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Comparative effects of parsley leaves aqueous extract and ramipril on gentamicin induced nephrotoxicity in rats

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ABSTRACT

BACKGROUND & OBJECTIVE: Rats are commonly used in experimental studies as they have a smaller body size, they are easily available, and their genetic profiles are similar to each other as compared to humans. Kidney pathologies are the result of an imbalance between reactive oxygen species and antioxidants. Parsley aqueous extract is rich in polyphenolic contents and has nephroprotective effects. The objective of this study is to observe the effects of ramipril and parsley leaves aqueous extract in gentamicin induced nephrotoxicity.

METHODOLOGY: An Experimental study consisting of 40 healthy male albino rats were randomly distributed into two categories after taking samples for baseline values of urea and creatinine: Category 1 consisted of a control group (Group 1) having 10 rats, while category 2 comprised of 30 rats, divided into three experimental groups after being given intraperitoneal injections of gentamicin (80mg/kg) per day. Group 2 was taken as disease control, while group 3 and group 4 were parsley treated and ramipril treated for 28 days, respectively. Biochemical markers (serum urea and creatinine) were done at day 35. SPSS version 22 was applied for Statistical analysis. One-way ANOVA test was used to determine any difference in mean values. Post hoc tuckeys test was applied for multiple comparisons amongst groups. $p<0.05$ was measured as significant.

RESULTS: The treatment decreased the levels of serum urea and creatinine in nephrotoxic rats in group 3 (parsley treated group) and group 4 (ramipril treated group) in comparison to group 2. Group 3 had significantly reduced biochemical markers ($p<0.05$).

CONCLUSION: Parsley leaves extract significantly reduced the serum urea and creatinine levels as compared to ramipril.

KEYWORDS: Parsley, Antioxidant, Ramipril, Gentamicin, Serum Urea, Serum Creatinine.

INTRODUCTION

During the rest period, an expected 20% of cardiac yield moves through the kidneys, where it is filtered and reconditioned [1]. The kidney is presented to huge concentration of drugs and toxins that are filtered through it [2]. Acute kidney injury (AKI) is a typical clinical issue, the epidemiological information suggests that the occurrence rate of chronic kidney disease (CKD) after an event of AKI was 7.8 per 100 patient-years, and the pace of end-stage renal disease (ESRD) was 4.9 per 100 patient-years. One of the significant reasons for AKI is toxin-incited nephrotoxicity [2]. Roughly 19-25% cases of nephrotoxicity in critically sick patients resulting from nephrotoxic medications all around the world [3]. In Pakistan, CKD is on the rise, Pakistan is ranked eighth in the world on the basis of the highest number of prevalent cases of CKD [4].

Aminoglycosides (AGs), which were created in the 1940s, are probably the most established antibiotics used to cure diseases caused by gram-negative and some gram-positive bacteria [5]. AGs perform their function by attaching to the 16S rRNA subunit of the bacterial ribosome at its A-site, obstructing the proper cooperating of aminoacyl-tRNAs to the anticodon. This prompts the formation of abnormal proteins with subsequent bacterial cell death [6].

Lipid peroxidation, DNA damage, and up-regulation of the caspase family of proteases with subsequent apoptotic

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cell death and increase in macrophage infiltrates mediated through reactive oxygen species are proximal events that occur in AGs nephrotoxicity [7]. All AGs typically produce a prolonged post-antibiotic effect (PAE) [8].

AGs are not metabolized and are eliminated unaffected in the urine by glomerular filtration [9]. The main adverse effect of AGs exposure is nephrotoxicity, it is related to a variable degree of malfunctioning of renal tubules that may, in the most critical patient, progress to non-oliguric acute kidney injury (AKI) [10]. Gentamicin is produced by the genus Micromonospora. Due to its wide spectrum of activity, rapid bacterial killing capacity, post-antibiotic effects, stable chemical profile, minimal expense, and effectiveness against microbes resistant to other drugs have proved that it is used as a drug of choice in variable clinical situations [7].

Angiotensin-converting enzyme inhibitors are considered ideal antihypertensive. Ramipril is a long-acting lipophilic medication. It has two significant methods of excretion. It not only efficiently regulates blood pressure (BP), but also affects the prognosis of patients with nephropathy because ramipril decreases the tone of efferent arterioles, and intraglomerular pressure prevents the proliferation of mesangial cells, thereby reducing the synthesis of mesangial matrix constituents and level of proteinuria. It has been shown that constant treatment with Angiotensin-converting enzyme inhibitors eventually leads angiotensin-II and aldosterone to their pretreatment levels therefore, it is necessary to search for alternative therapies [11].

There is a growing curiosity in the natural antioxidants found in different spices and their part in wellbeing and disease. Parsley belongs to the family Umbelliferae. It has been used medicinally in many European, Mediterranean, and Asian countries. It serves as medicinal herb and used worldwide because of its antioxidant activity, increases glutathione in the kidney and again builds up kidney tissue after nephrotoxicity [12]. The components of parsley which comprise ascorbic acid, carotenoids, flavonoids, coumarins, apiole, and tocopherol, have been artificially investigated [13]. The rationale of our study is to observe the nephroprotective effect of aqueous extract of parsley leaves in comparison with the antihypertensive drug ramipril.

METHODOLOGY

The present study was an experimental study performed at the Pharmacology Department of (ref #Riphah/IIMC/IRC/19/0354) in cooperation with the National Institute of Health (NIH) Islamabad for one year (September 2019-August 2020). Total 40 adult albino rats were obtained from the animal house of NIH and were kept in standard cages under regular laboratory settings. The care and management of animals was done in accordance with universally recognized standard rules for animal handling [14]. Animals had an easy approach to food and drinking water. Simple random sampling was used, and the rats were separated into four groups with 10 animals in each group. The research was started after approval of the research proposal from the Institutional Review Committee Islamic International Medical College. Healthy male albino rats weighing 300-350 grams were included in the study, while non-healthy or rats weighing less than 300 grams were excluded. Group 1 was a control group, it was kept as a reference for normal biochemical markers while comparing to other groups, and the others (group 2 to group 4) were experimental groups. Control group received a normal basal diet, and no treatment was given.

Baseline levels of serum urea and creatinine were done at day 0 before starting experiment. Research grade Gentamicin and Ramipril were procured from Sigma Aldrich. Gentamicin 80mg/kg [15] was injected into experimental group animals intraperitoneally (groups 2,3 and 4) (IP) to induce nephrotoxicity for eight consecutive days, which was checked through biochemical parameters after one day of the last gentamicin dose. RIFLE (risk injury failure loss end stage renal disease) criteria was followed to establish nephrotoxicity [16].

After induction of nephrotoxicity, treatment was instituted for rats of groups 3 and 4. Fresh Parsley plant was purchased from a local market, identification was done at herbarium section of National Agricultural Research Centre (NARC) Islamabad through proper taxonomical rules. The samples were deposited at the institute for future reference. Parsley leaves (100g) were chopped into small parts and then let to boil in water (1000 mL) for 1h and were filtered with Whatman filter paper no.1. This mixture was then left to cool and stored at 4C until use. This extract was freshly prepared twice a week [17]. Parsley leaves extract 2ml per oral to each rat [1] of group 3 was given daily for 4 weeks. Ramipril 10mg/kg/day orally in drinking water [18] was given to group 4 for four weeks period. At the end of the study, at day 35, the final blood samples were obtained through cardiac puncture. Measurement of serum urea was done By "Urease-GLDH": enzymatic UV test. Creatinine in serum was determined by Jaffe's reaction. Both parameters were measured via a commercially supplied kit (Merk Private Limited).

Statistical analysis was done by using SPSS 22. Results were interpreted as mean±SD. Quantitative parameters were compared among the four groups, and analysis was done using one-way ANOVA. Post hoc Tuckey's test was applied for multiple comparisons of these groups.

RESULTS

At the start of this study, serum urea and creatinine levels were comparable among the four groups. On administration of gentamicin I/P injection 80mg/kg/day for eight days, nephrotoxicity was induced in experimental groups' rats (groups 2, 3 and 4). To see the progress of the study and confirmation of nephrotoxicity induction, 2 rats from each group were taken randomly on day 9 before further intervention. Results showed the derangement of RFTs. In table-I day 35, on final sampling, mean serum creatinine was 0.64mg/dl for group 1, 1.78mg/dl in group 2, 0.98mg/dl in group 3, and 1.05 in group 4. A significant difference was observed among the groups.

Table-I: Serum urea & creatinine levels on day 35.

Group 1- Control (n=10)		Group 2-Disease (n=10)		Group 3-Parsley (n=10)			Group 4-Ramipril (n=10)
Serum Urea (mg/dl)	Serum Creatinine (mg/dl)	Serum Urea (mg/dl)	Serum Creatinine (mg/dl)	Serum Urea (mg/dl)	Serum Creatinine (mg/dl)	Serum Urea (mg/dl)	Serum Creatinine (mg/dl)
28	0.5	84	2	47	1.1	40	0.9
21	0.6	81	1.9	56	1.3	42	1.1
24	0.8	48	1.4	40	0.9	54	1.2
31	0.5	71	1.8	42	1	38	0.8
32	0.7	74	1.7	52	1.2	52	1.2
18	0.8	51	1.5	38	1	36	0.9
21	0.7	81	2.1	36	0.7	44	1
25	0.8	77	1.8	42	1.2	32	0.7
29	0.4	64	1.7	36	0.8	60	1.4
18	0.6	69	1.9	30	0.6	50	1.3

Table-II: Comparison of serum urea & creatinine between groups using ANOVA.

Groups	SOV	Sum of Squares	df	Mean Square	F	Sig.
Creatinine mg/dl	Between Groups	6.903	3	2.301	53.753	<0.001
	Within Groups	1.541	36	0.043		
	Total	8.444	39	-		
Urea mg/dl	Between Groups	10462.500	3	3487.500	43.344	<0.001
	Within Groups	2896.600	36	80.461		
	Total	13359.100	39	-		

Table-III: Post-Hoc Tukey test showing a comparison of creatinine between groups on day 35.

Creatinine			95%Confidence Interval	
Groups	Mean difference	p-value	Lower Bound	Upper Bound
2 vs 1	1.14	<0.001	0.8908	1.3892
2 vs 3	0.80	<0.001	0.5508	1.0492
2 vs 4	0.73	<0.001	0.4808	0.9792
4 vs 1	0.41	<0.001	0.1608	0.6592
4 vs 3	0.07	0.873	-0.1792	0.3192
3 vs 1	0.34	0.004	0.0908	0.5892

Table-IV: Post-Hoc Tukey test showing a comparison of urea between groups on day 35.

Urea			95%Confidence Interval	
Groups	Mean difference	p-value	Lower Bound	Upper Bound
2 vs 1	45.30	<0.001	34.4961	56.1039
2 vs 3	28.10	<0.001	17.2961	38.9039
2 vs 4	25.20	<0.001	14.3961	36.0039
4 vs 1	20.10	<0.001	9.2961	30.9039
4 vs 3	2.90	0.887	-7.9039	13.7039
3 vs 1	17.20	<0.001	6.3961	28.0039

Mean serum urea was 24.7 mg/dl for group 1, 70.0 mg/dl in group 2, 41.9 mg/dl in group 3 and 44.8 mg/dl in group 4. In (table-II) the ANOVA for comparison of means among groups and results shows a significant difference, there is evidence that at least two groups differ from each other, so further analysis to be carried out to check the significant difference among the different groups for this purpose post hoc test for the mean comparison test (Tuckey's test) was applied. In Tuckey's test, the values were compared with mean differences. In a comparison of creatinine between groups on day 35 result shows a non-significant difference between groups 4 vs 3 and 3vs 1, remaining all are significant (table-III). Similarly, a comparison of urea between groups on day 35 shows group 4 vs 3 have also non-significant results and the remaining show significant results (table-IV).

DISCUSSION

Kidneys are responsible for maintaining homeostasis in the body's circulatory system and act as a chief site for xenobiotic elimination and detoxification. Nephrotoxicity is caused by xenobiotic accumulation that is said to be responsible for ESRD [19]. Nephrotoxicity has become a major health problem, and there are reports of high incidence all over the world [20]. Drug induced toxicity is the reason of acute kidney injury in almost 20 to 60% of hospitalized patients. The most prevalent form of drug-induced nephrotoxicity is acute tubular necrosis (ATN) [21]. Parsley has many pharmacological activities, and it possesses diuretic, nephroprotective and antihypertensive properties. Its active constituents include flavonoids, coumarins, tocopherol and carotenoids. Parsley and its extract are used in various kidney diseases. Parsley has also been used as a treatment for urolithiasis [22].

In our Experimental study, we noticed measurable decline in serum urea and creatinine values after 28 days

administration of parsley leaves aqueous extract in the nephrotoxicity induced rat model. Our results of serum urea and creatinine are in accordance with the study of protective effects of parsley leaves against nephrotoxicity induced by gentamicin in rat [20]. Because of its antioxidant activity, parsley increases glutathione in the kidney and reconstructs kidney tissue after nephrotoxicity [12]. Our results of group 3 in comparison with group 2 are also supported by a study carried out by Pandit, who observed nephroprotective potential of Parsley against Cisplatin-induced Nephrotoxicity in rats. He also observed a decrease in serum markers [23]. Significant differences in means of biochemical parameters of group 4 (ramipril alone) 10mg/kg as compared to group 2 claimed that ramipril improved the signs of renal damage and setback inflammatory signs. These results are in accordance with study who observed decrease in serum markers after treatment with Ramipril [24]. Ramipril nephroprotective potential is due to its ability to inhibit indicators of oxidative damage. Ramipril decreases levels of NF-kB. It has antiapoptotic, anti-inflammatory, and antioxidative properties. Drug significantly lowered the levels of urea and creatinine [25,11].

The results of our study proved that nephroprotective potential of Parsley and Ramipril are supported by previous studies, and parsley can be used in renal ailments as an adjunct or alternative to nephroprotective drug ramipril.

Due to limited resources and time constraints, measurement of oxidative stress and GFR estimation could not be done. The other studies can be recommended with different doses of gentamicin, ramipril, and parsley leaves extract. Individual constituents present in parsley leaves can be explored for nephroprotection and compared with nephroprotective drugs. Histopathology of rat kidney tissue can be done to observe changes in renal architecture before and after treatment.

CONCLUSION

Parsley leaves aqueous extract, and ramipril has individual effects in improving renal status in gentamicin induced nephrotoxicity with statistically significant differences among disease control group and treatment groups ($p<0.05$). This study also concludes that when comparison was done to observe the effects of ramipril and parsley leaves aqueous extract individually, parsley extract is better in efficacy than ramipril.

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REFERENCES

1. Mahmoud NY, Salem ZA, El-Moslemany AM. Nephroprotective and antioxidant effects of parsley plant parts against gentamicin-induced nephrotoxicity in rats. Academic Journal of Nutrition. 2015;4:113-122. Doi: 10.5829/idosi.ajn.2015.4.3.10417
2. Chen Q, Cui Y, Ding G, Jia Z, Zhang Y, Zhang A, et al. PEA3 protects against gentamicin nephrotoxicity: role of mitochondrial dysfunction. American Journal of Translational Research. 2017;9(5):2153–2162.
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. Journal of Islamic International Medical College (JIIMC). 2018;13(4):184-188.
4. Rehman IU, Khan TM. Epidemiology of chronic kidney diseases (CKD) in Malaysia and Pakistan, pathophysiology of CKD-associated pruritus and other CKD-associated dermatological disorders. Progress In Microbes & Molecular Biology. 2020;3(1). Doi:10.36877/pmmb.a0000063
5. Wargo KA, Edwards JD. Aminoglycoside-induced nephrotoxicity. Journal of Pharmacy Practice. 2014;27(6):573-577. Doi:10.1177/0897190014546836
6. Labby KJ, Garneau-Tsodikova S. Strategies to overcome the action of aminoglycoside-modifying enzymes for treating resistant bacterial infections. Future Medicinal Chemistry. 2013;5(11):1285-1309. Doi: 10.4155/fmc.13.80
7. Ghaznavi H, Mehrzadi S, Dormanesh B, Tabatabaei SM, Vahedi H, Hosseinzadeh A, et al. Comparison of the protective effects of melatonin and silymarin against gentamicin-induced nephrotoxicity in rats. Journal of Evidence-based Complementary & Alternative Medicine. 2016;21(4):NP49-NP55. Doi:10.1177/2156587215621672
8. Krause KM, Serio AW, Kane TR, Connolly LE. Aminoglycosides: an overview. Cold Spring Harbor Perspectives in Medicine. 2016;6(6):a027029. Doi: 10.1101/cshperspect.a027029
9. Filazi A, Şireli UT, Pehlivanlar Önen S, Çadırcı Ö, Aksoy A. Comparative pharmacokinetics of gentamicin in laying hens. Kafkas Univ Vet Fak Derg. 2013;19 (3): 495-498. Doi: 10.9775/kvfd.2012.8138
10. McWilliam SJ, Antoine DJ, Smyth RL, Pirmohamed M. Aminoglycoside-induced nephrotoxicity in children. Pediatric Nephrology. 2017;32(11):2015-2025. Doi:10.1007/s00467-016-3533-z
11. Rutkowski B, Tylicki L. Nephroprotective action of renin-angiotensin-aldosterone system blockade in chronic kidney disease patients: the landscape after ALTITUDE and VA NEPHRON-D trails. Journal of Renal Nutrition. 2015;25(2):194-200. Doi:10.1053/j.jrn.2014.10.026
12. Gumaih H, Al-Yousofy F, Ibrahim H, Ali S, Alasbahy A. Evaluation of ethanolic seed extract of parsley on ethylene glycol induced calcium oxalate, experimental model. International Journal of Scientific Research. 2017;6:1683-1688.

13. Azab AE, Abushofa FA, Abdul Rahman HM. Nephroprotective effect of aqueous extract of parsley against nephrotoxicity induced by carbon tetrachloride in the male rats. Journal of Biotechnology & Bioengineering. 2019;3(4):16-26.
14. Tan B. Guidelines on the care and use of animals for scientific purposes. National Advisory Committee for Laboratory Animal Research. 2004.
15. Abdelsameea AA, Mohamed AM, Amer MG, Attia SM. Cilostazol attenuates gentamicin-induced nephrotoxicity in rats. Experimental and Toxicologic Pathology. 2016;68(4):247-253. Doi.org/10.1016/j.etp.2016.01.002
16. Kellum JA. Diagnostic criteria for acute kidney injury: present and future. Critical Care Clinics. 2015;31(4):621-632. Doi: 10.1016/j.ccc.2015.06.001
17. Alkhalaf AA, Alrusayni SA, Khalid A, Aloraifi A, AL-Shammari OA, Dhanarasu S. Efficacy of parsley (Petroselinum crispum) leaves extract and decoction on status of lipid profile and osmotic fragility in gentamicin-induced nephrotoxicity in rats. Journal of Chemical and Pharmaceutical Research. 2016;8(3):948-957.
18. Rubel D, Stock J, Ciner A, Hiller H, Girgert R, Müller GA, et al. Antifibrotic, nephroprotective effects of paricalcitol versus calcitriol on top of ACE-inhibitor therapy in the COL4A3 knockout mouse model for progressive renal fibrosis. Nephrology Dialysis Transplantation. 2014;29(5):1012-1019. Doi:10.1093/ndt/gft434
19. Weber EJ, Himmelfarb J, Kelly EJ. Concise review: current and emerging biomarkers of nephrotoxicity. Current Opinion in Toxicology. 2017;4:16-21. Doi: 10.1016/j.cotox.2017.03.002
20. Elkhamisy AE. Protective effect of parsley leaves and turmeric roots extracts against gentamicin induced nephrotoxicity in male rats. World Journal of Dairy & Food Sciences. 2015;10(1):1-8.
21. Downes KJ, Hayes M, Fitzgerald JC, Pais GM, Liu J, Zane NR, et al. Mechanisms of antimicrobial-induced nephrotoxicity in children. Journal of Antimicrobial Chemotherapy. 2020;75(1):1-13. Doi: 10.1093/jac/dkz325
22. Nirumand MC, Hajialyani M, Rahimi R, Farzaei MH, Zingue S, Nabavi SM, et al. Dietary plants for the prevention and management of kidney stones: preclinical and clinical evidence and molecular mechanisms. International Journal of Molecular Sciences. 2018;19(3):765. Doi:10.3390/ijms19030765
23. 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 Journal of Pharmacy and Pharmacology. 2018;4:680-685.
24. Darwish MY, El-Lateef A. Study of potential nephroprotective effects of Ramipril versus Alpha lipoic acid against Gentamicin induced nephropathy in rats. The Egyptian Journal of Hospital Medicine. 2018;73(10):7754-7760. Doi: 10.21608/ejhm.2018.20115
25. Adedapo AA, Ehizogie OR, Falayi OO, Oyagbemi AA, Ogunpolu BS, Omobowale TO, et al. Ramipril Blunt Glycerol-induced Acute Renal Failure in Rats Through its Anti-apoptosis, Anti-inflammatory, Anti-oxidant, and Renin-inhibiting Properties. The FASEB Journal. 2020;34(S1):1-. Doi:10.1096/fasebj.2020.34.s1.02037

Author's Contribution:

Eisha Akram: Substantial contributions to the conception and design of the work.
Akbar Waheed: Data collection, manuscript approval, and data interpretation.
Rukhsana Munawar: Data analysis, writing of the manuscript.
Sidra Mumal: Drafting the work and reviewing it critically for important intellectual content.
Abdul Azeem: Interpretation of data for the work.

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