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# The Nephroprotective Effects of Pomegranate Juice against Lithium-Induced Kidney Damage in Rats

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***ABSTRACT***

**Background***:* This study aims to investigate the effect of pomegranate juice on kidney damage caused by lithium in rats. **Methods**: Six groups of animals were studied, group one received neither lithium nor pomegranate juice, group two received only lithium, group three received only pomegranate juice, and groups four to six received both lithium and pomegranate juice at different doses. Kidney biomarkers were investigated as indicators of acute kidney failure. **Results**: Comparison between the studied groups regarding the amount of creatinine in the serum of rats showed a significant relationship between serum creatinine levels of rats in the group receiving lithium and the control group, and the group receiving lithium and those receiving pomegranate juice at a dose of 100 mg/kg. A significant relationship was observed in the study of serum urea amount in rats between the control group and the group receiving lithium, and the lithium group and the group receiving pomegranate juice at a dose of 100 mg/kg. **Conclusions** There was no significant difference between the groups when the amount of cystatin C in the serum of rats was compared. Pomegranate juice at 100 mg/kg and lithium (25mg/kg) led to a significant decrease in serum creatinine levels, which reduced kidney damage induced by lithium. Serum urea also decreased significantly, suggesting that pomegranate has anti-toxic effects on kidneys from lithium toxicity

**Keywords:** *Pomegranate Juice; Nephroprotective; Lithium; Rat*

## Introduction

Lithium is a medication commonly used to treat bipolar disorder, which has been proved effective in stabilizing mood swings, reducing the risk of suicide, and improving overall quality of life (Curran and Ravindran, 2014). However, long-term use of lithium has been associated with the development of chronic kidney disease, which can progress to end-stage renal disease if not properly managed (Pahwa *et al.*, 2021). Several studies have shown that chronic lithium use can lead to a decrease in glomerular filtration rate (GFR) and an increase in serum creatinine and blood urea nitrogen (BUN), all of which are markers of kidney dysfunction (Azab *et al.*, 2015, Tondo *et al.*, 2017, Turan *et al.*, 2002). Therefore, the use of antioxidants could be crucial for the treatment of lithium-induced nephrotoxicity (Ossani *et al.*, 2019). Pomegranate (*Punica granatum*) has been

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identified as a potential protective agent against kidney damage caused by various factors, including exposure to toxins, underlying medical conditions, infections, autoimmune disorders, and genetic factors (Adebisi *et al.*, 2022, Choi *et al.*, 2011, Doostan *et al.*, 2017). The protective effect of pomegranate juice extract is believed to be attributed to its antioxidant and anti-inflammatory properties, which can help mitigate the damage caused by these factors (Moneim *et al.*, 2011). Pomegranate juice is rich in antioxidants e.g. natural substances that help protect cells from the damage caused by free radicals. The antioxidant properties of pomegranate juice extract stem from its high concentration of polyphenols, including punicalagin, ellagic acid, and anthocyanin (Gil *et al.*, 2000, Seeram *et al.*, 2005). These compounds have been shown to scavenge free radicals and inhibit oxidative stress in kidneys (Amor *et al.*, 2020). In addition to its antioxidant properties, pomegranate juice extract has also been shown to have anti-inflammatory effects. Chronic inflammation is another contributing factor to many chronic diseases, and research has suggested that the anti-inflammatory properties of pomegranate juice extract may help to reduce inflammation in body (Asgary *et al.*, 2014, Basu and Penugonda, 2009). One study conducted on rats showed that pomegranate juice extract significantly reduced oxidative stress and inflammation in kidneys, leading to improved kidney function (Karwasra *et al.*, 2016). Another study on diabetic rats demonstrated that pomegranate juice extract decreased serum creatinine and BUN levels, indicating improved kidney function (Sun *et al.*, 2021). However, the potential of pomegranate juice extract to protect against lithium-induced kidney damage has not been extensively studied.

The current study aims to investigate the effect of pomegranate juice extract on kidney damage caused by lithium in rats. By assessing the changes in renal biomarkers in rats treated with lithium and pomegranate juice extract, the study will provide insights into the potential of pomegranate juice extract as a protective agent against lithium-induced kidney damage.

## Material and Methods

*Experimental animals:* Thirty-six male rats (Wistar) with an average weight of 275 grams were procured from Infertility Center of Shahid Sadoughi University of Medical Sciences, Yazd. The rats were fed with water and specialized food purchased from laboratory animal food factories. All the rats were kept in groups of 6 in separate cages under standard conditions- a photoperiod of 12 hours of darkness and 12 hours of light (6 am to 6 pm) with a room temperature of 23 ± 2 °C during the entire study. These conditions were maintained to minimize any external influences on the rats and ensure that they were in optimal physical health throughout the study. Diet was available at all times (libitum diet). Protocol of the study approved by the Institutional Animal Ethics Committee

*Pomegranate juice preparation:* Pomegranate juice was examined and extracted from the collected pomegranates using a juicer. The juice was then dried by placing 30 plates containing the juice in a refrigerator at -80 °C for one day and then in a freeze dryer for 72 hours. To perform gavage, different concentrations of pomegranate juice were needed depending on the weight of the mouse. For example, a concentration of 100 mg/kg was needed for a mouse weighing 250 g, while volumes of 0.5 ml and 0.25 ml were required for gavage regarding the mice weighing 50 g and 25 g, respectively. The required volume of injection was calculated based on the maximum volume, which was determined to be 1 ml per 1000 g of mouse weight. The prepared pomegranate juice was stored in the refrigerator, and before gavage, a volume of 30 ml was aliquoted from a volume of 100 ml every day, which was enough for 3 days of gavage.

*Experimental protocol:* 6 groups of rats were randomly divided, each containing six rats. Group 1 was the control group, receiving neither lithium nor pomegranate juice but the corresponding solvent. Group 2 was administered 25 mg/kg solution of lithium carbonate in 0.9% NaCl as an intraperitoneal injection for 14 days. Group 3 was



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given 50 mg/kg pomegranate juice per day for 2 weeks through gavage. Group 4 was given 25 mg/kg lithium and pomegranate juice per day for 1 hour after the prescription of lithium for 2 weeks through gavage. Group 5 was given 50 mg/kg lithium and pomegranate juice for 1 hour per day after the administration of lithium for 2 weeks through gavage. Group 6 was given 100 mg/kg lithium and pomegranate juice per day for 1 hour after the administration of lithium for 2 weeks through gavage. After cervical dislocation, blood samples were taken from retro-orbital plexus of all the animals. The blood was allowed to clot for 30 minutes and then centrifuged at 500 g for 15 minutes at 4 °C to separate the serum, which was then stored at -70 °C until analysis.

*Renal injury biomarkers:* The levels of serum urea and creatinine were measured using an automated analyzer (SYNCHRON LX20, Beckman Coulter Inc., Ireland) with the help of diagnostic kits from Becman Coulter. Cystatin-C (cat no: RAB0890) was measured by ELISA kit methods according to the instruction of the manufacturer.

*Ethical considerations:* The protocol of study was approved by ethical committee in Shahid Sadoughi University of Medical Sciences with Ethic code: IR.SSU.MEDICINE.REC.1398.337. It followed the NIH guidelines for the care and use of animals. Injectable-pentobarbitone sodium IP at 180 mg/kg was the method of the euthanasia, and there was no need for RCT registration number and not applicable

*Data analysis:* The data was presented as mean±SE, and statistical analysis was carried out through one-way ANOVA using SPSS version 23. The significance level between the two groups was determined by Dunnett's multiple comparison test and P-value < 0.05 was statistically significant.

## Results

The findings indicated that the rats in group 2 (receiving lithium) had the highest serum creatinine levels compared to other groups (0.97±0.12 mg/dl). The one-way ANOVA revealed a significant decrease in serum creatinine levels ($P$=0.017) in the treatment groups compared to group 1 (the control group). Moreover, group 3, which received empty pomegranate juice and lithium ($P$=0.011), and group 6, which received pomegranate juice at a dose of 100 mg/kg along with lithium ($P$=0.026) showed a significant difference in comparison with group 2 which only received lithium (**Figure 1**). These results suggested that pomegranate juice had a protective effect against lithium-induced increase in serum creatinine level.

The results of urea level in this study demonstrated that the serum urea level in the group receiving lithium was significantly higher than that of the other groups (58.93±3.93 µg/dl). One-way ANOVA indicated a significant decrease in serum urea level in rats ($P$<0.001). Moreover, significant differences were observed between the control group and the group receiving lithium ($P$<0.001), the control group and the group receiving pomegranate juice with 25 mg/kg lithium ($P$=0.008), the group receiving lithium and the group receiving pomegranate juice with 100 mg/kg lithium ($P$<0.001), the group receiving lithium and the group receiving empty pomegranate juice ($P$=0.031), the group receiving pomegranate juice with 25 mg/kg lithium, and the group receiving pomegranate juice with 100 mg/kg lithium ($P$=0.015, **Figure 2**). These findings suggested a significant relationship between lithium and serum urea levels, as well as the potential therapeutic effects of pomegranate juice in reducing urea levels.

The present study investigated the effect of pomegranate juice on serum level of cystatin C in rats. The results demonstrated that the highest level of cystatin C was observed in the group receiving 25 mg/kg pomegranate juice. However, the variable did not show a normal distribution, and the Kruskal-Wallis test showed no significant difference among the groups in terms of serum cystatin C level. These findings suggested that 25 mg/kg pomegranate juice may have a potential effect on cystatin C level, but further investigations were required to confirm this result (**Figure 3**).



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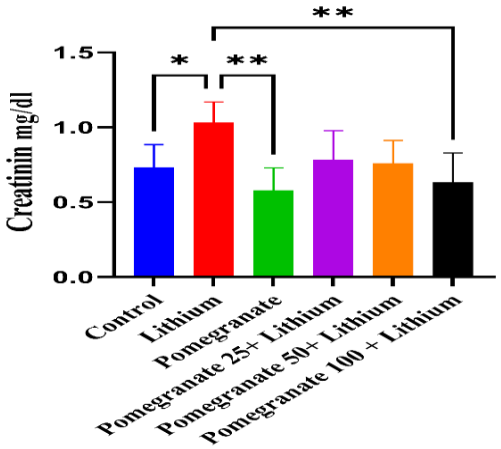


**Figure 1.** Comparing the studied groups regarding the amount of creatinine in the serum of rats.
* P < 0.05, ** P < 0.01.

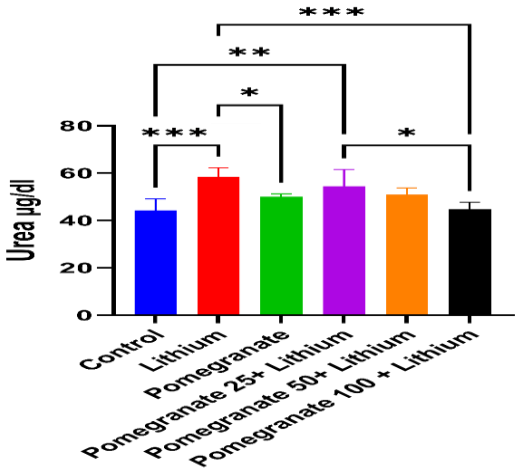


**Figure 2.** Comparing the studied groups regarding the amount of urea in the serum of rats.
* P < 0.05, ** P < 0.01, *** P < 0.001

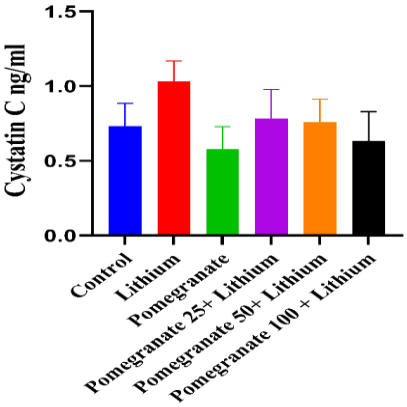


Figure.3. Comparison of the amount of cystatin C in the serum of rats between the studied groups



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## Discussion

Pomegranate (Punica granatum L.) is one of the oldest known edible fruit tree species, originated from Central Asia, but with a wide global geographical distribution, which indicates its adaptability to a wide range of climatic conditions (da Silva *et al.*, 2013). The biological medicinal activities of its compounds (tannins, flavonoids, alkaloids, organic acids, triterpenes, steroids, etc.) from different parts of the pomegranate plant reduce blood lipids; have antioxidant properties, antiviral, anti-neoplastic, anti-cancer antidiabetic, antidiarrheal, anthelmintic, vascular and digestive protective effects, and protect immune system (Chandra and Babu, 2010, Syed *et al.*, 2007). In this study, the effect of pomegranate juice on lithium-induced nephrotoxicity in rat's kidney biomarkers was investigated.

The results indicated a significant increase in serum creatinine level in rats receiving lithium, which revealed renal toxicity. However, the administration of 100 mg/kg pomegranate juice could overcome the increase in creatinine caused by lithium. These findings were in line with other studies which investigated the protective role of pomegranate juice against renal toxicity induced by steroids and cisplatin in rats (Ali *et al.*, 2017). The protective effect of pomegranate juice may be attributed to its high content of vitamin C and phenolic compounds, which act as strong antioxidants and free radical scavengers (Boroushaki *et al.*, 2015). These compounds can prevent the progression of oxidative kidney damage and provide renal protection against irreversible cellular damage caused by chemicals like lithium (Jilanchi *et al.*, 2014, Riezzo *et al.*, 2014). This effect may be attributed to the increase in the activity of antioxidant enzymes in the prescribed groups of pomegranate, which leads to an increase in the ability of the kidneys in eliminating toxic free radicals such as hydrogen peroxide and lipid peroxides.

The findings of the study revealed a significant decrease in the amount of urea in the serum of rats receiving 100 mg/kg pomegranate juice along with lithium compared to the other groups. However, the amount of urea did not decrease significantly in the group receiving 25 mg/kg pomegranate juice with lithium. These findings suggested that pomegranate juice may have a dose-dependent effect on reducing kidney's toxicity biomarkers.

The results of this study were consistent with previous studies that reported the beneficial effects of pomegranate seed oil and extract in reducing kidney toxicity markers (Boroushaki *et al.*, 2014). Moreover, the current study's assumption was based on the effect of antioxidant compounds in pomegranate seeds on reducing kidney toxicity markers.

Lithium-induced nephrotoxicity is associated with oxidative stress and free radical production, which leads to lipid peroxidation and tubular damage (Alkuraishy *et al.*, 2019). Furthermore, previous studies investigated the protective effect of different natural compounds against lithium-induced nephrotoxicity. For instance, rosemary extract improved the level of oxidative stress and biomarkers of kidney function in rats treated with lithium (Mwaheb *et al.*, 2016). These findings suggested that natural compounds may provide a promising approach for preventing and treating lithium-induced nephrotoxicity.

As a result of this study, there was no significant change in the amount of cystatin in the serum of rats. Cystatin C has a molecular weight of approximately 13.3 kDa and is removed from bloodstream by glomerular filtration in kidneys. Cystatin C levels in blood increase when kidney function and glomerular filtration rate decrease (Corrao *et al.*, 2006, Onopiuk *et al.*, 2015, Perkins *et al.*, 2005, Premaratne *et al.*, 2008).

## Conclusion

In this study significant kidney damage was caused by lithium via increasing serum creatinine levels. Similarly, the significant difference in urea biomarker levels between the group receiving 100 mg/kg pomegranate along with lithium, the group receiving only lithium, and the control group, supports this conclusion. These findings suggest that pomegranate may have a protective effect on kidneys when used in conjunction with lithium.



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## Authors' contributions

Each author participated sufficiently in the work to take public responsibility for appropriate portions of the content. Jamshidi HR, conceived, planned and analyzed data of the research project and Sedighi M conducted experiments.

## Conflict of interests

The authors declared no conflict of interests.

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