ORIGINAL ARTICLE

# RENO PROTECTIVE ROLE OF N ACETYL CYSTEINE AND AQUEOUS EXTRACT OF *BERBERIS LYCIUM* ROYALE ROOT BARK ON RATS

**Nimra Ijaz[1], Akbar Waheed[1], Mehwish Tayyab[2], Sidra Mumal[1], Rabia Iftikhar[1], Alia Rehman[1], Esha Akram[1]**

[1]Islamic International Medical College, Rawalpindi, [2]Hazrat Bari Sarkar Medical College, Rawalpindi-Pakistan

**Background:** N acetyl cysteine and *Berberis lycium Royale* root bark have been used to treat kidney diseases. Objectives of the study were to evaluate the individual and combined effect of N acetyl cysteine and aqueous extract of *Berberis lycium Royale* root bark in Gentamicin induced nephrotoxicity in rats. This **r**andomized control trial conducted at Islamic International Medical College, Rawalpindi in collaboration with NIH, Islamabad in 1 month from Sep to Oct 2020. **Methods:** Fifty Wister albino rats of 10-12 weeks old were divided into five groups with 10 in each group. Group 1 was normal control given food and water only and remaining 40 were in treatment groups. Nephrotoxicity was induced by intraperitoneal injection of Gentamicin (80mg/kg) for 6 days in group 2, 3, 4 and 5. After induction of nephrotoxicity, Group 3 was administered N acetyl cysteine 140mg/kg per oral, Group 4 was given aqueous extract of *Berberis lycium Royale* root bark 400 mg/kg per oral and Group 5 was given both N acetyl cysteine 140mg/kg per oral and aqueous extract of *Berberis lycium Royale* root bark 400 mg/kg per oral for 21 days. Serum uric acid was measured in all groups after 30 days to observe the reversal of renal injury. **Results:** The results of this study indicate that Group 3, Group 4 and Group 5 showed a decrease in serum uric acid level as compared to Disease Control Group (Group 2). However, Group 5 significantly reduced uric acid ($p$-0.05). **Conclusion:** Combined effect of N acetyl cysteine and aqueous extract of *Berberis lycium Royale* root bark showed improvement in uric acid level in Gentamicin induced nephrotoxicity in rats.

**Keywords:** N acetyl cysteine; Aqueous extract; *Berberis lyceum*

**Citation:** Ijaz N, Waheed A, Tayyab M, Mumal S, Iftikhar R, Salma, *et al*. Renoprotective role of N acetyl cysteine and aqueous extract of *Berberis lycium* Royale root bark on rats. J Ayub Med Coll Abbottabad 2021;33(4):553–7.

## INTRODUCTION

The kidney is an essential organ that performs several important functions in body. There are many causes of nephrotoxicity and drugs is one of them. Drug-induced nephrotoxicity arises either due to direct or indirect result of exposure to drugs.[1] Acute kidney injury due to drug-induced nephrotoxicity affects around 14–26% adult and 16% paediatric hospitalized patients.[2]

Many drugs are responsible for causing nephrotoxicity which includes antibiotics such as aminoglycosides, antifungals (amphotericin B), chemotherapeutics, immunosuppressive drugs, anti-inflammatory drugs among others.[3] The incidence of aminoglycoside induced nephrotoxicity has been increased progressively in about 10–25% in patients taking therapeutic doses.[4]

Gentamicin is widely used against many Gram-negative microorganisms. Therapeutic doses of gentamicin causes nephrotoxicity and acute kidney injury by generating reactive oxygen species (ROS), reactive nitrogen species, Na–K–ATPase inhibition and by inhibiting mitochondrial oxidative phosphorylation.[5] Gentamicin induced nephrotoxicity is primarily due to tubulopathy in which tubular damage and dysfunction are main reasons for kidney failure along with development of necrosis and inflammation.[6,7] Hence giving an antioxidant will prevent damage caused by gentamicin.

N acetyl Cysteine (NAC) is one of the most important antioxidant with sulfhydryl and glutathione group donor.[8] It is used to reverse the effects of nephrotoxic drugs by binding to reactive oxygen species and by replenishing glutathione pools.[9,10]

Medicinal plants are considered best for use in kidney damage due to their safety profile and being cost effective.[11] *Berberis lycium Royale* belongs to the family Berberidaceae found in temperate and subtropical regions of the world.[12] Every part of this plant its root, bark, stem and fruits has some medicinal value and is used to treat many diseases like diabetes, hyperlipidaemia, cancer, diarrhoea, arrhythmias, depression, infections and renal injuries.[13] Its root bark contains Berberine which is an alkaloid and it decreases the nitric oxide (NO) levels, restores glutathione GSH levels via its free radical scavenging antioxidant property, and helps ameliorate the renal impairment caused by the GM-induced oxidative stress.[11]

There are many pharmacological agents that can cause kidney damage. Treating nephrotoxicity

through the use of cheap and effective pharmacological agents decreases hospitalization costs as well as lowers the rate of disease morbidity and mortality. NAC and *Berberis lycium Royale* root bark extract has been reported to be nephroprotective.[15,16] However, there has been no previous comparative study concerning the synergistic nephroprotective effects of these two agents. Hence, this study was conducted in order to identify more effective and cheaper agents with fewer side effects.

## MATERIAL AND METHODS

This experimental study was carried out from September to October 2020 in animal house of NIH, Islamabad. After taking approval from ethical committee of Riphah International University, Rawalpindi, Pakistan, a total of 50 adult male albino rats were housed in animal house of NIH and divided into 5 groups, each containing 10 rats. Rats with no kidney disease, weighing 300–350 grams were included in the study. All others were excluded from the study. All animals were subjected to acclimatization for 2 days before the start of experiment. They were kept in plastic cages under the ambient temperature of 30±2°C provided with standard pelleted feed and water. *Berberis lycium Royale* was obtained from local market Islamabad. Root barks were dried under shade and grinded. About 500g of powder was soaked in 1000 ml of distilled water and macerated for 4 days at room temperature with constant stirring. The mixture was filtered using Whatmann filter paper no.1 which was then subjected under reduced pressure of rotary evaporator at 45 ºC.

After 30 days of treatment, blood was collected through cardiac puncture after giving anaesthesia to rats for biochemical determinations and histopathological samples were collected and preserved in 10% formaldehyde.

The enzymatic kinetic method was used for estimation of serum urea level at 600 nm by a UV-2500 Pharmaspec, Shimadzu spectrophotometer using a Human Gesellschaft fur Biochemical Germany Diagnostic Kit.

Rat plasma creatinine was estimated calorimetrically at 505 nm by a UV-2500 Pharmaspec, Shimadzu spectrophotometer using a creatinine standard from mdi Europa gmbh, Germany Diagnostic Kit.

Sections of the kidneys were fixed in 10% formalin, embedded in paraffin and cut at 5 µm thickness. Kidney sections were then processed and stained with haematoxylin and eosin dye for histological evaluation of renal injury, according to standard protocols. All sections were examined under light microscope. The criteria for kidney damage included proximal tubular necrosis, infiltration of inflammatory cells and vascular congestion[17]. Each specimen was scored using a scale from grade 1 to 4 as follows:[18]

**Grade zero** (no change observed in any field→normal)
**Grade 1**(changes observed in one field→minimal)
**Grade 2**(changes observed in two fields→slight)
**Grade 3**(changes observed in three fields→moderate)
**Grade 4**(changes observed in all four fields→ severe)

The analysis was done by using one-way ANOVA followed by post-hoc tukey test to make the comparison between disease control and gentamicin treated groups respectively. All data was expressed as mean standard deviation (Mean±SD). $p<0.05$ was considered statistically significant.

## RESULTS

Results showed a statistically significant difference in the level of renal biomarkers in disease control and experiment groups (Table-2). The levels of serum creatinine and urea were significantly ($p<0.05$) increased in gentamicin treated rats (toxic control) as compared to the normal control rats. However, treatment with N acetyl cysteine and aqueous extract of *Berberis lycium Royale* root bark showed significantly decreased ($p<0.05$) levels of serum creatinine and urea as compared to toxic control group. Results also indicated that combination of aqueous extract of *B. Lycium Royale* root bark and N acetyl cysteine was more effective to reverse gentamicin induced nephrotoxicity than the individual agents.

Histopathological examination showed that the control (normal) kidney of rat had normal glomerulus, proximal and distal tubules (Figure 3). All the vessels and interstitium revealed unchanged basement membrane and mesengium. In gentamicin-treated group, severe and widespread proximal tubular necrosis along with vascular congestion and infiltration of inflammatory cells like neutrophils and eosinophils were seen. In N acetyl Cysteine treated group slight and moderate changes were seen. In aqueous extract group slight and minimal changes in histopathology were observed, while in the combination group normal changes were observed like reversal of proximal tubular necrosis, no vascular congestion and no inflammatory cells.

Figure-4 is showing proximal tubular necrosis, vascular congestion and infiltration of inflammatory cells were present of severe grade in group 2 but significant difference is present in group 3, 4 and 5 as shown in bar chart.

**Table-1: Groups of rats and treatment**

| Groups | Treatment |
|---|---|
| **Group 1** | Distilled water 10 ml/kg p.o |
| **Group 2** | Gentamicin (80 mg/kg single i.p) for 6 days |
| **Group 3** | Gentamicin (80 mg/kg i.p) for 6 days then N acetyl cysteine (140 mg/kg/day) per oral for 21 days |
| **Group 4** | Gentamicin (80 mg/kg i.p) for 6 days then aqueous extract of *Berberis lycium Royale* root bark (400 mg/kg/day) per oral for 21 days |
| **Group 5** | Gentamicin (80 mg/kg i.p) for 6 days then aqueous extract of *Berberis lycium Royale* root bark (400 mg/kg/day) per oral and N acetyl cysteine (140 mg/kg/day) per oral for 21 days |

**Table-2: Group wise distribution of Mean Serum Urea and Creatinine on Day 30 among Control and Experimental groups of albino rats n=50 by ANOVA**

| Groups | Urea (mg/dL) | Creatinine (mg/dL) |
|---|---|---|
| Group 1 | 37.7±1.14 | 0.25±0.01 |
| Group 2 | 79.9±0.4 | 2.14±0.12 |
| Group 3 | 39.1±0.55 | 0.64±0.04 |
| Group 4 | 34.4±0.67 | 0.54±0.07 |
| Group 5 | 37.1±0.60 | 0.27±0.01 |
| $p \leq 0.05$ was considered statistically significant. | | |

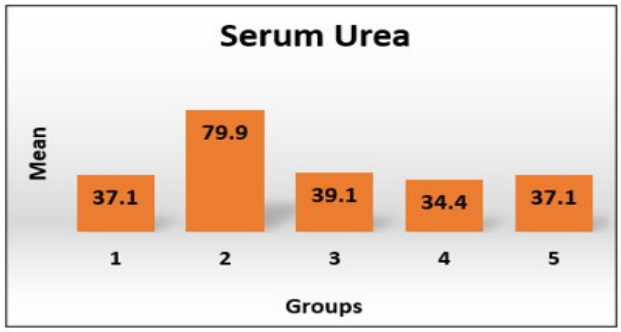


**Figure-1: Mean values of Serum Urea (mg/dL) on day 30 of all groups (n=10)**

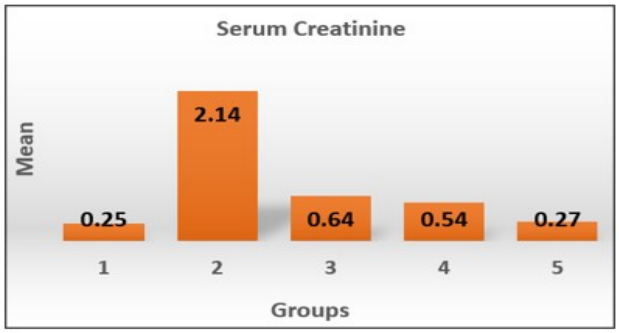


**Figure-2: Significant decrease in serum creatinine in groups 3, 4 and 5 after 30 days. Significant improvement is seen in group 5 where value has reached near to normal.**

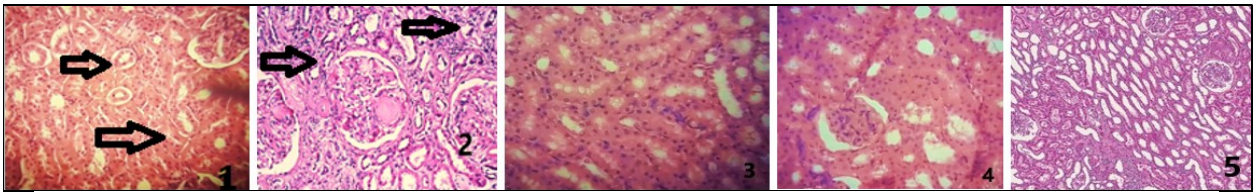


**Figure 3: Light photomicrograph of kidney tissue of rats treated with aqueous extract of *Berberis lycium Royale root bark* and N acetyl Cysteine in acute study. Haematoxylin and eosin, X100**

**1:** Normal control group showing normal Glomerulus and Proximal tubules.**2:** Disease control group showing proximal tubular necrosis, infiltration of inflammatory cells and vascular congestion. **3**: N acetyl Cysteine group showing moderate changes.**4:** *Berberis lycium Royale root bark* group showing minimal changes and **5:** Combination group showing reversal of histopathology to normal.

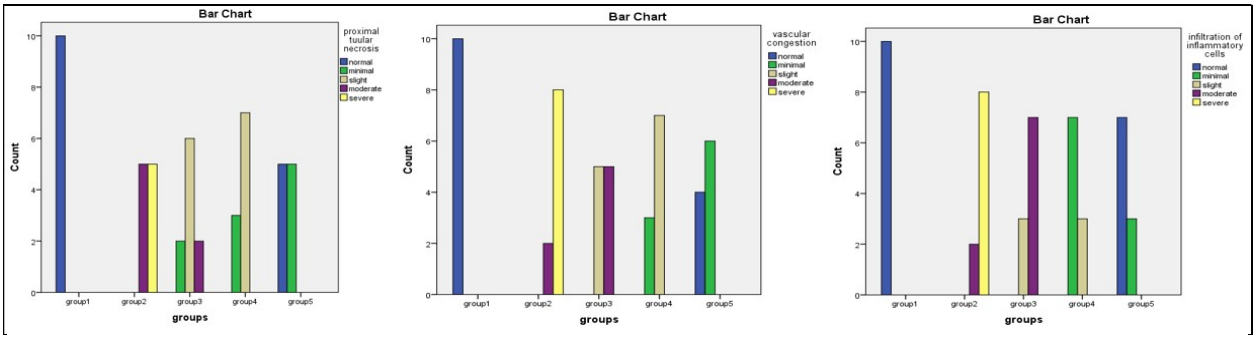


**Figure-4**: **Bar chart showing distribution of proximal tubular necrosis, vascular congestion and infiltration of inflammatory cells in all groups.**

## DISCUSSION

In the current study we observed that both aqueous extract of *Berberis lycium Royale* root bark and N acetyl Cysteine have nephroprotective effect as they cause a decrease in serum creatinine and urea levels and reversed the histopathological changes of nephrotoxicity which were caused by gentamicin. However, it was noticed that their combination have more potential to extenuate the renal damage and toxic effects. Gentamicin when administered intraperitoneally in a dose of 80mg/kg orally in drinking water produces nephrotoxicity by causing a rise in serum markers and brings histopathological changes like proximal tubular necrosis, vascular congestion and infiltration of inflammatory cells in kidneys.[17] In the current group 2 was given gentamicin with no treatment and all these changes were observed in rat kidneys.

Group 3 was treatment group and was given N acetyl Cysteine at dose of 140 mg/kg which showed a decrease in serum urea and creatinine when compared with group 2 which was only given gentamicin. This group also showed reversal of histopathological changes including proximal tubular necrosis and infiltration of inflammatory cells. N acetyl cysteine decreases the level of serum creatinine and urea in nephrotoxic rats hence recovering the renal injury.[19] the reversal of kidney damage is due to its antioxidant and anti-inflammatory effect.[15]

In the current study Group 4 was administered *Berberis lycium Royale* root bark aqueous extract 400mg/kg/day in drinking water which showed nephroprotective effect by decreasing serum creatinine and urea levels along with reversal of histological changes like proximal tubular necrosis, vascular congestion and infiltration of inflammatory cells[16].This is due to presence of alkaloid berberine found in its root bark mainly and possesses both antioxidant and anti-inflammatory properties.[20]

The third treatment group i.e group 5 was given combination of both N acetyl Cysteine and *Berebris lycium Royale* root bark aqueous extract which showed a significant decrease in serum urea and creatinine levels and reversing proximal tubular necrosis, vascular congestion and infiltration of inflammatory cells. There was minimal proximal tubular necrosis, no vascular congestion and no infiltration of inflammatory cells seen in this group after treatment. Hence it was proven that the combination has more potential in reversing the kidney damage as compared to individual components.

## CONCLUSION

Although previous studies have showed Reno protective effect of N acetyl Cysteine and aqueous extract of *Berberis lycium Royale* root bark but the results of above study indicate that synergistic use of N acetyl Cysteine and *Berebris lycium Royale* root bark aqueous extract show significant improvement in kidney damage caused by gentamicin. At given doses both showed reduction in serum creatinine and urea along with improvement in histopathological changes including proximal tubular necrosis, vascualar congestion and infiltration of inflammatory cells.

**Conflict of interest:** The authors declare that they have no competing interests

**Acknowledgement:** The authors are thankful to National institute of health, Islamabad for providing experimental facilities to conduct study.

## AUTHORS CONTRIBUTION

This manuscript is based on M.Phil thesis of first author. All other authors have contributed in performing analysis and writing the manuscript.

## REFERENCES

1. Fusco S, Garasto S, Corsonello A, Vena S, Mari V, Gareri P, *et al*. Medication-Induced Nephrotoxicity in Older Patients. Curr Drug Metab. 2016;17(6):608–25.
2. Awdishu L, Mehta RL. The 6R's of drug induced nephrotoxicity. BMC Nephrol. 2017;18(1):1–12.
3. Heidari-Soreshjani S, Asadi-Samani M, Yang Q, Saeedi-Boroujeni A. Phytotherapy of nephrotoxicity-induced by cancer drugs: An updated review. J Nephropathol [Internet]. 2017;6(3):254–63. Available from: http://dx.doi.org/10.15171/jnp.2017.41
4. Ustyol L, Demirören K, Kandemir I, Erten R, Bulan K, Kaba S, *et al.* Comparative Nephroprotective Effects of Silymarin, N-Acetylcysteine, and Thymoquinone Against Carbon Tetrachloride-Induced Nephrotoxicity in Rats. Iran Red Crescent Med J. 2016;19(1):1–8.
5. Ghaznavi H, Mehrzadi S, Dormanesh B, Tabatabaei SMTH, Vahedi H, Hosseinzadeh A, *et al*. Comparison of the Protective Effects of Melatonin and Silymarin Against Gentamicin-Induced Nephrotoxicity in Rats. J Evidence-Based Complement Altern Med. 2016;21(4):NP49–55.
6. Randjelović P, Veljković S, Stojiljković N, Sokolović D, Ilić I. Gentamicin nephrotoxicity in animals: Current knowledge and future perspectives. EXCLI J. 2017;16:388–99.
7. Shahani S, Behzadfar F, Jahani D, Ghasemi M, Shaki F. Antioxidant and anti-inflammatory effects of Nasturtium officinale involved in attenuation of gentamicin-induced nephrotoxicity. Toxicol Mech Methods [Internet]. 2017;27(2):107–14. Available from: http://dx.doi.org/10.1080/15376516.2016.1258748
8. Rababa`h AM, Hijjawi TB, Alzoubi KH, Al Demour S, Ababneh MA. The Nephroprotective Effect of N-Acetyl-L-Cysteine and Atorvastatin against Imipenem induced Nephrotoxicity. Curr Mol Pharmacol. 2017;11(2):155–61.
9. Ceylan B, Ozansoy M, Kılıç Ü, Yozgat Y, Ercan Ç, Yıldız P, *et al*. N-acetylcysteine suppresses colistimethate sodium-

induced nephrotoxicity via activation of SOD2, eNOS, and MMP3 protein expressions. Ren Fail [Internet]. 2018;40(1):423–34. Available from: https://doi.org/10.1080/0886022X.2018.1489286
10. Aldini G, Altomare A, Baron G, Vistoli G, Carini M, Borsani L, *et al*. N-Acetylcysteine as an antioxidant and disulphide breaking agent: the reasons why. Free Radic Res [Internet]. 2018;52(7):751–62. Available from: https://doi.org/10.1080/10715762.2018.1468564
11. Qureshi, Yaqoob E. Pakistan Veterinary Journal. Animals [Internet]. 2013;8318(2):85–92. Available from: http://www.pvj.com.pk/pdf-files/31_3/192-194.pdf
12. Madiseh RM, Heidarian E, Rafieian-kopae M. Biochemical components of Berberis lycium fruit and its effects on lipid profile in diabetic rats. J HerbMed Pharmacol [Internet]. 2014;3(1):15–9. Available from: http://herbmedpharmacol.com/index.php/herb/article/view/32
13. Parra SA, Gaur K, Ranawat LS, Rather MI. An Overview on Various Aspects of Plant Berberis Lycium Royale. Am J Pharmacol Sci. 2018;6(1):19–24.
14. Adil M, Kandhare AD, Dalvi G, Ghosh P, Venkata S, Raygude KS, et al. Ameliorative effect of berberine against gentamicin-induced nephrotoxicity in rats via attenuation of oxidative stress, inflammation, apoptosis and mitochondrial dysfunction. Ren Fail. 2016;38(6):996–1006.
15. Pei Y, Liu H, Yang Y, Yang Y, Jiao Y, Tay FR, *et al*. Biological activities and potential oral applications of N-acetylcysteine: Progress and prospects. Oxid Med Cell Longev. 2018;2018.
16. Anwar R, Sultan R, Batool F. Ameliorating effect of Berberis lycium root bark extracts against cisplatin-induced nephropathy in rat. Bangladesh J Pharmacol. 2018;13(3):248–54.
17. Ansari MA, Raish M, Ahmad A, Ahmad SF, Mudassar S, Mohsin K, *et al.* Sinapic acid mitigates gentamicin-induced nephrotoxicity and associated oxidative/nitrosative stress, apoptosis, and inflammation in rats. Life Sci [Internet]. 2016;165:1–8. Available from: http://dx.doi.org/10.1016/j.lfs.2016.09.014
18. Mann PC, Vahle J, Keenan CM, Baker JF, Bradley AE, Goodman DG, *et al*. International Harmonization of Toxicologic Pathology Nomenclature: An Overview and Review of Basic Principles. Toxicol Pathol. 2012;40(4):7S-13S.
19. Ustyol L, Demirören K, Kandemir I, Erten R, Bulan K, Kaba S, *et al*. Comparative nephroprotective effects of silymarin, N-acetylcysteine, and thymoquinone against carbon tetrachloride-induced nephrotoxicity in rats. Iran Red Crescent Med J. 2017;19(1):1–8.
20. Neag MA, Mocan A, Echeverría J, Pop RM, Bocsan CI, Crisan G, *et al.* Berberine: Botanical Occurrence, traditional uses, extraction methods, and relevance in cardiovascular, metabolic, hepatic, and renal disorders. Front Pharmacol. 2018;9(AUG):1–30.
21. Ceylan B, Ozansoy M, Kılıç Ü, Yozgat Y, Ercan Ç, Yıldız P, e*t al*. N-acetylcysteine suppresses colistimethate sodium-induced nephrotoxicity via activation of SOD2, eNOS, and MMP3 protein expressions. Ren Fail [Internet]. 2018;40(1):423–34. Available from: https://doi.org/10.1080/0886022X.2018.1489286

| *Submitted: March 8, 2021* | *Revised: June 21, 2021* | *Accepted: July 4, 2021* |
|---|---|---|

## Address for Correspondence:
**Nimra Ijaz,** Islamic International Medical College, Rawalpindi-Pakistan
**Email:** nimraijaz8725@gmail.com