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# Pomegranate juice attenuates acetaminophen induced hepatotoxicity in rat model of experiment

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## Abstract:

**Background:** Pomegranate fruit has high contents of various polyphenols and antioxidants due to which it possesses variety of therapeutic properties. In particular, pomegranate fruit peel and flowers have exhibited high antioxidant activity in different studies. Acetaminophen (APAP) overdose is the most common cause of drug induced liver toxicity including both accidental and intentional types. In our study, we investigated the protecting mechanism of pomegranate fruit juice (PJ) against toxicity caused by APAP in Wistar rats.

**Methods:** Rats were fed with 0.2% (w/v) pomegranate fruit extract as prophylaxis to counter single dose of APAP (2 g/kg, p.o). After that variation in levels of glutathione, glutathione peroxidase (GPx), and glutathione reductase (GR) were marked.

**Results:** A single dose of APAP elevated serum toxicity markers including lipid peroxidation. A simultaneous sharp depletion of glutathione and its metabolizing enzymes glutathione peroxidase (GPx), glutathione reductase (GR) was observed. Oral doses of PJ at (0.1% & 0.2% w/v) caused a significant ( $P < 0.001$ ) reduction in toxicity marker enzymes. A striking elevation in antioxidant armory was seen as in response to PJ.

**Conclusion:** The results provide a clear picture of the defensive effect of PJ against APAP induced hepatic toxicity.

## Introduction

*Punica granatum* grows mainly in the Mediterranean region, with many antioxidant and anti-inflammatory efficacies [1]. Various researchers have shown that antioxidant activity of active contents of pomegranate juice (PJ) is analogous with major reported antioxidants. Some reports reveal that it has more antioxidant properties than red wine as well. Pomegranate has anthocyanidins including delphinidin, cyanidin, and pelargonidin) which have been evaluated by many researchers for their antioxidant activities. A Fenton substance  $-OH$  producing scheme is arrested by anthocyanidins probably by means of ferrous ion [2]. Protective efficacy of anthocyanidins has been proved in rodent models through evidence of termination of  $H_2O_2$ -induced lipid peroxidation [3]. Recently, pomegranate has gained importance as anticancer agent, with many investigators showing interest to find out its protective efficacy. Acetaminophen (APAP) is a widely used antipyretic and analgesic drug. It can induce hepatotoxicity and even acute liver failure at high doses. APAP overdose is a common cause of adult and children liver toxicity all over the globe. APAP can be metabolized by cytochrome P450 enzymes (CYPs) to N-acetyl-P-benzoquinoneimine (NAPQI) [4]. APAP generates large number of NAPQIs, which deplete reduced glutathione (GSH) and are proficient enough to bind to mitochondrial proteins, ultimately leading to mitochondrial dysfunction and reactive oxygen species (ROS) generation, and finally hepatocellular damage with centrilobular hepatic necrosis. Present study engrossed to find out the defending properties of PJ in APAP induced toxicity using rat models, and to investigate if PJ can elevate antioxidant levels in rat at effective doses or not?

## Methods

### Chemicals

All the kits for serum toxicity markers and other reagents were bought from reputed companies of international quality standards. Mainly, kits provided by Human Gesellschaft fur Biochemical and Diagnostic mbH, Germany were used.

### Animals

Female Wistar rats (4-5 weeks old) were obtained from Jazan University's animal house facility of and all necessary conditions were provided. Animals were housed in an air-

conditioned room under controlled condition of illumination (12 h light/12 h darkness). All the rats were given laboratory feed, pellet diet and water *ad libitum* for 6 days (d). The animal diet was provided by central animal house, College of Medicine, Jazan University Gizan.

### Treatment Regimen

Group I Control	0.9% saline for 7 consecutive days
Group II Treatment	APAP (2g/kg p.o) on 7 <sup>th</sup> d
Group III PJ low dose+ APAP	PJ (0.1% w/v) +APAP (2g/kg p.o) on 7 <sup>th</sup> d
Group IV PJ high dose + APAP	PJ (0.2% w/v) +APAP (2g/kg p.o) on 7 <sup>th</sup> d
Group V only PJ at high dose	PJ (0.2% w/v)

All animals were killed after 24 hours of APAP treatment. 100% fresh pomegranate juice (PJ) was prepared before administration, no other beverage was mixed.

### Biochemical Assays

Blood was collected to check serum levels of enzymes i.e. alanine transaminase (ALT), aspartate transaminase (AST). Hepatic tissue was prepared for post-mitochondrial supernatant (PMS) formation. All the parameters were conducted as per instructions in assays.

### Post-mitochondrial supernatant and microsome preparation

Athar and Iqbal's modified methodology was followed for tissue processing and preparation of PMS [5]. Chilled phosphate buffer of 0.1 M, at pH 7.4 was used for hepatic tissue homogenization. The homogenized material was sifted in muslin cloth, and isolation of nuclear debris was done by further centrifugation at  $800\times g$  for 10 minutes at  $4^\circ C$ . The aliquot was centrifuged at 12,000 rpm for 20 minutes by maintaining the temperature at  $4^\circ C$  to prepare PMS for assessing biochemical enzymes. PMS was further centrifuged at 34,000 rpm at  $4^\circ C$  for 60 minutes to prepare microsomes. Within 24 hours of rats' sacrifice, all the biochemical experiments were carried out for valid results.

### Catalase activity

Claiborne's method was used to estimate catalase activity [6].

### Assay for glutathione peroxidase (GPx) activity

Mohandas *et al*, methodology was taken into consideration for estimating GPx activity [7].

### Estimation of reduced glutathione

Jollow's method was used to carry out the assay for reduced glutathione [8].

### Assay for glutathione reductase (GR) activity

Carlberg and Mannervick's method was used to find out GR activity [9].

### Assay for lipid peroxidation (LPO)

LPO was assessed by method developed by Wright *et al* [10].

### Estimation of protein

The protein concentration in all samples was determined by the Lowry's method [11].

### Statistical analysis

Analysis of variance (ANOVA) was used to find variance between the groups further Dunnett's multiple comparison test was applied. Data is represented as the treatment groups mean  $\pm$  standard error.

### Results

Treatment with single dose of APAP resulted in the depletion of glutathione content and reduction in the activities of GPx and GR significantly ( $p < 0.001$ ). PJ effected and restored the APAP mediated suppressed glutathione metabolizing enzymes like GR and GPx as shown in table 1.

The effect of prophylactic treatment with PJ on APAP induced reduction in the activity of catalase is shown in table 1. APAP alone treatment caused reduction in its activity as compared to saline-treated control group. Treatment with PJ at both the doses caused the recovery of the catalase enzyme significantly ( $p < 0.001$ ) as compared with APAP treated control group.

Figure 1(a) shows that APAP treatment enhances the susceptibility of hepatic microsomal membrane for iron-ascorbate induced lipid peroxidation as compared to controls. PJ treatment caused reduction in hepatic microsomal lipid peroxidation significantly ( $p < 0.001$ ) as compared with APAP treated group as shown in figure 1b.

However, PJ alone group produced results near to saline control values. Figure 2 & 3 illustrates the effect of pretreatment of PJ on APAP induced serum toxicity markers. A sharp decrease was noted in SGOT and SGPT

levels in PJ pretreated groups in comparison to only APAP group.

Treatment Groups	Catalase (n moles H <sub>2</sub> O <sub>2</sub> consumed/min /mg protein)	Glutathione reductase (nmol NADPH oxidized/min /mg protein)	Glutathione peroxidase (nmol NADPH oxidized/min /mg protein)
Group I Control	13.4 $\pm$ 0.59	180.2 $\pm$ 11.5	98.4 $\pm$ 31.7
Group II APAP	7.049 $\pm$ 0.30 <sup>a</sup>	116.2 $\pm$ 22.9 <sup>a</sup>	76.5 $\pm$ 3.5 <sup>a</sup>
Group III PJ (0.1%) + APAP	9.05 $\pm$ 0.14 <sup>b</sup>	151.5 $\pm$ 2.17 <sup>b</sup>	79.0 $\pm$ 25.4 <sup>b</sup>
Group IV PJ (0.2%) + APAP	9.92 $\pm$ 0.13 <sup>b</sup>	158.0 $\pm$ 21.0 <sup>b</sup>	84.7 $\pm$ 8.35 <sup>b</sup>
Group V PJ (0.2%)	12.84 $\pm$ 0.17	174.1 $\pm$ 11.9	97.8 $\pm$ 3.1

Table 1: APAP mediated depletion in the activity of protecting enzymes and their restoration by PJ treatment: Significant observations were made ( $P < 0.001$ ) to control group: Significant observations were made ( $P < 0.001$ ) to APAP treated group

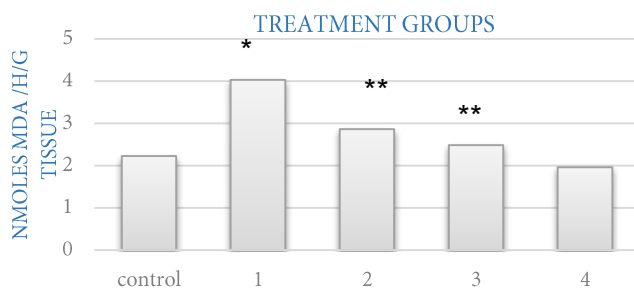


Figure 1(a): Effect of PJ on APAP induced Lipid peroxidation in rat liver

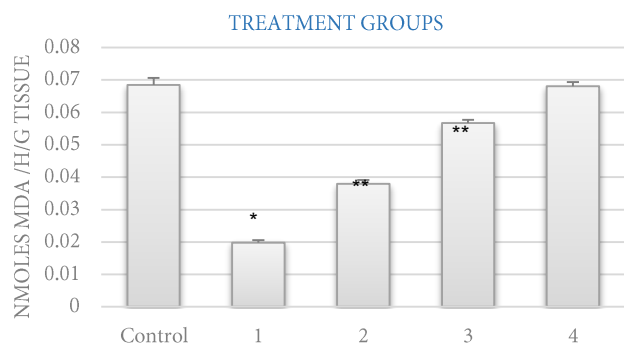
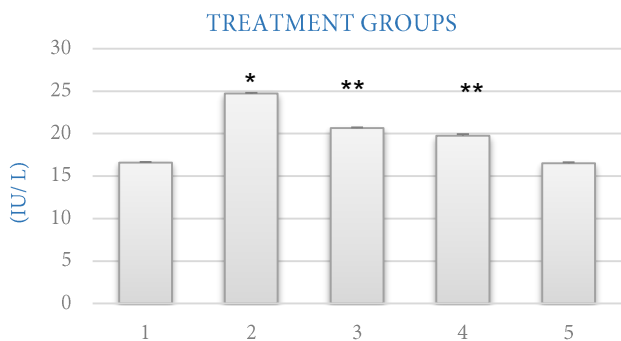
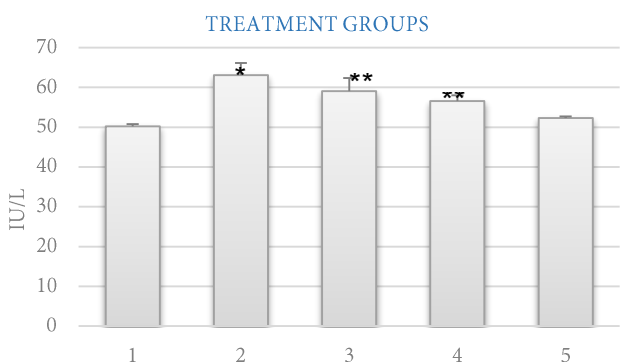


Figure 1(b): PJ attenuated APAP induced alterations of hepatic GSH content. Figure represent mean $\pm$  S.E of five groups (control, 1-treated, 2-PJLD +APAP, 3-PJHD+APAP, 4-only PJ)



**Figure 2: Influence of PJ on APAP induced SGPT activity in serum.** Figure shows mean± S.E of five group, (1-control, 2-APAP treated, 3-PJLD+APAP, 4-PJHD+APAP, 5-only PJ). Significant results were noted ( $P < 0.001$ ) for both control group and APAP treated group



**Figure 3: Impact of PJ on APAP induced serum SGOT activity.** Figure shows mean± S.E of five group, (1-control, 2-APAP treated, 3-PJLD+APAP, 4-PJHD+APAP, 5-only PJ). Significant results were noted ( $P < 0.001$ ) for both control group and APAP treated group.

## Discussion

Pomegranate has recently gained much attention, with emphasis on its beneficial effects against many dreadful diseases including cancer [12,13]. In vitro and in vivo studies have proved it as a potential antioxidant with major role in inhibiting various tumor formations [14]. This motivated us to investigate the effects of pomegranate juice treatment against hepato-cellular injury and serum toxicity induced by APAP.

APAP high dose significantly increases the serum levels of ALT, AST while PJ pretreated groups showed marked lowering in these groups [15]. APAP induced elevation in oxidative degradation of lipids with simultaneous depletion of antioxidant armory of hepatic tissue. PJ prophylactic groups revealed its protective efficacy by induction of glutathione redox cycle enzymes and lowering of lipid

peroxidation. Several studies have shown that natural plant products have potent antitumor promoting activities which could be attributed to presence of flavonoids in them [16]. APAP treatment limits the activities of antioxidant enzymes i.e. GPx, GR and catalase, while PJ was able to restore their values back to normal levels. PJ neutralize the oxidant effects of APAP, may be, through its antioxidant effects. This study shows induction of liver glutathione content and its redox cycle enzymes with PJ prophylaxis treatment. Enhancement in tri-peptide reduced glutathione has been considered to be the foremost contrivance for fortification against many carcinogens and xenobiotic. Many environmental carcinogens entail to be metabolized to their ultimate noxious forms. Phase I enzymes break down these xenobiotic to proximate carcinogens by oxidative reactions catalyzed by CYPs [17]. Detoxifying phase enzymes abolish these metabolites into the forms that are relatively less toxic, water soluble and excrete-able [18]. Oxidative stress and inflammation are adjunctive processes and ultimately causes toxicity [19]. The results show that APAP persuades oxidative damage by induction of lipid peroxidation and squelching antioxidants. Glutathione (GSH) levels are considered to be the basic resistance contraction against oxidative damage as it works as a free radical scrounger and as a cofactor in reclamation of toxins [20]. It has been evident from this study that GR levels are decreased on pretreatment of organisms with PJ which produce low amounts of GSH. Similarly, low levels of GSH indicate less incorporation of NADPH in cellular detoxification processes hence, normal cycles of Glutathione oxidation and reduction. Substantial upsurge was noted in GSH level in PJ-pretreated groups. Dose-dependent sharp decrease was noted in iron-ascorbate induced lipid peroxidation markers namely malondialdehyde levels in the PJ treated groups through lessened creation of unstable molecules. In addition, PJ prophylactic treatment also reversed APAP mediated suppression of antioxidant enzymes. PJ restored them approximately to the normal control levels. Dysregulation of these antioxidant enzymes has been reported as the cause of induction of various dreadful diseases including Cancer [21]. It can be concluded from this study that PJ at different doses was able to attenuate APAP induced toxicity. It also gives an insight and paves a path for PJ efficacy as antioxidant and anti-toxicant. However, further work needs to be conducted

for confirmation of PJ as antioxidant. Free radical scavenging effect of PJ is shown by depletion of LPO and simultaneous induction of antioxidant armory. Protective efficacy of PJ may be attributed to presence of anthocyanins and tannins. We urge and recommend the evaluation studies of protective effects of PJ in hepatic tissue through histopathology and electron microscopy.

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