DOI: https://doi.org/10.53350/pjmhs20231711359

ORIGINAL ARTICLE

Evaluating Synergistic Nephroprotective Effect of Parsley Extract and Ramipril in a Rat Model of Gentamicin-Induced Nephrotoxicity

EISHA AKRAM[1], SADIA LODHI[2], AYESHA AFTAB[3], ABDUL AZEEM[4], ANUM USMAN[5], AYESHA SARWAR[6], AHSANULLAH MIRBAHAR[7]
[1]*Assistant Professor of Pharmacology Department Watim Medical and Dental College, Rawat Islamabad*
[2]*Assistant Professor of Pharmacology Department Watim Medical and Dental College, Rawat Islamabad*
[3]*Professor of Pharmacology Department Al Nafees Medical College and Hospital, Islamabad*
[4]*Associate Professor of Pharmacology Department Watim Medical and Dental College, Rawat Islamabad*
[5]*Associate Professor of Pathology Department Al Nafees Medical College and Hospital, Islamabad*
[6]*Associate Professor of Pathology Department of HBS Medical & Dental College, Islamabad*
[7]*Senior Research Officer, HRI-NIH Research Centre, National Institute of Health, Islamabad*
Correspondence to: Ayesha Aftab, Email: drayeshah1986@gmail.com

ABSTRACT

Background: The kidney has an essential function in maintaining homeostasis by carrying out functions such as blood filtration, hormone synthesis, and drugs metabolism. This study is done to examine the combined effects of ramipril and parsley Leaves water-soluble extract on nephrotoxicity induced by gentamicin.

Methods: A case control study was carried out at the Pharmacology Department Islamic International Medical College in collaboration with NIH Islamabad, from September 2019 to August 2020. This study was conducted with a sample of 50 healthy male albino rats. After collecting baseline measurements of urea and creatinine randomly, rats were divided into two groups. Group 1 was designated as the control group and remaining four groups were experimental groups. On day 35, the results were analyzed using biochemical parameters and histopathological evaluations. The statistical analysis was conducted using SPSS version 22. The One-way ANOVA test was applied to ascertain any variations in mean values. The post hoc Tukey's test has been used to perform several comparisons among the groups. A significance level was $p<0.05$.

Results: The treatment group 5, which received both parsley and ramipril, exhibited a significant decrease in serum creatinine and urea levels in nephrotoxic rats, in comparison to group 3 (Group treated with parsley) and group 4 (Group treated with Ramipril). It also exhibited significant improvements in histological evaluation. The group reported reduced indications of renal injury, including minimal infiltration of inflammatory cells, minimal vacuolation and necrosis.

Conclusion: The current investigation has determined that the aqueous extract of parsley leaves and the drug ramipril both independently improve the nephrotoxic effects caused by gentamicin.

Keywords: Gentamicin, Nephrotoxicity, Ramipril, Parsley leaves extract, Serum Creatinine & Urea level.

INTRODUCTION

The kidney, a vital organ in the human body, has an essential function in maintaining homeostasis by carrying out functions such as blood filtration, hormone synthesis, and drugs metabolism. The kidney receives about 20% of cardiac output, which puts it at significant risk of damage due to drugs and toxin exposure[1]. Acute kidney damage (AKI), a prevalent and serious clinical issue, is distinguished by a high rate of morbidity and mortality. Epidemiological research suggests that there is a significant occurrence of chronic kidney disease (CKD) after an episode of acute kidney injury (AKI), with drug-induced nephrotoxicity being a prominent contributing factor[2]. Worldwide, between 19% and 25% of cases of kidney damage in critically ill patients are caused by nephrotoxic drugs, which can be identified through elevated levels of urea and creatinine in the blood[3-4]. There has been a worldwide increase in the prevalence of CKD. CKD ranked as the 27th most common cause of mortality worldwide in 1990, but surged to the 18th position by 2010. CKD stages 1–5 have a global prevalence of around 23.4%, and Pakistan has experienced a significant rise in CKD cases due to inadequate healthcare facilities[5].

Aminoglycosides, including gentamicin, have played a vital role in the treatment of severe infections since the 1940s. Although they are highly effective, one important disadvantage is nephrotoxicity, which mostly damages the renal tubular cells[6-7]. Angiotensin-converting enzyme inhibitors (ACEIs), including ramipril, are currently considered as optimal antihypertensive drug has demonstrated effectiveness in decreasing proteinuria and tubular damage[8]. Simultaneously, there is increasing interest with natural antioxidants derived from herbs such as parsley (Petroselinum crispum) due to their potential health benefits, including nephroprotection. Parsley exhibits notable therapeutic qualities, such as anti-inflammatory, anti-edematous, and antioxidant activity, which are essential for the regeneration of kidney tissue following nephrotoxicity[9-10].

Received on 23-04-2023
Accepted on 05-10-2023

The aim of our study is to evaluate the combined nephroprotective effect of a water-based extract of parsley leaves and Ramipril on gentamicin-induced kidney injury in rats. This study proposes that the collaboration between these agents might improve their particular protective characteristics while potentially minimizing adverse effects. The research aims to support nephroprotective measures, particularly in areas such as Pakistan where the prevalence of CKD is increasing and healthcare resources are frequently limited.

MATERIAL AND METHODS

This research was a case control study carried out at the Department of Pharmacology (reference number Riphah/IIMC/IRC/19/0354) in collaboration with the National Institute of Health (NIH) Islamabad, extending a duration of one year from September 2019 to August 2020. Forty adult albino rats were bought from the animal house of NIH and housed in conventional cages under standard laboratory settings. The rats were subsequently segregated into four groups, and with each group including 10 animals. The study included healthy male albino rats with a weight range of 300-350 grams. Rats that were unhealthy or weighed below 300 grams were excluded from the study. Group 1 was the control group, providing a point of reference for regular biochemical parameters in comparison to the other experimental groups (group 2 to group 5). The control group was administered a standard baseline diet without any therapy.

The trial started with the measurement of blood creatinine and urea levels on day 0. High-quality Gentamicin and Ramipril for research purposes were obtained from Sigma Aldrich. The experimental group animals (groups 2, 3, 4, 5) were administered with Gentamicin at a dosage of 80mg/kg intraperitoneally (IP) for eight consecutive days to produce nephrotoxicity. The nephrotoxicity was assessed by measuring biochemical parameters one day after the final dose of Gentamicin. The RIFLE criteria, which stands for risk injury failure loss end stage renal disease, were employed to assess nephrotoxicity. Treatment was administered to rats in groups 3, 4, and 5 following the

development of nephrotoxicity. Total 100 grams of parsley leaves were finely chopped and boiled in 1000 ml of water for one hour. The solution was further purified by passing it through the Whatman filter paper No.1. Subsequently, the concoction was allowed to cool and preserved at a temperature of 4°C till it was ready for use. This extract was made biweekly. Each rat in group 3 and 5 received a daily oral dose of 2ml of parsley leaf extract for a duration of 4 weeks. Group 4 and 5 received a dosage of Ramipril at a rate of 10mg/kg of body weight per day, administered orally by drinking water, for a duration of four weeks. On the 35th day, the study was completed and the last blood samples were obtained by conducting a cardiac puncture. The serum urea level was measured using the Urease-GLDH enzymatic UV test. The measurement of serum creatinine was conducted using Jaffe's reaction. Histopathological sample were collected which were preserved in 10% formaldehyde. To assess the kidney injury, following parameters were considered. Tubular necrosis, infiltration of inflammatory cells and Vacuolization.

SPSS 22 was used to conduct statistical Analysis. The findings were interpreted as the average value ±the standard deviation (SD). One-way ANOVA was used in the analysis to compare the quantitative parameters among the five groups. Multiple comparisons between these groups were performed using the post hoc Tukey's test.

RESULTS

Biochemical parameters evaluation at first, the four groups in the current study had similar serum urea and creatinine levels. The rats in groups 2, 3, 4, and 5 experienced kidney impairment after receiving an intraperitoneal injection of gentamicin at a dose of 80 mg/kg/day for eight days. In order to assess the advancement of the study and verify the production of nephrotoxicity, two rats were randomly selected from each group on day 9 prior to any additional intervention.

The results indicated an impairment of renal function tests (RFTs). On day 35, at the last sample, the average serum creatinine levels for groups 1, 2, 3, 4 and 5 were 0.64 mg/dl, 1.78 mg/dl, 0.98 mg/dl, 1.05 mg/dl and 0.86 mg/dl respectively. Among the groups, a significant difference was noted. The mean serum urea levels were 24.70 ± 1.63 mg/dl in 1st group, 70.00 ± 3.92 mg/dl in 2nd group, 41.90 ± 2.48 mg/dl in 3rd group, 44.80 ± 2.81 mg/dl in 4th group and 36.30 ± 2.59 mg/dl in 5th group. Comparing the levels of urea between groups on day 35, no statistically significant differences were seen between group 3 versus 4, group 3 versus 5, and group 4 versus 5. However, significant differences were observed in the remaining groups as shown in (Table I).

Table 1: Post-hoc Tukey test was conducted to compare the levels of urea among different groups on day 35.

Urea		
Groups	Mean difference	p value
1 vs 2	-45.30000*	.000
1 vs 3	-17.20000*	.001
1 vs 4	-20.10000*	.000
1 vs 5	-11.60000*	.039
2 vs 3	28.10000*	.000
2 vs 4	25.20000*	.000
2 v 5	33.70000*	.000
3 vs 4	-2.90000	.947
3 vs 5	5.60000	.619
4 vs 5	8.50000	.216

The ANOVA analysis in table-II indicates a significant difference in means between groups, suggesting that at least two groups are distinct from each other. To further investigate this difference, a post hoc test for mean comparison (Tuckey's test) was conducted. Mean differences were used to compare the results in Tuckey's test. It was found that there was no significant difference between the groups in creatinine levels on day 35. The assessment of creatinine levels across groups on day 35 showed an insignificant statistical difference between the groups 1 vs 3, 1 vs 5, 3 vs 4, 3 vs 5 and 4 vs 5, which are all significant. (Table II).

Table 2: Post-hoc Tukey test was conducted to compare the levels of creatinine among different groups on day 35

Creatinine		
Groups	Mean difference	p value
1 vs 2	-1.14000*	.000
1 vs 3	-.34000*	.007
1 vs 4	-.41000*	.001
1 vs 5	-.22000	.161
2 vs 3	.80000*	.000
2 vs 4	.73000*	.000
2 v 5	.92000*	.000
3 vs 4	-.07000	.947
3 vs 5	.12000	.717
4 vs 5	.19000	.286

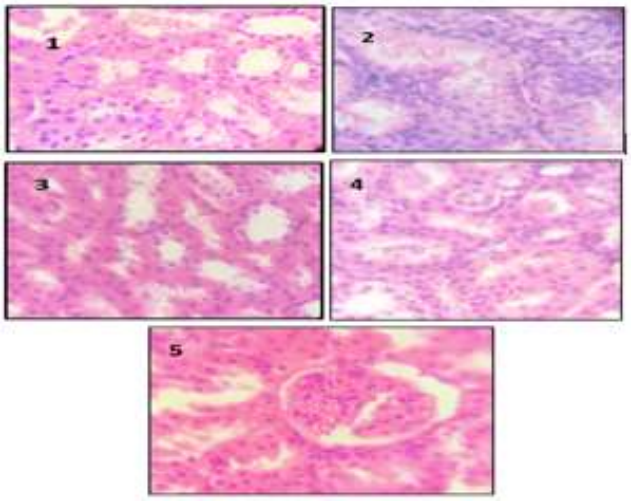

Figure 1: The histological characteristics indicate (1) Negligible (2) Severe (3) Minimal (4) Minimal (5) Minimal infiltration of inflammatory cells.

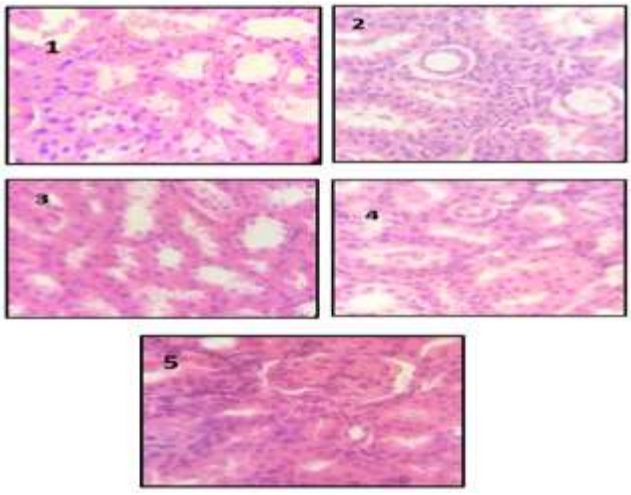

Figure 2: The histological characteristics indicate (1) Negligible (2) Moderate to Severe (3) Minimal (4) Mild to minimal (5) Minimal to negligible vacuolation of cells.

Histological evaluation for infiltration of inflammatory cells shown in Figure 1. The histological characteristics indicate all rats in group 1 had minimal infiltration of inflammatory cells. Within group 2, 40% of the rats had a moderate level of infiltration of inflammatory cells, while the remaining 60% showed a severe level of infiltration. A minimum penetration of 70% was observed in

group 3. Within group 4, 50% of the rats had low infiltration of inflammatory cells, while 40% displayed mild infiltration. Group 5 exhibited 30% negligible infiltration and 60% minimal penetration. The differences in infiltration grades were statistically significant among all groups, with a p-value of < 0.05.

Vacuolation showed in Figure 2, all rats in group 1 had normal renal architecture without vacuolation. Within group 2, 60% of the rats exhibited mild vacuolation, while 40% displayed severe vacuolation. Within group 3, 60% exhibited minimal Vacuolation, whereas 30% mild Vacuolation. Within group 4, 40% exhibited minor vacuolation, whereas the remaining 60% had mild vacuolation. Group 5 demonstrated 40% negligible vacuolation and 60% minimal vacuolation. The vacuolation of all groups exhibited a p-value of less than 0.05 indicates a significant difference.

The renal tubular necrosis showed in Figure 3, within group 1, 90% of the rats had a typical structure of the renal cortex. Within group 2, 60% exhibited moderate whereas the remaining 40% displayed severe tubular necrosis. Within group 3, 20% rats exhibited negligible, whereas 40% showed minimal and the remaining 40% displayed mild necrosis. Within group 4, 40% of the rats exhibited minor, whereas the remaining 60% displayed mild tubular necrosis. Group 5 exhibited 60% insignificant and 30% minor tubular necrosis. The tubular necrosis varied significantly among the groups, with a p-value of <0.05.

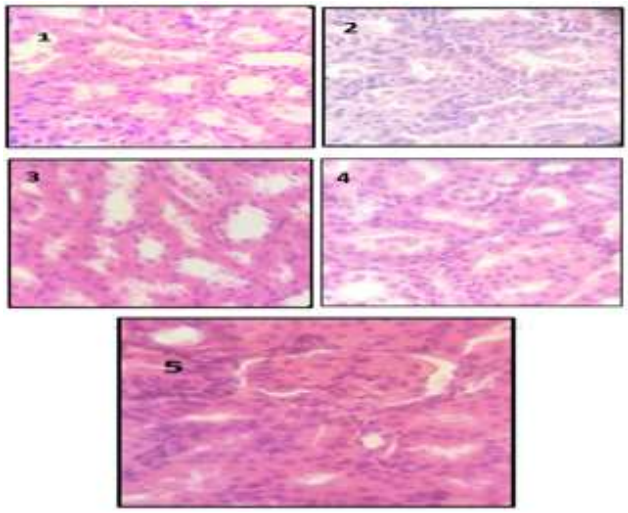

Figure 3: The histological characteristics indicate (1) Negligible (2) Moderate to Severe (3) Mild to Minimal (4) Mild to minimal (5) Minimal to negligible tubular necrosis.

DISCUSSION

The kidneys have a vital function in maintaining homeostasis within the circulatory system of the body, particularly by acting as the primary site for the removal and filtration of foreign substances (xenobiotics). Nephrotoxicity is caused by the buildup of these foreign substances, and is recognized as a primary factor in the development of end-stage renal disease (ESRD) [11]. Gentamicin, an aminoglycoside antibiotic, is used for its efficacy against gram-negative aerobic bacteria, however it is known to have significant adverse effects such as ototoxicity and nephrotoxicity. Its accumulation in the kidneys, results in brush border damage, acute tubular necrosis, and a reduced glomerular filtration rate (GFR). The main cause of gentamicin's adverse reactions is oxidative stress, which is characterized by a reduction in antioxidants and an elevation in the production of toxic substances. Free radicals, leading to lipid peroxidation and cellular necrosis in renal tubules. As a consequence, there is an increase in the amounts of urea and creatinine in the blood, which suggests damage to the kidneys[12].

The study demonstrated the fatal consequences of gentamicin poisoning by analyzing histology specimens and biochemical markers. Group 2 rats had notable tubular necrosis, nuclear alterations, and the presence of apoptotic cells. This discovery aligns with prior study conducted by Azouz[13], Erseçkin[14], Merdana[15], and Mehanna[16], who also reported comparable observational studies on kidneys. Parsley contains flavonoids, coumarins, tocopherol, and carotenoids, which provide it diuretic, nephroprotective, and antihypertensive effects. It is effective in treating kidney diseases, such as the formation of urinary stones[17]. The trial also includes Ramipril, an antihypertensive drug that improves the outcomes of nephropathy by decreasing intraglomerular pressure and proteinuria. The research findings demonstrate that Ramipril, whether administered alone or in conjunction with parsley, effectively enhances kidney functioning. This underscores its potential as a treatment for gentamicin-induced nephrotoxicity in rats[18].

The study reinforces the findings of Elkomy et al, demonstrating the nephroprotective properties of parsley against gentamicin-induced nephrotoxicity[19]. This is evidenced by the reduction in serum urea and creatinine levels. Additionally, there was seen improvement in the histopathological findings, in line with Pandit's study on the nephrotoxic effects of Cisplatin, which emphasizes the renal protective properties of parsley[20]. The study highlights the synergistic effects of the aqueous extract of parsley leaves and ramipril in group 5. The data demonstrate significant differences in urea and creatinine concentrations between group 5 and group 2, suggesting a protective effect on the kidneys during acute inflammation. The histological measures exhibited improvement, as evidenced by the presence of inflammatory cell infiltrate in 30% of rats in group 5, and the absence of vacuolation in 40% of rats. Group 5 rats experienced improved renal function, as evidenced by enhanced blood indicators and histology, following the simultaneous treatment of parsley and ramipril after nephrotoxicity.

CONCLUSION

The current investigation has determined that the aqueous extract of parsley leaves and the drug ramipril both independently improve the nephrotoxic effects caused by gentamicin. Furthermore, it has been established that the concurrent administration of ramipril and parsley have a synergistic impact on enhancing kidney function in cases of nephrotoxicity caused by gentamicin. This study also finds that when comparing the effects of ramipril and parsley leaves aqueous extract individually, parsley extract demonstrates superior efficacy to ramipril. Overall combination of parsley and ramipril is best among all in improving nephrotoxicity induced by gentamicin.

Disclosure Statement: No potential conflict of interest was reported by the author(s).

Funding: The author(s) reported there is no funding associated with the work featured in this article.

REFERENCES

1. Akram E, Waheed A, Munawar R, Mumal S, Azeem A. Comparative effects of parsley leaves aqueous extract and ramipril on gentamicin induced nephrotoxicity in rats. J Univ Med Dent Coll. 2022 Nov 3;13(4):478-82. DOI: https://doi.org/10.37723/jumdc.v13i4.729
2. El Latif AA, Zahra AE, Badr A, Elbialy ZI, Alghamdi AA, Althobaiti NA, Assar DH, Abouzed TK. The potential role of upregulated PARP-1/RIPK1 expressions in amikacin-induced oxidative damage and nephrotoxicity in Wistar rats. Toxicology Research. 2023 Oct;12(5):979-89. https://doi.org/10.1093/toxres/tfad091
3. Tahir M, Sadiq N, Ahmed S, Ali A, Rajpoot NN, Riaz U. Effects of aqueous and methanolic extracts of cichorium intybus seeds on gentamicin induced nephrotoxicity in rats. J Islam Int Med Coll 2018;13(4):184-8. https://journals.riphah.edu.pk/index.php/jiimc/article/view/1054
4. Sultana MS, Saha RR, Islam SN, Afrin T, Afroz R, Lotus MJ. Nephroprotective Activity of Aqueous Extract of Carica Papaya Seeds in Carbon Tetrachloride Induced Nephrotoxic Rats. Ibrahim Cardiac Medical Journal. 2022;12(1):9-17. https://doi.org/10.3329/icmj.v12i1.69540

5. Imtiaz S, Salman B, Qureshi R, Drohlia MF, Ahmad A. A review of the epidemiology of chronic kidney disease in Pakistan: a global and regional perspective. Saudi J Kidney Dis Transpl. 2018;29(6):1441-51. DOI: 10.4103/1319-2442.248307
6. Sravani M, Krishnamurthy S, Parameswaran N, Rajappa M. Assessment of causality in hospitalized children With aminoglycoside-related nephrotoxicity. Indian Pediatr. 2022 Mar;59(3):226-9. PMID: 34992186.
7. Elgazzar D, Aboubakr M, Bayoumi H, Ibrahim AN, Sorour SM, El-Hewaity M, Elsayed AM, Shehata SA, Bayoumi KA, Alsieni M, Behery M. Tigecycline and gentamicin-combined treatment enhances renal damage: oxidative stress, inflammatory reaction, and apoptosis interplay. Pharmaceuticals. 2022 Jun 10;15(6):736. doi: 10.3390/ph15060736.
8. Sanidas E, Papadopoulos D, Chatzis M, Velliou M, Barbetseas J. Renin angiotensin aldosterone system inhibitors in chronic kidney disease: a difficult equation. Am J Cardiovasc Drugs. 2021 Nov 21(6):619-27. https://doi.org/10.1007/s40256-021-00467-9
9. Gelen V, Şengül E. Protective effects of resveratrol on kidney function tests and renal histopathology in carbon tetrachloride-induced renal toxicity in rats. World J Advanc Res Rev. 2021;10(1):156-61. https://doi.org/10.30574/wjarr.2021.10.1.0155
10. Ahmed KM, Mahmud SA. Curative Effects of Ethanol Extract of Prosopis Farcta (Syrian Mesquite) Against Ethylene Glycol Induced Urolithiasis in Male Albino Rats. Science Journal of University of Zakho. 2021 Jun 30;9(2):89-96. DOI: https://doi.org/10.25271/sjuoz.2021.9.2.802
11. Campbell RE, Chen CH, Edelstein CL. Overview of Antibiotic-Induced Nephrotoxicity. Kidney Int Rep. 2023 Aug 25;8(11):2211-25. doi: 10.1016/j.ekir.2023.08.031.
12. Iqbal K, Aslam R, Iqbal SS, Munir N, Abdullah I, Rasheed S, et al. Analyzing the impact of hemodialysis on urea and creatinine analytes in renal failure patients from Lahore-Pakistan. Nat J Med Health Sci. 2023;5(1):27-32. DOI: https://doi.org/10.52587/njmhs.v5i1.65.
13. Azouz AA, Hanna DA, Abo-Saif AA, Messiha BA. Interference with megalin expression/endocytic function by montelukast mitigates gentamicin nephrotoxicity: Downregulation of ClC-5 expression. Saudi Pharma J. 2022 Feb 1;30(2):150-61. https://doi.org/10.1016/j.jsps.2021.12.013
14. Erseçkin V, Mert H, İrak K, Yildirim S, Mert N. Nephroprotective effect of ferulic acid on gentamicin-induced nephrotoxicity in female rats. Drug and chemical toxicology. 2022 Mar 4;45(2):663-9. https://doi.org/10.1080/01480545.2020.1759620
15. Merdana IM, Watiniasih NL, Sudira IW, Arjana AAG, Gunawan IWNF, Sudimartini LM, et al. The effect of ethanolic extract of Myrmecodia pendans on gentamicin induced nephrotoxicity in Wistar Rats. Int J Veterinary Sci. 2021;10(2):96-101. https://doi.org/10.47278/journal.ijvs/2020.025
16. Mehanna ET, Khalaf SS, Mesbah NM, Abo-Elmatty DM, Hafez MM. Anti-oxidant, anti-apoptotic, and mitochondrial regulatory effects of selenium nanoparticles against vancomycin induced nephrotoxicity in experimental rats. Life Sci. 2022 Jan 1;288:120098. doi: 10.1016/j.lfs.2021.120098.
17. Avila-Carrasco L, García-Mayorga EA, Díaz-Avila DL, Garza-Veloz I, Martinez-Fierro ML, González-Mateo GT. Potential therapeutic effects of natural plant compounds in kidney disease. Molecules. 2021 Oct 9;26(20):6096. https://doi.org/10.3390/molecules26206096
18. Wei M, Liu X, Li M, Tian X, Feng M, Pang B, et al. The role of Chinese herbal medicine in the treatment of diabetic nephropathy by regulating endoplasmic reticulum stress. Frontiers in Pharmacology. 2023 Jun 26;14:1174415. https://doi.org/10.3389/fphar.2023.1174415
19. Elkomy A, Aboubakr M, Elsayed F, Medhat Y. Protective effect of cinnamon and/or parsley oils against carbon tetrachloride (CCl4) induced hepatotoxicity in rats. J Pharm Clin Res 2020;8(1):555734.
20. Pandit SB, Pagar HJ, Patel TR, Darwade AP, Jadhav SA. In vivo study of Nephroprotective potential of Shilajit by using Cisplatin induced nephrotoxicity model. Asian J Pharm Pharmacol 2018;4(5):680-5.

This article may be cited as: Akram E, Lodhi S, Aftab A, Azeem A, Usman A, Sarwar A, Mirbahar A: Evaluating Synergistic Nephroprotective Effect of Parsley Extract and Ramipril in a Rat Model of Gentamicin-Induced Nephrotoxicity. Pak J Med Health Sci, 2023;17(11):359-362.