

Full Length Research Paper

# Studies on the effect of pomegranate (*Punica granatum*) juice and peel on liver and kidney in adult male rats

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The pomegranate has been traditionally used as medicines in many countries. The study aimed to investigate the antioxidant properties of pomegranate in hepatic and renal tissues of rats. Eighteen adult male albino rats were randomly divided into three groups, six rats of each. The first group served as control and received saline (0.2 ml saline/rat) by oral administration *via* epigastric tube. The second group received oral administration of 3 ml/kg pomegranate juice for 21 days and served as pomegranate juice (PJ) group. The third group received oral administration of 200 mg/kg methanol extract of pomegranate peel for 21 days and served as methanol extract of pomegranate peel (MEPP) group. Pomegranate has no effect on liver and kidney functions. The present data demonstrate that PJ and MEPP reduced lipid peroxidation and nitric oxide in both liver and kidney tissue homogenate. A significant increase in superoxide dismutase and catalase activities of rats received pomegranate was observed. These findings demonstrate that pomegranate has a potent anti-oxidative effect.

**Key words:** *Punica granatum*, oxidants/antioxidants status, liver, kidney, rat.

## INTRODUCTION

*Punica granatum* L. (*Punicaceae*), commonly called pomegranate, recently described as nature's power fruit, is a plant used in folkloric medicine for the treatment of various diseases (Abdel Moneim, 2011; Ajaikumar et al., 2005) widely cultivated in the Mediterranean region.

Pomegranate has strong antioxidant and anti-inflammatory properties. Recent studies have demonstrated its anti-cancer activity in several human cancers (Adhami and Mukhtar, 2007; Longtin, 2003). Apart from their antioxidant capacity, there have been numerous reports on the *in vivo* properties of pomegranates, namely on anti-atherosclerotic capacity (Aviram and Dornfeld, 2001; Kaplan and Aviram, 2001), anti-proliferative and pro-apoptotic activities of pomegranate tannin extract (Seeram et al., 2005), anti-inflammatory activity (Adams et al., 2006), as well as chemopreventive and chemotherapeutic potential towards prostate cancer by pomegranate juice (PJ) (Malik et al., 2005).

The reduction of platelet aggregation, of atherogenic low density lipoprotein (LDL) modifications and of macrophage oxidative state has been demonstrated, establishing a relationship between the consumption of PJ and cardiovascular protection in rats (Faria et al., 2007; Rozenberg et al., 2006). In addition, pomegranate peel extract with an abundance of flavonoids and tannins has been shown to have a high antioxidant activity

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**Abbreviations:** PJ, Pomegranate juice; MEPP, methanol extract of pomegranate peel; LDL, low density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase;  $\gamma$ GT,  $\gamma$ -glutamyl transpeptidase; TB, total bilirubin; UA, uric acid, BNU, blood nitrogen urea; Cr, serum creatinine; MDA, malondialdehyde; NO, nitric oxide; GSH, glutathione; DTNB, 5,5'-dithiobis (2-nitrobenzoic acid); CAT, catalase; DHBS, 3,5-dichloro-2-hydroxybenzene sulfonic acid; AAP, 4-aminophenazone; SOD, superoxide dismutase; GST, glutathione-S-transferase; GPx, glutathione peroxidase; GR, glutathione reductase; DNA, deoxyribonucleic acid; GSSG, glutathione disulfide; H<sub>2</sub>O<sub>2</sub>, hydrogen peroxide.

(Abdel Moneim, 2011). Still, the lack of whole organism studies on pomegranate juice and peel and the growing amount of PJ consumption substantiates the need to investigate the effect of prolonged PJ ingestion on general oxidative status of liver and kidney to establish the potential roles that may make pomegranate juice and peel one of the most chosen foods of the future.

## MATERIALS AND METHODS

### Experimental animals

Adult male albino wistar rats weighing 120 – 150 g were obtained from The Holding Company for Biological Products and Vaccines (VACSERA, Cairo, Egypt). Animals were kept in wire bottomed cages in a room under standard condition of illumination with a 12-h light-dark cycle at  $25\pm 1^\circ\text{C}$ . They were provided with water and balanced diet *ad libitum*. The experiments were approved by the state authorities and followed Egyptian rules on animal protection.

### Pomegranate juice preparation

The fresh pomegranate fruits, free of blemishes or obvious defects were purchased in August 2010, dried and powdered before extraction. 10 kg of pomegranates (*P. granatum*) were washed and manually peeled, without separating the seeds. Juice was obtained using a commercial blender (Braun, Germany), filtrated with a buchner funnel and immediately diluted with distal water to volume of 1:3 and stored at  $20^\circ\text{C}$  for no longer than 2 months (Faria et al., 2007).

### Pomegranate peel extracts preparation

An aqueous extract of the pomegranate peel prepared by mashing in a proportion of 1:2:2 (w peel/v water/v methanol) and left for about 48 hrs in refrigerator. After Mashing, the resulting extract was filtered and then the solvent was evaporated under reduced pressure at  $40 - 50^\circ\text{C}$ . It was stored at  $3 - 4^\circ\text{C}$  until used and designated as methanol extract of pomegranate peel (Abdel Moneim, 2011).

### Experimental protocol

To study the effect of pomegranate peel, eighteen adult male albino rats were randomly divided into three groups, six rats of each. Group I served as control and received saline (0.2 ml saline/ rat) by oral administration *via* epigastric tube. Group II received oral administration of 200 mg/kg (Parmar and Kar, 2008) methanol extract of pomegranate peel for 21 days and served as MEPP group.

Group III received oral administration of 3 ml/kg pomegranate juice for 21 days and served as PJ group. The animals of all groups were cervically dislocated and blood samples were collected from retro-orbital plexus. Blood stranded for half an hour and then centrifuged at 500 g for 15 min at  $4^\circ\text{C}$  to separate serum and stored at  $-70^\circ\text{C}$  until analysis.

Pieces of liver and kidney were weighed and homogenized immediately to give 50% (w/v) homogenate in ice-cold medium containing 50 mM Tris-HCl and 300 mM sucrose (Tsakiris et al., 2004). The homogenate was centrifuged at 500 g for 10 min at  $4^\circ\text{C}$ . The supernatant (10%) was used for the various biochemical determinations.

## Biochemical estimations

### Liver function test

Colorimetric determination of alanine aminotransferase (ALT) or aspartate aminotransferase (AST) was estimated by measuring the amount of pyruvate or oxaloacetate produced by forming 2, 4-dinitrophenylhydrazine according to the method of Reitman and Frankel (1957), the color of which was measured at 546 nm.  $\gamma$ -glutamyl transpeptidase ( $\gamma$ GT) and alkaline phosphatase were assayed in liver homogenate using kits provided from Biodiagnostic Co. (Giza, Egypt) according to the method described by Szasz (1969) and Belfield and Goldberg (1971), respectively. Also, Total bilirubin (TB) in serum was assayed according to the method of Schmidt and Eisenburg (1975).

### Kidney function test

Uric acid (UA), blood nitrogen urea (BNU) and serum creatinine (Cr) were assayed in serum using kits provided from Biodiagnostic Co. (Giza, Egypt) according to the methods described by Fossati et al. (1980), Fawcett and Soctt (1960) and Szasz et al. (1979), respectively.

### Determination of malondialdehyde and nitric oxide

Malondialdehyde (MDA) and nitric oxide (NO) were assayed colorimetrically in liver and kidney homogenate according to the methods of Ohkawa et al. (1979) and Berkels et al. (2004), respectively. MDA was determined by using 1 ml of trichloroacetic acid 10% and 1 ml of thiobarbituric acid 0.67% and were then heated in a boiling water bath for 30 min. Thiobarbituric acid reactive substances were determined by the absorbance at 535 nm and expressed as MDA formed. NO was determined in acid medium and in the presence of nitrite the formed nitrous acid diazotise sulphanilamide is coupled with N-(1-naphthyl) ethylenediamine.

The resulting azo dye has a bright reddish – purple color which can be measured at 540 nm.

### Estimation of reduced glutathione and anti-oxidant enzymes

The hepatic and renal reduced glutathione (GSH) level were determined by the methods of Ellman (1959). The method based on the reduction of Ellman's reagent (5, 5' dithiobis (2-nitrobenzoic acid) "DTNB") with GSH to produce a yellow compound. The reduced chromogen was directly proportional to GSH concentration and its absorbance can be measured at 405 nm. In addition, the activity of hepatic and renal antioxidant as catalase (CAT) reacts with a known quantity of  $\text{H}_2\text{O}_2$  according to the method of Aebi (1984).

The reaction is stopped after exactly 1 min with CAT inhibitor. In the presence of peroxidase (HRP), the remaining  $\text{H}_2\text{O}_2$  reacts with 3,5-Dichloro-2-hydroxybenzene sulfonic acid (DHBS) and 4-aminophenazone (AAP) to form a chromophore with a color intensity inversely proportional to the activity of CAT in the original sample. Superoxide dismutase (SOD) activity was assayed by the method of Nishikimi et al. (1972).

This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye. Also, the activity of glutathione-S-transferase (GST), glutathione peroxidase (GPx) and glutathione reductase (GR) activities were determined by the methods of Habig et al. (1974), Paglia and Valentine (1967), and Factor et al. (1998), respectively.

**Table 1.** Effect of methanol extract of pomegranate peel and pomegranate juice on liver function of male rats.

Group	ALT (U/L)	AST (U/L)	$\gamma$ -GT (U/L)	ALP (IU/L)	Bilirubin (mg/dl)
Control	70.15±0.88	56.08±0.30	3.88±0.27	147.73±9.07	2.64±0.09
PJ	72.32±1.14	56.25±0.16	3.89±0.16	93.75±3.11 <sup>a</sup>	2.28±0.05 <sup>a</sup>
MEPP	70.95±0.63	56.17±0.21	3.23±0.15	55.40±3.51 <sup>ab</sup>	1.76±0.09 <sup>ab</sup>

Values are means±SE (n=6). a: significant change at  $p < 0.05$  with respect to control group. b: significant change at  $p < 0.05$  with respect to pomegranate juice group.

**Table 2.** Effect of methanol extract of pomegranate peel and pomegranate juice on kidney function of male rats.

Group	Uric Acid (mg/dL)	Urea (mg/dL)	Creatinine (mg/%)
Control	69.63±4.01	4.10±0.19	0.50±0.013
PJ	65.05±1.59	4.99±0.08 <sup>a</sup>	0.39±0.006 <sup>a</sup>
MEPP	67.58±1.50	4.48±0.08 <sup>b</sup>	0.35±0.007 <sup>ab</sup>

Values are means±SE (n=6). a: significant change at  $p < 0.05$  with respect to control group. b: significant change at  $p < 0.05$  with respect to pomegranate juice group.

**Table 3.** Malondialdehyde and nitric oxide content in liver and kidney of male rats treated with methanol extract of pomegranate peel and pomegranate juice.

Group	Hepatic MDA (nmol/g)	Renal MDA (nmol/g)	Hepatic NO ( $\mu$ mol/g)	Renal NO ( $\mu$ mol/g)
Control	1027.20±47.79	1302.82±46.81	128.54±2.39	183.89±5.35
PJ	647.06±18.93 <sup>a</sup>	873.55±7.44 <sup>a</sup>	100.42±5.24 <sup>a</sup>	118.23±7.44 <sup>a</sup>
MEPP	489.08±8.31 <sup>ab</sup>	835.18±32.76 <sup>a</sup>	113.06±2.28 <sup>ab</sup>	94.83±1.80 <sup>ab</sup>

Values are means±SE (n=6). a: significant change at  $p < 0.05$  with respect to control group. b: significant change at  $p < 0.05$  with respect to pomegranate juice group.

### Statistical analysis

The obtained data were presented as means  $\pm$  standard error. Statistical analysis was performed using an unpaired Student's t-test using a statistical package program (SPSS version 17.0).

## RESULTS

### Potential role of pomegranate in liver function

The results of the present study showed that pomegranate caused a significant reduction ( $p < 0.05$ ) in both of alkaline phosphatase and bilirubin when administered in the form of juice or peel extracts (Table 1). Pomegranate has no significant effects on ALT, AST or  $\gamma$ -GT (Table 1), but it significantly lowers ( $p < 0.05$ ) the level of alkaline phosphatase (Table 1).

### Beneficial effect of pomegranate in kidney function

The results of kidney function tests in PJ group (Table 2)

showed that pomegranate administration caused significant increased in urea (4.99±0.08 mg/dl) and a significant decrease in Cr (0.39±0.006 mg%). Kidney function in rats of MEPP Group was similar to that of PJ group (Table 2).

### Protective effect of pomegranate in oxidant/antioxidant status

Pomegranate administration to rats of PJ group induced a significant reduction in MDA of liver and kidney (37 and 33%, respectively), indicating that pomegranate has antioxidant properties (Table 3). MDA was more significantly reduced in hepatic and renal tissues of rats of MEPP Group, indicating that pomegranate peel extracts has more antioxidant properties (Table 3). PJ could also induce a significant reduction of NO in both of the hepatic and renal tissues of rats by approximately 22% and 35%, respectively (Table 3). Pomegranate extract from peel was able to significantly decrease the NO level in hepatic and renal tissues of rats by 12% and

**Table 4.** Reduced glutathione, catalase and superoxide dismutase levels in liver and kidney of male rats treated with methanol extract of pomegranate peel and pomegranate juice.

Group	Hepatic GSH (mmol/g)	Renal GSH (mmol/g)	Hepatic CAT (U/g)	Renal CAT (U/g)	Hepatic SOD (U/g)	Renal SOD (U/g)
Control	36.69±1.10	53.02±0.87	1.21±0.04	1.51±0.02	1.06±0.01	0.74±0.01
PJ	35.59±0.63	54.09±2.45	1.12±0.02	2.47±0.08 <sup>a</sup>	1.41±0.02 <sup>a</sup>	0.85±0.03
MEPP	38.19±0.78	52.07±0.59	1.11±0.03	1.52±0.01 <sup>u</sup>	1.75±0.01 <sup>au</sup>	2.89±0.01 <sup>au</sup>

Values are means±SE (n=6). a: significant change at  $p < 0.05$  with respect to control group. b: significant change at  $p < 0.05$  with respect to pomegranate juice group.

**Table 5.** Effect of methanol extract of pomegranate peel and pomegranate juice on hepatic and renal glutathione reductase, glutathione peroxidase and glutathione-S-transferase of male rats.

Group	Hepatic GR (µmol/g)	Renal GR (µmol/g)	Hepatic GPx (U/g)	Renal GPx (U/g)	Hepatic GST (µmol/h/g)	Renal GST (µmol/h/g)
Control	10.25±8.97	15.87±3.07	1722.43±69.21	1125.77±83.03	0.61±0.04	0.65±0.02
PJ	10.89±2.47	13.87±5.23 <sup>a</sup>	1702.00±71.95	1128.42±83.19	0.64±0.01	0.54±0.02 <sup>a</sup>
MEPP	10.16±7.31	13.73±7.86 <sup>a</sup>	1792.55±76.87	1589.94±85.49 <sup>au</sup>	0.66±0.02	0.51±0.03 <sup>a</sup>

Values are means±SE (n=6). a: significant change at  $p < 0.05$  with respect to control group. b: significant change at  $p < 0.05$  with respect to pomegranate juice group.

48%, respectively (Table 3). Data in (Table 4) demonstrate the potential effect of pomegranate as an antioxidant agent. Glutathione level in both of hepatic and renal tissues of rats were not affected by pomegranate treatment. Pomegranate induced a significant increase in CAT level in the renal tissues of rats administered either pomegranate juice or the aqueous extract of the pomegranate peel. CAT level showed no significant change in hepatic tissues of rats. There was a significant increase in the level of superoxide dismutase in the hepatic tissue of rats of PJ group (1.41±0.02 U/g) and MEPP group (1.75±0.01 U/g) when compared to the control group (1.06±0.01 U/g). The level of SOD was significantly increased by about 4 folds in the renal tissue of rats administered aqueous extract of the pomegranate peel (Table 4).

GR was not altered in hepatic tissues of rats treated with pomegranate while in the renal tissues the level was significantly reduced in PJ and MEPP groups by 13.87±5.23 and 13.73±7.86 µmol/g, respectively (Table 5). Renal GPx was significantly increased (1589.94±85.49 U/g) in MEPP group when compared to the control group (1125.77±83.03 U/g). Pomegranate did not affect GST in both of hepatic tissues of rats although it has been decreased in the renal tissues (Table 5).

## DISCUSSION

Pomegranate is an important source of anthocyanins, hydrolysable tannins punicalagin and punicalin (Afaq et al., 2005), ellagic and gallic acids (Lansky and Newman,

2007) and also contains vitamin C (Turk et al., 2008). The antioxidant and free radical scavenging activity of phenolic compounds derived from pomegranates (Rosenblat et al., 2006) and vitamin C (Sonmez et al., 2005) have been reported. In this study, it was observed that PJ and MEPP had significant effect on some oxidants/antioxidants enzymes of liver and kidney when compared to the control.

It has been reported that oxidizing biological material leads to a rapid burst of ROS, such as superoxide ( $O_2^{\cdot-}$ ), hydrogen peroxide ( $H_2O_2$ ) and hydroxyl ( $\cdot OH$ ) generated primarily because of the ionizing of water molecules (Agrawal et al., 2001), which then interact with biological target molecules, causing lipid peroxidation and Deoxyribonucleic acid (DNA) damage, and subsequently resulting in cell killing and mutations (Abdel Moneim, 2011). Plants, vegetables, herbs and spices used in folk and traditional medicine have been accepted currently as one of the main sources of chemo preventive drug discovery and development (Aruoma, 2003). It has been observed that many plant polyphenols, such as ellagic acid, catechins, and chlorogenic, caffeic and ferulic acids act as potent antioxidant, antimutagenic and anticarcinogenic agents (Ayrton et al., 1992).

In this study, the antioxidant activity of pomegranate juice and methanol extract of peel was evaluated on liver and kidney of rats. The present data demonstrate that PJ and MEPP reduced lipid peroxidation and nitric oxide in both liver and kidney tissue homogenate. The ability of pomegranate to reduce the oxidant molecules seems likely by scavenging the reactive oxygen radicals. Because of its rich concentration of diverse, free-radical-scavenging

bioflavonoid, pomegranate has been recommended in the treatment of acquired immune deficiency syndrome (AIDS). Our results are in agreement with the studies which have demonstrated that *P. granatum* peel extract decreased lipid peroxidation in hepatic, cardiac, and renal tissues (Parmar and Kar, 2008) and had a facilitator effect on the scavenging ability of superoxide anion and hydrogen peroxide. Previously, Toklu et al. (2007) have shown that chronic pomegranate peel extract supplementation alleviated oxidative injury of the liver and improved the hepatic structure and function in rats exposed to bile duct ligation. Another study in rats with carbon tetrachloride-induced liver damage demonstrated that pretreatment with pomegranate peel extract resulted in the reduction of lipid peroxidation, while the free-radical scavenging activity of CAT, SOD, and peroxidase were significantly enhanced (Chidambara et al., 2002).

In the present study, MEPP were more potent than PJ as antilipoxidant. Singh et al. (2002) investigated the antioxidative activity of methanol, water and acetone extracts of pomegranate peel using  $\beta$ -carotene-linoleate model system and found a positive correlation between the phenolic content and the antioxidant activity of the three extracts. Kulkarni et al. (2004) compared the antioxidative activity of pomegranate peel extracts with punicalagin, a major pomegranate polyphenol. They found that the extract had higher antioxidative activity than punicalagin, which showed a synergetic effect between different phenolic compounds present in the peel extract.

In the present study, the GSH level that was not changed in PJ and MEPP groups. In our opinion, observed GSH levels could be translated into one of two hypothetical scenarios: (1) an increase in oxidative stress status supported by reduced GSH levels or (2) interference of PJ with GSH synthesis or metabolism. The first hypothesis seems unlikely as a decrease in oxidative damage to biomolecules has been observed following PJ ingestion. Both protein and DNA damage were reduced in mice that ingested PJ, which strongly disagrees with this assumption. The second and more probable explanation needed further biochemical exploration. For this purpose, the activity of GSH related enzymes was assessed.

GR is responsible for recycling glutathione disulfide (GSSG) formed during oxidation events reducing it back to GSH. After pomegranate intake, a decrease in GR activity was observed. The reduction of this enzyme's activity could be a result of the decreased total GSH levels. Less GSH levels will indubitably require less GR activity. It has also been described that certain polyphenols, namely tannic acid and coumarins (Perez-Vicente et al., 2002), are able to reduce GR activity (Zhang et al., 1997) and the presence of these or related polyphenols, such as tannic acid, in pomegranate (Adams et al., 2006; Perez-Vicente et al., 2002) may account for the GR inhibition observed. Another GSH related enzyme is GST, a phase-II enzyme responsible

for the detoxification of several substrates. This enzyme can also conjugate altered proteins and mediates protein repair mechanisms. Decreased renal GST activity was observed in the groups that ingested pomegranate. GST inhibition could reflect the decrease in protein damage, which will, most likely, translate into less GST activity. Another explanation could be, as for GR, the regulation of this enzyme is in a competitive manner by polyphenols from pomegranate (for example ellagic and tannic acid), as has been previously proposed (Das et al., 1986).

Finally, the effect of pomegranate on GSH level is due to its polyphenols, where it is known to be able to modulate the transcription and expression of proteins related to the endogenous antioxidant defense by interacting with antioxidant response elements in gene promoter regions of genes encoding proteins related to oxidative injury management (Moskaug et al., 2005; Myhrstad et al., 2002). The main detoxifying system for peroxides is CAT. CAT is an antioxidant enzyme, which destroys  $H_2O_2$  that can form a highly reactive OH in the presence of iron as a catalyst. By participating in the GSH redox cycle, GSH together with GPx converts  $H_2O_2$  and lipid peroxides to non-toxic products (Sanocka and Kurpysz, 2004). Phenolic compounds derived from pomegranate (Sumner et al., 2005), have been used as antioxidant agents to prevent various lipid-peroxidation-induced damages in different organs. A significant decrease in MDA level, by-product of lipid peroxidation, and increases in SOD and CAT activities of liver and kidney samples of rats received pomegranate were observed in the present study. These findings demonstrate that pomegranate has a potent anti-oxidative effect.

Finally, according to the results presented above, the methanol extract of peel and juice of *P. granatum* has antioxidant properties with a nutritive value.

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