ORIGINAL ARTICLE

ANNALS OF PUNJAB MEDICAL COLLEGE

Effect of N Acetyl Cysteine and Aqueous Extract of Berberis Lycium Royale Root Bark on Uric Acid of Albino Rats

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Submitted for Publication: 21-04-2021
Accepted for Publication 30-06-2021

How to Cite: Ijaz N, Iftikhar J, Tayyab M, Javed S, Aslam H, Salim S. Effect of N Acetyl Cysteine and Aqueous Extract of Berberis Lycium Royale Root Bark on Uric Acid of Albino Rats. APMC 2021;15(2):101-4. **DOI: 10.29054/APMC/2021.1222**

ABSTRACT

Background: N acetyl cysteine and *Berberis lycium Royale* root bark have been used to treat kidney diseases. **Objectives:** 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. **Study Design:** Randomized control trial. **Settings:** Islamic International Medical College, Rawalpindi in collaboration with NIH, Islamabad. **Duration:** 1 month from September 2020 to October 2020. **Methodology:** 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: *Berberis lycium Royale, Nephrotoxicity, Gentamicin, N acetyl Cysteine.*

INTRODUCTION

Kidneys are major organs of human body which are responsible for maintaining homeostasis and excreting unwanted substances from the body. Drugs produce 19-25% of kidney damage.[1] Many risk factors contribute in development of drug induced kidney injury which include factors related to the patient, disease and care of the patient.[2] Mechanisms involved in inducing nephrotoxicity include direct DNA damage, stimulation of oxidative stress which directly or indirectly causes increase in reactive oxygen species, inflammation, mitochondrial damage and kidney cell death due to apoptosis and necrosis.[3]

Many drugs produce renal damage which include antibiotics like aminoglycosides, antifungals, anticancer drugs, immunosuppressive drugs, anti-inflammatory drugs etc.[3] The incidence of aminoglycoside induced nephrotoxicity has been increased progressively occurring about 10–25% in patients taking therapeutic doses.[4]

Gentamicin belongs to aminoglycoside which is used against many Gram-negative microorganisms. Therapeutic doses of gentamicin produces nephrotoxicity and acute kidney injury by generating reactive oxygen and reactive nitrogen species.[1] The prevalence of this complication is 15-30% in patients taking this antibiotic.[5] Primary cause of Gentamicin induced nephrotoxicity is due to tubular damage, proteinuria and electrolyte imbalance along with development of necrosis and inflammation.[6,7] Histologically it is characterized by tubular fibrosis, edema in epithelial cells, glomerular hypertrophy and proximal tubular necrosis leading to an increase of monocyte/macrophage infiltration and kidney failure.[1]

N acetyl Cysteine (NAC) is an antioxidant which is a sulfhydryl and glutathione group donor.[8]It is the acetylated form of L-cysteine which is used to reverse nephrotoxicity by binding with reactive oxygen species[9] and by replacing glatathione pools.[10] Hence, it possess both antioxidant and antinflmmatory properties.[12]

About 80% of the world population rely on plant based medicines to cure their basic health problems.[13] Medicinal plants provide better safety profile than drugs and are cost effective. *Berberis lycium Royale* belongs to the family Berberidaceae found in temperate and subtropical regions of the world. In Pakistan it is found in northern areas, Sawat and Murree valley. It is also known as Sumbloo, Barbery, Ziarlargay in local language.[14] Every part of this plant its root, bark, stem and fruits has some medicinal value and is used to treat different diseases like diabetes, hyperlipidemia, cancer, diarrhea, arrhythmias, depression, infections and renal injuries.[13] Its root bark contain an alkaloid Berberine which decreases the NO levels and restores GSH levels via its free radical scavenging antioxidant property, and hence it amends the renal impairment caused by the GM-induced oxidative stress.[15]

Many studies have been directed on nephroprotective effect of N acetyl cysteine and *Berberis lycium Royale* root bark extract but comparative and synergistic effect of these agents have not been explored before. In present study comparative effect of NAC and aqueous extract of *Berberis lycium Royale* root bark has been observed.

METHODOLOGY

Study Design: Comparative Randomized Control Trial.

Settings: Pharmacology Department of Islamic International Medical College, Rawalpindi in collaboration with National Institute of Health (NIH), Islamabad, Pakistan.

Duration: One month from September 2020 to October 2020.

Sample Technique: Non probability consecutive sampling technique was used.

Sample Size: 50 male albino rats.

Inclusion Criteria: Healthy male albino rats weighing 300-350 grams with normal serum uric acid levels and no kidney disease.

Exclusion Criteria: Female unhealthy albino rats weighing less than 300 grams with abnormal serum uric acid levels and kidney disease.

Data Collection Procedure: Male adult 50 Wister albino rats (300-350g) were procured from animal house of NIH. Rats were housed in steel cages in the animal house. 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.

Collection and preparation of plant extract:

Berberis lycium Royale root bark was procured from local market in Islamabad and was identified by Dr. Amir Sultan from the herbarium section of National Agriculture Research Centre (NARC) Islamabad.

Root barks of plant were dried under shade and grinded. About 500 g of powder was soaked in 1000 ml of distilled water. The powder was macerated for 4 days at room temperature with constant stirring. The mixture was filtered using Whatmann filter paper no.1. The filtrate was subjected under reduced pressure of rotary evaporator at 45^{o}C at the research laboratory of Riphah Institute of Pharmaceutical Sciences (RIPS), Islamabad. The extract obtained was as a dark brown semi-solid form which was placed in an oven to get dried. It was stored in air tight glass bottles to be used throughout the experiment.

The study was found in accordance with the international ethical guidelines and approved by Departmental Animal Ethics Committee, Faculty of Pharmacology and Therapeutics, Riphah International University, Rawalpindi, Pakistan.

All animals were divided into 5 groups with 10 animals in each group (n=10). Group 1 received distilled water and diet for 30 days. Group 2, 3, 4 and 5 was administered gentamicin (80mg/kg/day) intraperitoneally for 6 days. After 6 days, Group 3 was given N acetyl cysteine (140 mg/kg/day) per oral, Group 4 was given aqueous extract of *Berberis lycium Royale* root bark (400 mg/kg/day) per oral and Group 5 was given both NAC and aqueous extract of *Berberis lycium Royale* root bark (400 mg/kg/day) per oral for 21 days.

After 4 weeks, blood was collected through cardiac puncture under anesthesia for determination of uric acid.

Determination of Uric Acid: Determinations was made using the automated Siemens Advia 1200 analyzer.

Data Analysis: Statistical analysis was done by applying the statistical package for Social Sciences version 25 (SPSS 25). Results were documented as Mean ± SEM. Comparisons of quantitative parameters among the five groups were analyzed by using One way ANOVA (post hoc tukey test). P - value of less than 0.05 was considered as significant.

RESULTS

At the start of study serum uric acid levels were normal among all rats. After 6 days significant increase in uric acid was observed in gentamicin induced groups i.e; group 2, 3, 4 and 5. Treatment was given in group 3 (NAC), Group 4 (*Berberis lycium Royale*) and Group 5 (NAC and *Berberis lycium Royale*). Serum uric acid was measured in all groups after 30 days. All 3 treatment groups showed a significant decrease in uric acid as compared to group 2 which was given no treatment. However, Group 5 which was given combination of both NAC and *Berberis lycium Royale* showed more significant

decrease in uric acid ($p<0.05$) as compared to other groups.

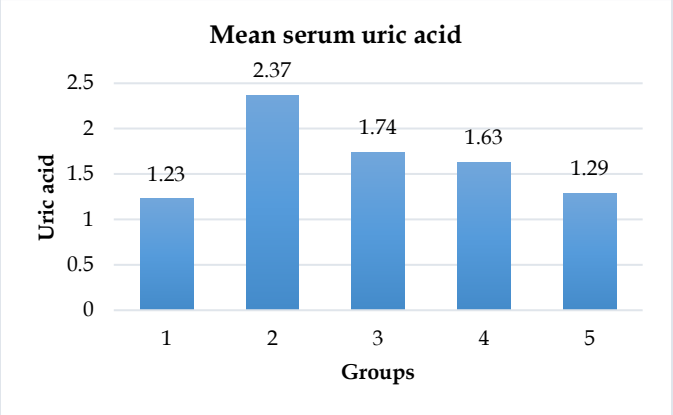
Table 1: Comparison of Mean Serum Uric acid + SEM on Day 30 among all groups of albino rats (n=50) by ANOVA

Groups	Group 1	Group 2	Group 3	Group 4	Group 5
Mean	1.23	2.37	1.74	1.63	1.29
SEM	0.02	0.23	0.23	0.32	0.09
P value	< 0.001*				

Table 2: Comparison of Mean + SEM of uric acid (mg/dl) among all groups on day 30 (n=50) by Post hoc Tukey

Groups	Mean Difference	P-value
1 vs 2	1.14 ± 0.93	0.000*
1 vs 3	0.51 ± 0.93	0.000*
1 vs 4	0.40 ± 0.09	0.001*
1 vs 5	0.06 ± 0.09	0.965
2 vs 3	0.63 ± 0.09	0.000*
2 vs 4	0.74 ± 0.09	0.000*
2 vs 5	1.08 ± 0.0	0.000*
3 vs 4	0.11 ± 0.09	0.762
3 vs 5	0.45 ± 0.09	0.000*
4 vs 5	0.34 ± 0.09	0.0006

Figure 1: Bar chart showing mean serum uric acid (mg/dl) at day 30



DISCUSSION

Many studies have been conducted to reverse the renal damage using N acetyl cysteine (11) but no study has demonstrated the combined effect of NAC and *Berberis lycium Royale* on uric acid till now. In this study it was observed that both *Berberis lycium Royale* root bark aqueous extract and N acetyl Cysteine have nephroprotective effect as they cause a decrease in serum uric acid level which was raised by gentamicin. However, their combination have more potential to extenuate the renal damage and toxic effects. They decrease the levels of serum uric acid to normal.

In the current study, gentamicin when administered intraperitoneally in a dose of 80mg/kg produced nephrotoxicity by causing a rise in serum uric acid level.[16] N acetyl cysteine decreases the level of serum uric acid in rats hence recovering the renal injury.[12] Renal damage due to oxidative stress is one of the mechanism by which gentamicin induces kidney damage.[17]*Berebris lycium Royale* root bark extract shows nephroprotective effect due to presence of alkaloid berberine.[18] It posseses antioxidant property.[19] The main focus of this study was to find the protective strategy to reverse nephrotoxicity through assessment of uric acid levels. Kidney damage causes an increase in uric acid. *Berebris lycium Royale* root bark aqueous extract showed reversal of kidney damage by causing a significant decrease in serum uric acid when compared with the group which was given only gentamicin.[19]

CONCLUSION

In summary, the indications and mechanism of NAC and *Berebris lycium Royale* is still being discovered. The present study demonstrated the potentially beneficial nephroprotective effect of N acetyl Cysteine and *Berebris lycium Royale* root bark aqueous extract in gentamicin induced kidney injury. In future, this observation may extend the official clinical indication for the use of this combination in treating kidney patients. Nevertheless, it should be emphasized that the results of our study have to be verified in clinical trials to confirm the suitability of NAC and *Berebris lycium Royale* in nephrotoxic patients.

LIMITATIONS

Owing to time constrains, cost and availability of resources, our study was based on selected parameter. We did not determine the electrolyte, bicarbonate or glucose levels.

SUGGESTIONS / RECOMMENDATIONS

Aqueous and methanolic extract of *Berebris lycium Royale* can be used in combination with NAC to reverse renal damage caused by other nephrotoxic drugs.

CONFLICT OF INTEREST / DISCLOSURE

None to declare.

ACKNOWLEDGEMENTS

I am gratefully acknowledged to Sir Brig (Ret) Akbar Waheed and my department for their support and confidence.

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