IJKD KIDNEY DISEASES

Protective Effect of the Hydroalcoholic Extract of *Pelargonium Graveolens L.* on Rats with Acetaminophen-Induced Nephrotoxicity

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Keywords. pelargonium, acetaminophen, kidney, antioxidants, toxicology.

Original Paper

Introduction. Geranium has various antioxidant, anti-inflammatory, and anti-microbial effects. Prescribing glutathione probably enhances the protective mechanisms of nephrons against oxidative stresses. This study aimed to evaluate the protective effect of geranium on acetaminophen-induced nephrotoxic rats.

Methods. In the present study, 70 mice were divided into seven groups. In five groups (T1, T2, T3, T4, and T5), different doses of geranium were given by gavage to the mice for seven days, then on the 8th day, a high dose of acetaminophen was administered intraperitoneally. Group T5 only received geranium extract. The control group received neither acetaminophen nor the extract while the last group received only a toxic dose of acetaminophen. Twenty-four hours after the last drug administration, blood samples were taken to check the levels of uric acid, blood nitrogen, and creatinine. The data were analyzed in SPSS version 25. To investigate the between-group factors' effects, one-way ANOVA with Tukey's post hoc test was performed at the alpha level of < 0.05.

Results. The differences between the levels of blood creatinine, urea, and uric acid were significant ($P < .001$) among the groups. The mean blood urea for groups T3 and T4 were similar, and they had a significant difference in comparison with the control group ($P < .05$). The mean creatinine levels were similar between T4, T5, and the control groups and were significantly different from the other groups ($P < .05$). Blood uric acid for groups T1 and T2 were similar to Group B and higher than the other groups ($P < .05$).

Conclusion. The results showed that by strengthening cell protection mechanisms against oxidative stress, geranium extract reduces the toxic effects of acetaminophen on mice's kidney function and thus ameliorates the damage. As a result, the geranium extract has no adverse effects on kidney function.

IJKD 2022;16:108-14
www.ijkd.org
DOI: 10.52547/ijkd.6679

INTRODUCTION

The few side effects of Acetaminophen (paracetamol) at therapeutic doses and its accessibility have made it a very common medication for relieving headaches and reducing fevers worldwide. However, the use of this drug at high doses causes serious damage to different organs such as the kidney and liver. The easy access to this drug may explain the

high prevalence of its toxicity. Based on the annual report of the US AAPCC, more than 50000 cases of pure acetaminophen toxicity and more than 17000 cases of toxicity in combination with other drugs, were reported in the US in 2018.[1] Toxicity with this drug in the UK is the most common cause of liver failure requiring liver transplantation. Acetaminophen, at the therapeutic dose, is usually metabolized by sulfation and glucuronidation, and less than 5% of it is oxidized by the P450 cytochrome system. However, when it is taken at high doses, the glutathione and sulfate reserves are decreased, and acetaminophen metabolism shifts towards the P450 oxidase system, thereby increasing the N- Acetyl –P- benzoquinonimini (NAPQI) metabolite.[2] By activating the lysosomal enzymes, it starts cell apoptosis and death, eventually leading to tissue necrosis and organ dysfunction.[3,4] Hepatic acetaminophen toxicity has been extensively studied,[5-7] but its toxic effects on other tissues have received less attention. Acetaminophen increases the risk of kidney dysfunction by 23 to 37%,[8-12] and acute kidney failure has been reported in 2% of patients with acetaminophen toxicity.[13] Acetaminophen toxicity is observed at doses > 150 mg/kg. Currently, the drug of choice for treating acetaminophen toxicity is N-Acetylcystein, available in the injectable and oral form.[14],[15] Recently, the use of medicinal herbs has received more attention in treatment and control of different diseases because of their fewer side effects and lower cost. Sweet-scented geranium (*Pelargonium graveolens*) is a genus belonging to the family Graniaceae. It is a slow-growing and perennial plant, whose brewed form is used in Iran as a tranquilizer. The hydro-alcoholic extract of the leaf of this plant has flavonoid compounds and vitamins A&E with antioxidant effects.[16] This plant has numerous therapeutic effects, such as antibacterial, analgesic, anti-itching, and cholesterol- and blood glucose-reducing.[2, 17-22]

Today, the use of medicinal plants is growing worldwide because they have fewer side effects than chemical drugs. However, the use of acetaminophen is increasing daily, and there is little knowledge about the renal side effects of acetaminophen toxicity and the details of its treatment. *Pelargonium graveolens L.* has proved antioxidant properties; no studies has been done on the effect of it on acetaminophen-induced nephrotoxicity. Therefore, the aim of the present study was to evaluate the effects of *the hydroalcoholic extract of Pelargonium graveolens L.* on renal damages induced byacetaminophen in rats.

MATERIALS AND METHODS

This case-control study was approved by the Ethics Committee (IR.IAU.REC.1399.069) of the Islamic Azad University of Kazerun, and examined the protective effects of geranium on renal toxicity resulting from acetaminophen in mice.

Animals And Treatments

The mice were kept in the same laboratory conditions and were divided into seven groups. Five groups received different doses of *P. graveolens* extract for 7 days: one group was monitored to examine the possible side effects of the extract, and the other four groups received a toxic dose of acetaminophen on the 8th day to induce nephrotoxicity. The control group received neither acetaminophen nor the extract, while the last group received only a toxic dose of acetaminophen.

Extract Preparation

Geranium leaves were separated, dried at 25 °C (room temperature) in shade, and powdered by a mechanical mill. Afterward, 15 mg of the powder was placed in 1 Liter of 80% ethanol for 72 hours. This mixture was filtered and placed on the rotary device, and then the solvent was separated. The mixture was placed in a Petri dish under a hood for a week, and the resulting extract remaining at the bottom of the dish was used.

Acetaminophen Preparation

Based on the manufacturer's (Sigma-Aldrich) guidelines, acetaminophen powder was dissolved in normal saline solution at 50 °C. Subsequently, the temperature was lowered to 37 °C, and acetaminophen was injected at a dose of 600 mg/kg to the mice intraperitoneally.

Mice Selection

Seventy mice weighing between 20 to 25 g were selected. During the study, all the mice were kept in one room, received standard laboratory food, and had ad libitum access to water. The room temperature was 25 to 27 °C with a relative humidity of 60 ± 5%. The mice were kept under a 12:12 light/dark cycle.

Treatment

The mice were randomly divided into seven groups of 10 each (A, B, T1 to 5).

- Group A: Received physiological serum for 7 days.
- Group B: Had the routine diet and received acetaminophen at a dose of 600 mg/kg on the eighth day.
- Group T1: Received 50 mg/kg of geranium leaf extract daily for 7 days and received acetaminophen at a dose of 600 mg/kg on the eighth day.
- Group T2: Received 100 mg/kg of geranium leaf extract daily for 7 days and received acetaminophen at a dose of 600 mg/kg on the eighth day.
- Group T3: Received 200 mg/kg of geranium leaf extract daily for 7 days and received acetaminophen at a dose of 600 mg/kg on the eighth day.
- Group T4: Received 300 mg/kg of geranium leaf extract daily for 7 days and received acetaminophen at a dose of 600 mg/kg on the eighth day.
- Group T5: Received 200 mg/kg of geranium leaf extract daily for 7 days.

Twenty-four hours after the last administration of the drug, the mice were anesthetized by using chloroform and their heart blood samples were taken. Subsequently, the blood urea nitrogen (BUN), creatinine and uric acid levels of the samples were measured by an auto-analyzer via spectrophotometry. After sampling and during the time mice were under anesthesia, they were transferred to a CO_2 box to ensure an easy demise.

Data Analysis

The data were analyzed in IBM SPSS statistics software, version 25. To investigate the between-group factors' effects, one-way ANOVA with Tukey's post hoc test were performed at the alpha level of < 0.05.

RESULTS

Mice, in seven groups of 10, were compared in terms of renal function. A comparison of the means of the data showed that the highest level of blood urea nitrogen, creatinine, and uric acid was found in a group receiving the toxic dose of acetaminophen without geranium extract. In the groups receiving the extract, despite receiving acetaminophen at a high dose, the kidney function tests were less disturbed. We still observed that some groups did not significantly differ from the control group. The ANOVA test result was significant for BUN differences ($P < .001$), indicating unequal means of BUN between the groups. By using post hoc tests, we examined the pairwise comparisons. We found that groups A, T3, T4, and T5 had the lowest mean levels of BUN, and the difference was significant in comparison with the other groups. The level of BUN in T1 was similar to group B; therefore, the administration of a low dose of the plant showed no significant protective effects on renal function (Table 1). As shown in Table 2, groups receiving 200 and 300 mg/kg of geranium extract (T3, T4) had similar mean BUN levels to the control group, and the mean levels were significantly different from group B, which was not receiving the extract. Mean blood creatinine was significantly different between the groups according to the results of ANOVA test ($P < .000$). In Tukey's test, the mean blood level of creatinine was similar in groups T4, T5, and the control group, showing with no significant differences, while the mean levels significantly differed from the other groups ($P < .001$, Table 3).

The comparison of blood uric acid levels yielded similar results. The uric acid levels were higher for groups T1 and T2 than the other groups, and

Table 1. Mean Values of Uric Acid, BUN, and Creatinine in Different Groups

Groups	BUN (mg/dL)	Uric Acid (mg/dL)	Creatinine (mg/dL)
A (Control)	20.40 ± 6.29^{a}	7.40 ± 0.39^{a}	0.60 ± 0.06^{a}
B (Acetaminophen)	50.40 ± 3.64^{d}	12.82 ± 0.70^{d}	1.61 ± 0.11^{d}
T1 (50mg/kg)	46.30 ± 3.314^{cd}	11.97 ± 0.883^{cd}	1.54 ± 0.093^{cd}
T2 (100mg/kg)	40.50 ± 3.152^{c}	10.43 ± 0.553^{bc}	1.41 ± 0.110^{bc}
T3 (200mg/kg)	32.10 ± 1.728^{b}	9.01 ± 0.495^{ab}	1.10 ± 0.148^{ab}
T4 (300mg/kg)	26.90 ± 3.816^{ab}	8.68 ± 0.504^{a}	0.928 ± 0.125^{ab}
T5 (Sh 200mg/kg)	24.00 ± 2.37^{ab}	8.26 ± 0.68^{a}	0.67 ± 0.119^{a}

Table 2. Comparing the BUN Levels Across the Groups

Group	N	Subset for Alpha = 0.05		
		1	2	3
A	10	20.40		
T5	10	24.00		
T4	10	26.90		
T3	10	32.10	32.10	
T2	10		40.50	40.50
T1	10			46.30
B	10			50.40
Sig*		0.42	0.42	0.23

*Comparison based on Tukey's test

Table 3. Comparison of the Mean Creatinine Levels Across the Groups

Group	N	Subset for Alpha = 0.05				
		1	2	3	4	5
A	10	0.60				
T5	10	0.67	0.67			
T4	10	0.92	0.92	0.92		
T3	10		1.10	1.10	1.10	
T2	10			1.41	1.41	1.41
T1	10				1.54	1.54
B	10					1.61
Significance*		0.39	0.11	0.52	0.11	0.87

*Tukey's test

similar to the group receiving a toxic dose of acetaminophen without the extract (group B). The ANOVA showed a significant difference in the uric acid levels across the groups ($P < .000$). Turkey's post hoc test indicated a significant difference in the comparison of these three groups with the other groups. The uric acid levels were minimum for T3, 4, and 5 groups which did not significantly differ from the control group ($P > .05$, Table 4).

No significant difference was found in examining all three above-mentioned variables between T5 and the control groups; therefore, it seems improbable that the geranium extract should have negative effects on renal function ($P > .05$; Tables 2, 3, 4).

Table 4. Comparison of the Mean Uric Acid Levels Across the Groups

group	N	Subset for Alpha = 0.05		
		1	2	3
A	10	7.40		
T5	10	8.26	8.26	
T4	10	8.68	8.68	
T3	10	9.01	9.01	
T2	10		10.43	10.43
T1	10			11.97
B	10			12.82
Significance*		0.53	0.19	0.11

*Tukey's test

DISCUSSION

Acetaminophen is a commonly used drug for relieving headache and fever. Excessive use of this drug reduces the glutathione and sulfate reserves in the organs in charge of its metabolism. In addition, by activating the P450 cytochrome system, acetaminophen is converted into the toxic metabolite NAPQI.[23] By binding to glutathione, this metabolite turns into water-soluble mercapturic and is excreted by kidneys. In cases of acetaminophen toxicity, NAPQI is produced excessively and damages nephrons by binding to the proteins in these cells. The severity of the damage depends on the glutathione reserves and P450 cytochrome, which is mostly in the proximal tubules of kidneys.[24,25] Due to their beneficial compounds and few side effects, medicinal herbs have recently received attention by researchers who are discovering their further therapeutic uses. The antioxidant effect of medicinal substances has been demonstrated in different studies. Various studies have investigated different medicinal herbs such as ginger, licorice, *Taraxacum syriacum*, and green tea to find a way to reduce the toxic effects of acetaminophen on kidneys.[26-30] Geranium is traditionally used in Iran as a brew to alleviate gastric symptoms, some topical diseases, and serves as a tranquilizer as well.[31,32] Various studies have examined different effects of this plant. Researchers have reported the antioxidant effects of *G. sanguineum* on improving the reproductive system function against oxidative stresses by reducing lipid peroxidase;[33] its anti-inflammatory and anti-allergic effects and its effects on improving the respiratory function after induced asthma, proposing it as a new treatment for respiratory system inflammation;[34] its significant effect on blood glucose reduction in diabetes at a 150 mg/kg dose;[22] its analgesic effect due to its flavonoid compounds at the doses of 200 and 600 mg/kg;[35] and its antioxidant effect on the cell membrane. This plant also reduces the osmotic hemolysis of red blood cells and increases their resistance against H_2O_2. Its ability to reduce lipid peroxidation has been also reported as its protective mechanism and effect against influenza infection.[36] This study is among the few studies examining the protective effect of geranium on

renal function after inducing renal toxicity by acetaminophen in mice. The results revealed that the administration of acetaminophen at a dose of 600 mg/kg clearly disrupted the mice's kidney function. The administration of geranium extract for 7 days at a dose of 200 mg/kg did not have a negative effect on the kidneys (T5). At doses of 200 and 300 mg/kg, (T3 and T4), it even had a protective effect against the stress resulting from acetaminophen induced renal toxicity. The renal function tests of groups T3 and T4 were similar to the control group, which did not receive acetaminophen, and were significantly better than the other groups. This difference was not observed in the groups receiving lower doses of the extract (Tables 2, 3, 4).Although the levels of glutathione were not evaluated in this study, glutathione reserve-increasing effects have been reported after the consumption of geranium in the AFAF study; it seems that this extract reduces the toxic effect of acetaminophen on the kidneys of the examined miceand ameliorates renal damage with the same mechanism.[36]

CONCLUSION

In this study, we investigated the protective effect of the hydroalcoholic extract of *Pelargonium graveolens L.* on rats with acetaminophen-induced nephrotoxicity. In the groups receiving the extract, despite receiving acetaminophen at a high dose, the kidney function tests were less disturbed. Still, we observed that some groups did not significantly differ from the control group. The results showed that the groups receiving 200 and 300 mg/kg of geranium extract (T3 and T4) had similar mean BUN levels to the control group and significantly differed from group B receiving only the extract. This study is one of the few studies that has examined the protective effect of geranium on renal function after inducing renal toxicity by acetaminophen in mice. It seems that, by strengthening cell protection mechanisms against oxidative stress, geranium extract reduces the toxic effect of acetaminophen on mice's kidney function loss and thus ameliorates the damage.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All the experiments were carried out in accordance with the guidelines of the Ethics Committee (IR.IAU.REC.1399.069) of the Islamic Azad University of Kazerun.

HUMAN AND ANIMAL RIGHTS

No humans were involved in this study and the reported experiments on animals were in accordance with the guidance of ethical committee for research on laboratory animals of Islamic Azad University of Kazerun, which is in accordance with the Helsinki Declaration.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

All data presented by this paper.

FUNDING

This research was supported by the Islamic Azad University of Kazerun (#1399.069).

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

We thank Edris Hoseinzadeh, PhD, from Saveh University of Medical Sciences (www.savehums.ac.ir), for valuable comments.The authors are grateful to the Deputy for Research, the Islamic Azad University of Kazerun for administrative assistance.

SUPPLEMENTARY MATERIAL

There is no supplementary material.

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Received September 2021
Revised November 2021
Accepted January 2022