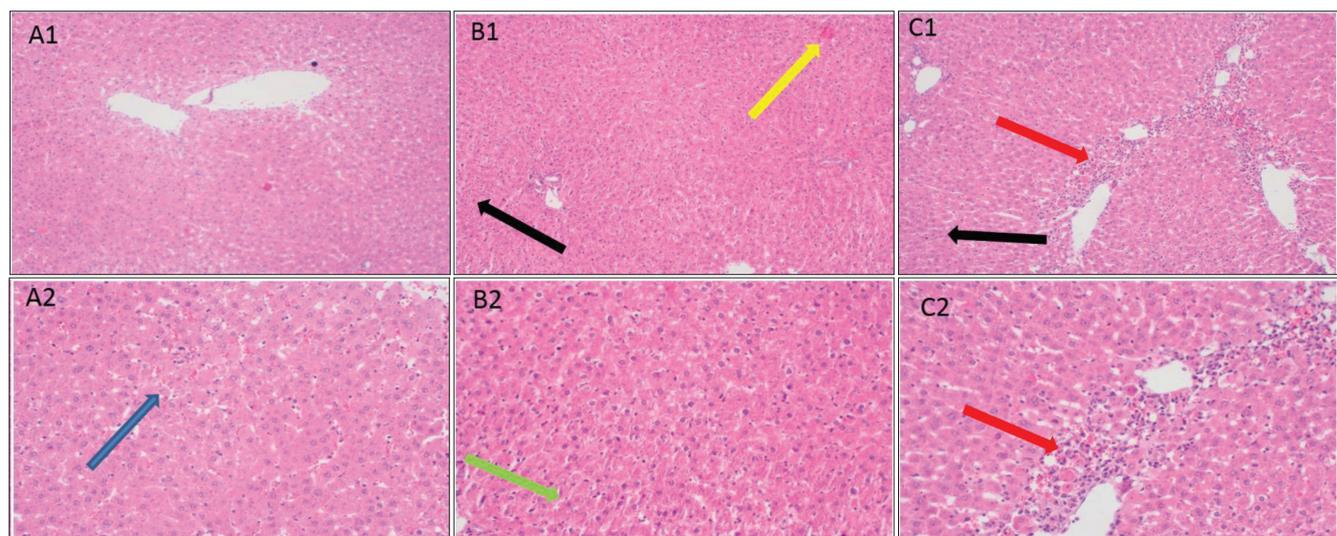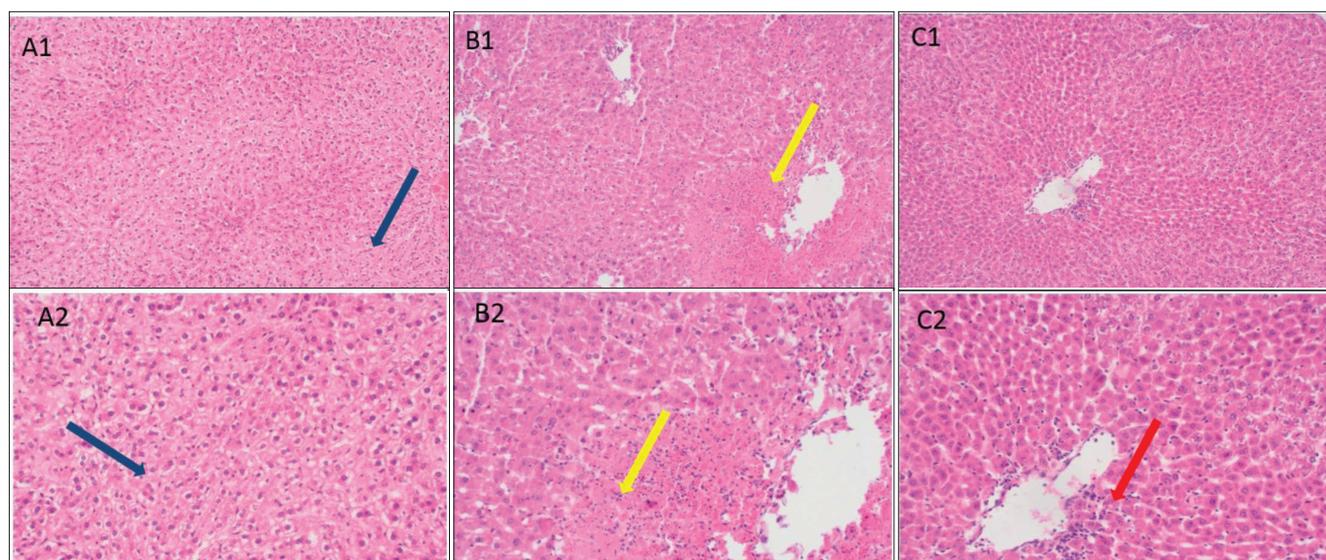

Fig 6. A) 1-40x, 2-100x, H-E staining results of PARA+NAC, B) 1-40x, 2-100x, H-E staining results of PARA+PSO1, C) 1-40x, 2-100x, H-E staining results of PARA+PSO2. *blue arrow:* minimal necrosis, *yellow and green arrow:* congestion, *red arrow:* portal inflammation, *black arrow:* mitotic cells

our study, liver SOD activities decreased significantly in the paracetamol group by the exposure of duration. In the PSO groups which is given protectively, SOD activities increased significantly (P<0.05). And again low dose PSO application increased the SOD activity more than the high dose. The protective effect of NAC increased SOD activity at the same level as low dose PSO administration (P<0.05). And again we observed that, administration of low dose PSO increased the activity of SOD more than that of the NAC group. In treatment groups, SOD activities were found to increase in the same amount in low dose PSO and NAC groups. In high dose PSO treatment, SOD activity is increased, but this increase was not as high as in the low dose treatment. there is a significant decrease in paracetamol toxicity in tissue GSH levels compared to control. In the treatment groups, NAC and PSO treatment significantly increased GSH levels. High dose PSO therapy increased GSH levels, but this increase was not as high as other treatment groups. By the protective effect, low dose PSO and NAC application significantly increased GSH levels (P<0.05). High dose PSO administration increased GSH levels but did not increase as much as other anti toxic groups *(Fig. 2)*.

Tissue TNF-a Levels and mRNA Expression Analyses

Paracetamol-induced toxicity is accompanied by oxidative stress and inflammation leading to increased exacerbation. The most important cytokines that causes inflammation is TNF-α. TNF-α, a proinflammatory cytokine, is significantly

Fig 7. A) 1-40x, 2-100x, H-E staining results of PSO1+PARA, B) 1-40x, 2-100x, H-E staining results of PSO2+PARA, C) 1-40x, 2-100x, H-E staining results of NAC+PARA. *blue arrow:* cellular damage, *yellow arrow:* necrosis, *red arrow:* portal inflammation

increased due to damage in paracetamol toxicity. In our study, tissue TNF-α level significantly increased with time depending on the application of paracetamol (P<0.05). It has been shown that low dose PSO treatment reduces tissue TNF-α level more than NAC and high dose PSO treatment. Tissue TNF-α levels in all treatment groups were significantly decreased more than the toxicity groups (P<0.05). Tissue TNF-α levels significantly decreased in PSO and NAC application. Difference of Increase in tissue TNF-α levels causes to be the low dose PSO and NAC inhibitory effect is higher than that of the high dose PSO.

TNF-α mRNA expression levels significantly increased in paracetamol applied groups in directly proportional to the duration of exposure relative to control. Low dose PSO therapy significantly reduced TNF-α mRNA expression levels caused by paracetamol (P<0.05). High dose PSO treatment did not showed any significant effect on TNF-α mRNA expression levels. NAC treatment significantly reversed the increase in TNF-α mRNA expression levels. Low doses of PSO given as protective against paracetamol toxicity significantly reduced TNF-α mRNA expression levels, while high-dose PSO administration was ineffective. NAC significantly reduced the levels of TNF-α. MRNA expression. Decreased inflammation due to PSO shows the antiinflammatory activity of the PSO and has an important place in the prevention and treatment of damage *(Fig. 4)*.

Histopathological Analyses

No pathology was observed in the liver tissues of the control group. At the 24th h of paracetamol administration, chronic inflammation, hemorrhage, and focal lithic necrosis were seen around portal areas. At the 48th h of paracetamol application, moderate to severe chronic inflammation, apoptotic cells, and double nucleus formation were observed around portal areas *(Table 2)*. İn treated groups, Chronic inflammation and focal lithic necrosis observed. In some portal areas in the treated groups with NAC in the treatment groups, while mild inflammation, focal necrosis areas, necrotic areas were observed around the portal area in low dose PSO treatment. However, in high-dose PSO treatment, regenerative findings (increased mitotic activity) in hepatocytes, showed chronic inflammation around the portal areas, and dilatation of the sinusoids. While administration of low dose PSO resulted in mild regenerative atypia in hepatocytes against paracetamol toxicity, chronic inflammation around the portal areas were seen with high dose PSO administration. In the group given NAC, inflammation around the portal areas and a few focal lytic necrosis centers were observed *(Fig. 5,6,7)*.

DISCUSSION

In our study, the possible protective and therapeutic effects of PSO in liver toxicity caused by paracetamol were demonstrated. However, the therapeutic effect was seen especially in low dose PSO administration, at high dose all parameters showed lower activity.

Paracetamol when it converts to NAPQI by the CYP2E1 enzyme in the liver can be tolerated by the endogenous antioxidants in our body when taken in therapeutic doses. However, in the case of high dose paracetamol ingestion, the active metabolite NAPQI, which cannot be tolerated due to insufficient endogenous antioxidant deposits, accumulates in the liver leading to damage. The CYP2E1 enzyme level plays an important role in the formation of the amount of active metabolite responsible for paracetamol toxicity and in indirectly evolved liver damage. The high level enzyme paracetamol purifies the active and harmful metabolites [18]. In our study, the CYP2E1 enzyme induced

by paracetamol toxicity, causes more NAPQI formation. For reverse paracetamol toxicity, the PSO reduced the level of CYP2E1 enzyme and prevented further NAPQI formation. ALT and AST are serum markers of the liver damage.

After 12 h of paracetamol poisoning ALT and AST levels increases due to severity of damage [19]. İn our study showed that when paracetamol given ALT and AST levels increased proportional with time. our protective and treatment groups showed that ALT and AST levels decreased significantly, especially when low dose PSO was given. At the same time at paracetamol toxicity teatment there is no difference between currently used NAC and PSO application. Previous studies showed that increasing ALT and AST levels due to toxicity are significantly reduced by application of *Punica granatum* juice extract [12].

Oxidative stress is primer mechanism and responsible from resulting damage. When Oxidative stress depended peroxidation starts it spreads leading to membrane damage [20]. The MDA production is the result of the peroxidation of the membrane fatty acids and is used to determine the degree of lipid peroxidation [21]. Our study showed that Increased MDA levels due to paracetamole have been shown to significantly decrease with PSO application. Again in study, it has been shown that PSO reduces oxidative stress in parallel with our findings [22].

There are many antioxidants in our body that compensate for the harmful effects of increasing free radicals. Free oxygen radicals and antioxidant defense systems are normally in balance. Antioxidants are activated in response to increased oxidative stress in pathological conditions like as toxicity and infection. As we know GSH and SOD are the most powerful antioxidans. GSH detoxifies paracetamol by conjugating with the active metabolite NAPQI. However, increased NAPQI level causes damage by consuming glutathione deposits. Free O⁻ radicals were increased by NAPQI are scavenging by SOD. Because of the inadequate antioxidant response in the developing toxicity situations, agents that strengthen the antioxidant defense system are needed exogenously.

Today, *Punica granatum* juice is one of the most important antioxidant sources. Antidiarrheic [23], antifungal [24], anti-ulcer [25], antitumoral [26], antibacterial [27] effects have been shown in studies with *Punica granatum*. The core part of the *Punica granatum* fruit strengthen these antioxidants and antiinflammatory systems are rich in Punikalagins and ellagic acids and taken place at etnobotanic studies for many years. In our study decreased antioxidant defense systems due to high dose paracetamol increased significantly with PSO application. Here we describe the therapeutic and prophylactic efficacy of PSO, both by reducing the level of CYP2E1 and by exhibiting increased oxidative stress and reduced antioxidant response. Previous studies have shown that PSO inhibits damage by

strengthening antioxidant defense mechanisms in organ toxins like liver and kidney.

Another important parameter which lead to paracetamol induced hepatotoxicity is inflammation. TNF-α, a pro-inflammatory cytokine, stimulates the acute phase reaction. İt induces Apoptotic cell death and inflammation during toxicity [28]. TNF-α regulates the initiation and maintenance of events related to inflammatory reactions by increasing the release of inflammatory cytokines, especially interleukin 1 beta, 6 and 8 [29,30]. In previous studies, It has been demonstrated that increased TNF-α levels correlate with paracetamol induced hepatotoxicity. Also, Ferah et al.[31] demonstrated protective effect of TNF-α inhibitors in paracetamol induced hepatotoxicity. Therefore, TNF-α is an another important parameter developing paracetamol induced toxicity. In our study, levels of TNF-α, which is associated with oxidative stress and inflammation increased in paralelly with paracetamol administration. At the same time, TNF-α expression up regulated in liver tissues via paracetamol administration. In this study, Increased TNF-α levels reduced with a strong antiinflammatory effect of PSO. Low dose PSO administration has strong inhibitory effect according to NAC and high dose PSO administration. In previous studies also have shown that PSO has TNF-α mediated antiinflammatory activity in various organ toxicities. Based all of these information, PSO has exert protective and therapotic effect by antiinflammtory activity

Punica granatum seed oil with strong antiinflammatory and antioxidant activity was statitistically significant improved hepatic necrosis due to toxicity with serum ALT, AST and tissue MDA levels by decrasing CYP2E1 enzyme, which is the primary role in the development of paracetamol toxicity. At the same time *Punica granatum* seed oil prevented the severity of damage by strengthening the antioxidant defense system and preventing inflammation and demonstrated protective effect. It has been demonstated that therapotic effects of *Punica granatum* seed oil is promising in the treatment of paracetamol intoxication when compared to NAC used routinly in paracetamol intoxications.

CONFLICT OF INTEREST

We wish to draw the attention of the Editor to the following facts which may be considered as potential conflicts of interest and to significant financial contributions to this work.

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