



BJP

Bangladesh Journal of Pharmacology

Research Article

**Protective and curative effects of
Cestrum nocturnum on rabbit kidney**

Protective and curative effects of *Cestrum nocturnum* on rabbit kidney

Uzma Saleem¹, Nauman Ali¹ and Bashir Ahmad²

¹Faculty of Pharmaceutical Sciences, Govt. College University, Faisalabad, Pakistan; ²Riphah Institute of Pharmaceutical Sciences, Riphah International University, Lahore Campus, Lahore, Pakistan.

Article Info

Received: 1 May 2017
Accepted: 2 July 2017
Available Online: 10 August 2017
DOI: 10.3329/bjp.v12i3.32410

Cite this article:

Saleem U, Ali N, Ahmad B. Protective and curative effect of *Cestrum nocturnum* on rabbit kidney. Bangladesh J Pharmacol. 2017; 12: 284-91.

Abstract

The objective of current study was to evaluate the nephroprotective and nephrocurative effects of methanolic extract of *Cestrum nocturnum* leaves in rabbits. Gentamicin (80 mg/kg/day) was administered intramuscularly to induce nephrotoxicity. The induction was assessed by biochemical and histopathological analysis. Methanolic extract (100, 300 or 500 mg/kg) was given orally with gentamicin for 25 days in nephroprotective study whereas in nephrocurative study, gentamicin was administered intramuscularly for first 10 days and for next 15 days only methanolic extract (100, 300 or 500 mg/kg, orally) was administered. A significant decrease was found in total oxidant status, blood urea nitrogen, serum creatinine and uric acid levels whereas albumin level increased significantly. Histopathological studies also revealed nephroprotective and nephrocurative effects. In conclusion, the methanolic extract of *C. nocturnum* leaves exhibited significant nephroprotective and nephrocurative effects in experimental animal.

Introduction

Gentamicin is an aminoglycoside antibiotic that has broad spectrum activity and is extensively used in the treatment of infections caused by Gram negative bacteria (Ali, 1995). However, its use is restricted due to severe adverse effects such as nephrotoxicity and ototoxicity (Mathew, 1992). The potential of aminoglycosides nephrotoxicity has been graded as streptomycin < netilmicin < tobramycin < kanamycin < sisomicin < gentamicin < neomycin (Parker et al., 1982). Nephrotoxicity induced by aminoglycoside is accompanied by enhanced formation of free radicals. Moreover, this toxicity is inhibited with free radical scavengers or antioxidants like dimethyl sulfoxide, dimethylthiourea, deferoxamine or sodium benzoate (Walker and Shah, 1988).

Various investigations show that anti-oxidant like vitamin E does not defend against aminoglycoside-induced damage (Ramsammy et al., 1988). The intention for this inconsistency is not known and the exact

part of the lipid peroxidation in nephrotoxicity induced by gentamicin remains unclear.

Cestrum nocturnum commonly called Night Cestrum, Queen of Night, Night Blooming Jasmine and Lady of Night (Perez-Saad and Buznego, 2008), belongs to the family "Solanaceae". It's analgesic, anti-inflammatory (Mazumder et al., 2010), antimicrobial (Khan et al., 2011), antiepileptic, anticonvulsant (Perez-Saad and Buznego, 2008), piscicidal (Punctatus, 2012), antioxidant (Rashed, 2013), wound healing (Nagar et al., 2016), CNS depressant (Zeng et al., 2003), anti-hyperglycemic, anti-hyperlipidemic (Sahane et al., 2014), anti-arrhythmic (Zou et al., 2009), anti-tumor (Zhao et al., 2013), larvicidal and insecticidal (Jawale and Dama, 2010).

Various studies were organized to achieve agents that can ameliorate the nephrotoxicity of aminoglycoside such as *Nigella sativa* (Saleem et al., 2012), *Orthosiphon stamineus* (Ramesh et al., 2014), *Mentha arvensis* (Gautam et al., 2014), *Bauhinia variegata* (Prusty et al., 2012),



Samanea saman (Patel et al., 2013), *Foeniculum vulgare* (Shaheen et al., 2014), *Moringa pterygosperma* (Lakshmana et al., 2013), *Carica papaya* (Olagunju et al., 2009) and *Withania somnifera* (Kushwaha et al., 2016).

Nephrotoxicity is the 9th leading cause of mortality all over world. Many metabolic and physiological complications are the major causes of nephrotoxicity. Various medical literatures has described about enhance incidence of acute renal failure and the major cause is the prolonged use of antibiotics especially aminoglycoside are common. Increase levels of serum creatinine and urea were considered as the important parameters of nephrotoxicity. Therefore, in this study, blood urea nitrogen, uric acid, serum creatinine, total oxidant status, and albumin were planned to be assessed to examine the nephroprotective and curative effects of methanolic leaf extract of *C. nocturnum* in rabbits against gentamicin-induced nephrotoxicity. Gentamicin has been revealed to increase the production of hydrogen peroxide and superoxide anion by the renal cortical mitochondria.

Materials and Methods

Gentamicin sulfate (Gentacin® 80 mg/2 mL), silymarin (Silliver® 200 mg, Abbott Laboratories Ltd., Pakistan) and Randox kits (Randox Laboratories, UK) were purchased locally.

Plant collection

C. nocturnum leaves were collected from the suburb of Faisalabad, Pakistan. The plant leaves were identified and authenticated by a Taxonomist of the Department of Botany, University of Agriculture, Faisalabad-Pakistan. Leaves were cleaned with tap water and permitted for the shade drying. Dried leaves were then ground into powder using a mechanical grinder.

Preparation of the extract

The 2,000 g powder was accurately weighed and then macerated with 70% methanol (1: 4) for seven days with random shaking in the air tight vessels at room temperature. The macerates were filtered by a muslin cloth and the further filtration was done by the filter paper. Extract that obtained after the filtration, was concentrated into semi-solid thick mass through rotary evaporator at 40°C. The extract was soluble in water. It was stored in air-tight container at room temperature.

Experimental animals

Sixty healthy rabbits, weighing between 1-1.8 kg, were purchased from the local market. These animals were placed in the animal house at the Department of Pharmacology, G.C.U Faisalabad, Pakistan, under controlled room temperature. Green fresh fodder was given thrice daily to the animals with water *ad libitum*.

Study design

Nephroprotective and nephrocurative studies were performed as per under given study design.

Nephroprotective study

Thirty healthy rabbits were divided into six groups, each with five animals. First group served as the normal control and second group (disease control) was injected gentamicin 80 mg/kg I/M once daily for 25 days. Third group received gentamicin (80 mg/kg I/M) and after 3 hours, this group was treated with silymarin 200 mg/kg, orally daily for 25 days. Remaining three groups were injected gentamicin (80 mg/kg I/M) and after 3 hours given methanolic leaf extract of *C. nocturnum* at doses of 100, 300 and 500 mg/kg, orally respectively daily for 25 days.

Nephrocurative study

Thirty healthy rabbits were divided into six groups, each with five animals. First group served as the normal control and second group (disease control) received gentamicin 80 mg/kg I/M for once daily 10 days. Third group was administered gentamicin (80 mg/kg I/M) for first 10 days and for the next 15 days administered silymarin 200 mg/kg, orally. Remaining three groups were given gentamicin (80 mg/kg I/M) for the first 10 days and for the next 15 days was given methanolic leaf extract of *C. nocturnum* at doses of 100, 300 and 500 mg/kg, orally respectively.

Collection of blood samples

The blood samples were collected from the marginal vein of the rabbit's ear at day 0, 10, 20 and 26 of the study period in the non-heparinized collecting tubes. These tubes were tending to undisturbed for 25-30 min to permit the blood clot. Then these tubes were centrifuged at 4,000 rpm for 20 min to isolate the serum that was moved to the serum tubes for biochemical analysis.

Biochemical analysis

Blood urea nitrogen, uric acid, serum creatinine, total oxidant status and albumin were measured by using standard procedures. Blood urea nitrogen and serum creatinine were measured by using Randox kits. Total oxidant status was measured using previously described method (Erel, 2005). Serum uric acid was measured by using Uric Acid Assay Kit (Abnova) Cat # KA1651. Albumin was measured by using Diasys Diagnostic Systems. The samples were measured at day 0, day 10 and day 26 of the study period. The values on day 0 were counted as the normal.

Histopathological analysis

On day 26, all the animals were sacrificed and kidneys were isolated, washed and then preserved in 10% neutral buffered formalin for the histopathological

study. Sections were stained and prepared with eosin and hematoxylin for the examination under light microscope.

Statistical analysis

The results were represented as mean \pm SD (standard deviation) for the blood urea nitrogen, uric acid, serum creatinine, total oxidant status, and albumin of the animals. The data were evaluated by two-way ANOVA by using GraphPad Prism version 5. The statistical implication was measured at p value \leq 0.001.

Results

Total oxidant status decreased on day 26 of the study in both nephroprotective and nephrocurative groups which was expressing the anti-oxidant power of the *C. nocturnum*. In disease state i.e. day 10 reading of nephrocurative groups, total oxidant status increased which indicated oxidative stress in the body (Table I).

A significant increase ($p < 0.001$) was found in body weight and albumin levels of all the treated groups as compared to disease control group on day 26 of the study. There was dose-dependent decrease in blood urea nitrogen, serum creatinine and uric acid levels in extract-treated groups and the maximum reduction (36.6, 32.2 and 35.7% respectively) was at 500 mg/kg concentration but this decrease was less than the standard group values (Table II). These results suggested the nephroprotective effect of *C. nocturnum*.

In nephrocurative study, there was decrease in body weight and albumin levels and increase in blood urea nitrogen, serum creatinine and uric acid levels on day 10 reading which confirmed the induction of nephrotoxicity. Body weight and albumin levels increased on day 26 whereas blood urea nitrogen, serum creatinine

and uric acid levels decreased with the treatment and the highest decline was obtained at 500 mg/kg concentration that was indicative of nephrocurative effect of *C. nocturnum* (Table III).

Histopathological analysis

The renal parenchyma was normal in appearance. The nuclei of the tubular epithelial cells were normal indicated by nucleolus and fine chromatin material. The epithelium of the tubules and glomeruli was intact. Urinary space was clear and dilated (Figure 1A).

The renal parenchyma in disease control was indicating the moderate to severe degree of nuclear changes indicated by condensed and pyknotic nuclei of the tubular epithelium cells. Mild degree of congestion was also present throughout the renal parenchyma. This was an indication of acute tubular necrosis (Figure 1B).

The renal parenchyma in standard group was normal in appearance at few places while at other places it was indicating the mild degree of necrotic changes indicated by condensed nuclei of the tubular epithelium cells. The urinary space was clear. It indicated ameliorative effect of silymarin (Figure 1C).

In nephroprotective study, the renal parenchyma in methanolic extract (500 mg/kg) was indicating the mild degree of nuclear changes shown by the condensed nuclei of the tubular epithelial cells. This was an indication of ameliorative effect of plant extract and it was almost normal renal parenchyma (Figure 1D).

In nephrocurative study, the renal parenchyma in methanolic extract (500 mg/kg) was showing almost normal appearance. Nuclei of tubular epithelium cells have nucleolus and chromatin material. However, at few places condensed nuclei were also present that is an indication of mild degree of nuclear changes. The urinary spaces were clear. Mild degree of congestion

Table I

Total oxidant status levels in nephroprotective and nephrocurative evaluations

| Days | Treatment Groups | | | | | |
|------------------------------------|----------------------------|-----------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| | Normal control | Gentamicin (80 mg/kg) | Silymarin (200 mg/kg) | Extract (100 mg/kg) | Extract (300 mg/kg) | Extract (500 mg/kg) |
| Nephroprotective evaluation | | | | | | |
| Pre-treatment values (0) | 2.5 \pm 0.1 | 2.5 \pm 0.2 | 2.5 \pm 0.1 | 2.4 \pm 0.2 | 2.4 \pm 0.2 | 2.7 \pm 0.1 |
| 10 | 2.4 \pm 0.3 ^b | 3.4 \pm 0.3 | 2.3 \pm 0.2 ^b | 2.9 \pm 0.1 ^{ns} | 2.7 \pm 0.2 ^a | 2.5 \pm 0.1 ^a |
| 26 | 2.4 \pm 0.2 ^b | 4.5 \pm 0.1 | 1.8 \pm 0.1 ^b | 3.3 \pm 0.2 ^b | 2.8 \pm 0.1 ^b | 2.4 \pm 0.3 ^b |
| Nephrocurative evaluation | | | | | | |
| Pre-treatment values (0) | 2.2 \pm 0.0 | 2.4 \pm 0.1 | 2.2 \pm 0.1 | 2.3 \pm 0.1 | 2.3 \pm 0.1 | 2.3 \pm 0.1 |
| 10 | 2.5 \pm 0.1 ^b | 3.5 \pm 0.1 | 3.6 \pm 0.2 ^{ns} | 3.6 \pm 0.1 ^{ns} | 3.6 \pm 0.1 ^{ns} | 3.6 \pm 0.1 ^{ns} |
| 26 | 2.4 \pm 0.2 ^b | 4.3 \pm 0.0 | 2.4 \pm 0.1 ^b | 3.1 \pm 0.1 ^b | 2.9 \pm 0.1 ^b | 2.7 \pm 0.1 ^b |

Values were presented as mean \pm SEM; ^a $p < 0.01$, ^b $p < 0.001$ with respect to disease control group, ns = non-significant with respect to disease control group

| Table II | | | | | | |
|--|-------------------------|-----------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Nephroprotective effect of methanol extract of <i>C. nocturnum</i> | | | | | | |
| Days | Treatment Groups | Treatment Groups | Treatment Groups | Treatment Groups | Treatment Groups | Treatment Groups |
| | Normal control | Gentamicin (80 mg/kg) | Silymarin (200 mg/kg) | Extract (100 mg/kg) | Extract (300 mg/kg) | Extract (500 mg/kg) |
| Body weight (kg) | | | | | | |
| Pre-treatment values (0) | 1.8 ± 0.0 | 2.0 ± 0.0 | 1.9 ± 0.0 | 1.9 ± 0.0 | 1.9 ± 0.0 | 1.8 ± 0.0 |
| 10 | 1.9 ± 0.0 ^c | 1.6 ± 0.0 | 2.0 ± 0.0 ^c | 1.8 ± 0.0 ^c | 1.9 ± 0.0 ^c | 1.9 ± 0.0 ^c |
| 26 | 1.8 ± 0.0 ^c | 1.5 ± 0.0 | 2.1 ± 0.0 ^c | 1.8 ± 0.0 ^c | 1.9 ± 0.0 ^c | 2.0 ± 0.0 ^c |
| Blood urea nitrogen (BUN, mg/dL) levels | | | | | | |
| Pre-treatment values (0) | 14.5 ± 0.1 | 15.4 ± 0.2 | 14.4 ± 0.2 | 16.5 ± 0.2 | 16.4 ± 0.2 | 15.5 ± 0.2 |
| 10 | 15.2 ± 0.1 ^c | 21.3 ± 0.2 | 16.5 ± 0.2 ^c | 19.4 ± 0.1 ^c | 18.2 ± 0.1 ^c | 16.9 ± 0.1 ^c |
| 20 | 14.3 ± 0.2 ^c | 25.3 ± 0.2 | 16.5 ± 0.1 ^c | 20.3 ± 0.1 ^c | 19.5 ± 0.3 ^c | 18.3 ± 0.2 ^c |
| 26 | 15.2 ± 0.1 ^c | 30.4 ± 0.0 | 17.3 ± 0.2 ^c | 22.3 ± 0.2 ^c | 20.3 ± 0.2 ^c | 19.3 ± 0.2 ^c |
| Serum creatinine (mg/dL) levels | | | | | | |
| Pre-treatment values (0) | 0.7 ± 0.0 | 0.8 ± 0.0 | 0.9 ± 0.0 | 0.7 ± 0.0 | 0.8 ± 0.02 | 0.9 ± 0.01 |
| 10 | 0.7 ± 0.0 ^c | 1.0 ± 0.0 | 0.8 ± 0.0 ^c | 0.9 ± 0.0 ^c | 0.9 ± 0.0 ^c | 0.9 ± 0.0 ^c |
| 20 | 0.7 ± 0.0 ^c | 1.1 ± 0.0 | 0.8 ± 0.0 ^c | 0.9 ± 0.0 ^c | 0.8 ± 0.0 ^c | 0.8 ± 0.0 ^c |
| 26 | 0.8 ± 0.0 ^c | 1.1 ± 0.0 | 0.7 ± 0.0 ^c | 0.9 ± 0.0 ^c | 0.8 ± 0.0 ^c | 0.8 ± 0.0 ^c |
| Albumin (g/dL) levels | | | | | | |
| Pre-treatment values (0) | 4.5 ± 0.2 | 3.6 ± 0.2 | 3.5 ± 0.1 | 4.4 ± 0.2 | 4.5 ± 0.1 | 4.4 ± 0.1 |
| 10 | 4.4 ± 0.2 ^c | 3.5 ± 0.2 | 4.3 ± 0.0 ^c | 3.1 ± 0.1 ^{ns} | 4.0 ± 0.2 ^a | 4.1 ± 0.1 ^b |
| 20 | 4.2 ± 0.1 ^c | 3.3 ± 0.2 | 4.5 ± 0.0 ^c | 3.8 ± 0.3 ^a | 4.1 ± 0.2 ^c | 4.2 ± 0.2 ^c |
| 26 | 4.4 ± 0.3 ^c | 2.4 ± 0.2 | 4.6 ± 0.2 ^c | 3.9 ± 0.2 ^c | 4.0 ± 0.3 ^c | 4.2 ± 0.2 ^c |
| Uric acid (mg/dL) levels | | | | | | |
| Pre-treatment values (0) | 2.4 ± 0.0 | 2.4 ± 0.0 | 2.5 ± 0.0 | 2.4 ± 0.0 | 2.5 ± 0.0 | 2.4 ± 0.0 |
| 10 | 2.5 ± 0.0 ^c | 4.2 ± 0.0 | 2.9 ± 0.0 ^c | 3.3 ± 0.0 ^c | 3.2 ± 0.0 ^c | 3.2 ± 0.0 ^c |
| 20 | 2.4 ± 0.0 ^c | 4.5 ± 0.0 | 2.8 ± 0.0 ^c | 3.4 ± 0.0 ^c | 3.3 ± 0.0 ^c | 3.1 ± 0.0 ^c |
| 26 | 2.5 ± 0.0 ^c | 4.6 ± 0.0 | 2.8 ± 0.0 ^c | 3.3 ± 0.0 ^c | 3.2 ± 0.0 ^c | 3.0 ± 0.0 ^c |

Values were presented as mean ± SEM; ^ap<0.05; ^bp<0.01; ^cp<0.001 with respect to disease control group, ns = non-significant with respect to disease control group

was also present (Figure 1E).

Discussion

Total oxidant status declined in methanolic extract-treated groups at the end of study in both nephroprotective and curative experiments indicating antioxidant potential of *C. nocturnum*. Blood urea nitrogen, serum creatinine, and uric acid levels decreased dose-dependently, the maximum decline was seen at 500 mg/kg dose. Albumin levels were raised with extract treatment indicating protective and curative effect on kidney.

The findings of this study are showing the trend which is revealed in results of Bag and Mumtaz (2013). Due to species difference, the values for blood urea nitrogen and creatinine are different in present study but the

trend of effect in preventing and improving gentamicin caused toxicity by *C. nocturnum* is almost identical. A similar study had been carried out by Ullah et al., (2013) who investigated the nephroprotective activity of *Cinnamomum zeylanicum*. The data of present study is in agreement with the findings of Ullah et al., (2013) in respect of two parameters i.e. blood urea nitrogen and serum creatinine, though the actual values of these parameters differ from our data. This difference can be attributed to the strains of animals being different in two studies.

Gentamicin causes nephrotoxicity by generating oxidative stress due to production of free radicals in renal cortical mitochondria (Balakumar et al., 2010; Walker et al., 1999). Gentamicin triggers platelet activation factor causing local vasoconstriction and as a result, impedes renal blood flow that leads to fall in glomerular filtration rate. Many studies support this

| Table III | | | | | | |
|--|-------------------------|-----------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Nephrocurative effect of methanol extract of <i>C. nocturnum</i> | | | | | | |
| Days | Treatment groups | | | | | |
| | Normal control | Gentamicin (80 mg/kg) | Silymarin (200 mg/kg) | Extract (100 mg/kg) | Extract (300 mg/kg) | Extract (500 mg/kg) |
| Body weight (kg) | | | | | | |
| Pre-treatment values (0) | 1.5 ± 0.0 | 1.5 ± 0.0 | 1.5 ± 0.0 | 1.5 ± 0.0 | 1.5 ± 0.0 | 1.5 ± 0.0 |
| 10 | 1.5 ± 0.0 ^c | 1.3 ± 0.0 | 1.3 ± 0.0 ^{ns} |
| 26 | 1.5 ± 0.0 ^c | 1.2 ± 0.0 | 1.6 ± 0.0 ^c | 1.4 ± 0.0 ^c | 1.5 ± 0.0 ^c | 1.6 ± 0.1 ^c |
| Blood urea nitrogen (BUN, mg/dL) levels | | | | | | |
| Pre-treatment values (0) | 13.4 ± 0.2 | 16.2 ± 0.1 | 17.2 ± 0.1 | 19.3 ± 0.2 | 16.4 ± 0.1 | 14.4 ± 0.3 |
| 10 | 13.3 ± 0.2 ^c | 29.3 ± 0.2 | 28.4 ± 0.2 ^{ns} | 29.8 ± 0.3 ^{ns} | 28.3 ± 0.2 ^{ns} | 29.7 ± 0.2 ^{ns} |
| 20 | 15.4 ± 0.2 ^c | 31.5 ± 0.1 | 21.3 ± 0.2 ^c | 25.4 ± 0.2 ^c | 24.4 ± 0.1 ^c | 22.3 ± 0.2 ^c |
| 26 | 14.4 ± 0.2 ^c | 33.5 ± 0.3 | 18.2 ± 0.1 ^c | 22.3 ± 0.1 ^c | 21.2 ± 0.1 ^c | 20.2 ± 0.1 ^c |
| Serum creatinine (mg/dL) levels | | | | | | |
| Pre-treatment values (0) | 0.8 ± 0.0 | 0.6 ± 0.0 | 0.9 ± 0.0 | 0.8 ± 0.0 | 0.7 ± 0.0 | 0.7 ± 0.0 |
| 10 | 0.8 ± 0.0 ^c | 1.1 ± 0.0 | 1.1 ± 0.0 ^{ns} | 1.1 ± 0.1 ^{ns} | 1.1 ± 0.0 ^{ns} | 1.1 ± 0.0 ^{ns} |
| 20 | 0.7 ± 0.0 ^c | 1.1 ± 0.0 | 0.7 ± 0.0 ^c | 0.9 ± 0.0 ^c | 0.9 ± 0.0 ^c | 0.8 ± 0.0 ^c |
| 26 | 0.8 ± 0.0 ^c | 1.1 ± 0.0 | 0.6 ± 0.0 ^c | 0.9 ± 0.0 ^c | 0.8 ± 0.0 ^c | 0.7 ± 0.0 ^c |
| Albumin (g/dL) levels | | | | | | |
| Pre-treatment values (0) | 4.6 ± 0.1 | 4.4 ± 0.1 | 4.4 ± 0.1 | 4.4 ± 0.1 | 4.4 ± 0.1 | 4.5 ± 0.1 |
| 10 | 4.4 ± 0.1 ^c | 2.5 ± 0.1 | 2.5 ± 0.2 ^{ns} | 2.5 ± 0.2 ^{ns} | 2.5 ± 0.1 ^{ns} | 2.5 ± 0.1 ^{ns} |
| 20 | 4.6 ± 0.2 ^c | 2.9 ± 0.0 | 3.9 ± 0.1 ^c | 3.3 ± 0.1 ^{ns} | 3.5 ± 0.1 ^b | 3.7 ± 0.1 ^c |
| 26 | 4.4 ± 0.2 ^c | 2.6 ± 0.1 | 4.1 ± 0.1 ^c | 3.3 ± 0.1 ^c | 3.4 ± 0.1 ^c | 3.8 ± 0.1 ^c |
| Uric acid (mg/dL) levels | | | | | | |
| Pre-treatment values (0) | 2.4 ± 0.0 | 2.5 ± 0.0 | 2.4 ± 0.0 | 2.4 ± 0.0 | 2.4 ± 0.0 | 2.5 ± 0.0 |
| 10 | 2.5 ± 0.0 ^c | 4.6 ± 0.0 | 4.6 ± 0.0 ^{ns} | 4.6 ± 0.1 ^{ns} | 4.5 ± 0.0 ^{ns} | 4.7 ± 0.2 ^{ns} |
| 20 | 2.5 ± 0.0 ^c | 4.6 ± 0.0 | 2.9 ± 0.0 ^c | 3.9 ± 0.0 ^c | 3.6 ± 0.0 ^c | 3.3 ± 0.0 ^c |
| 26 | 2.5 ± 0.0 ^c | 4.5 ± 0.1 | 2.5 ± 0.0 ^c | 3.4 ± 0.0 ^c | 3.2 ± 0.0 ^c | 2.8 ± 0.0 ^c |

Values were presented as mean ± SEM; ^ap<0.05; ^bp<0.01; ^cp<0.001 with respect to disease control group, ns = non-significant with respect to disease control group

view that the oxidative stress is main contributor in nephrotoxicity induced by gentamicin (Palmer, 2002; Schoolwerth et al., 2001). Urea is nitrogen containing final product of the protein catabolism. The levels of urea are increased when glomerular filtration rate is reduced. Furthermore, the serum urea levels start to rise after the damage of parenchymal tissues (Safa et al., 2010). Creatinine originates from the endogenous sources by tissue breakdown. In the initial phases of renal disease, the level of plasma creatinine is important parameter than that of level of urea (Reddy et al., 2012). In the present study, both BUN and creatinine levels increased with gentamicin treatment showing the damage to renal cortical mitochondria, consequently decreased renal blood flow and reduce GFR. The treatment with methanolic extract of *C. nocturnum* brought the levels of these two parameters to normal values suggesting nephro-protective and -curative

activity of plant extract. Albumin is synthesized in liver and is major parameter for plasma osmotic pressure. Level of albumin reduces in malnutrition, renal diseases, reduced absorption in GIT diseases and enhanced loss of fluid in burns (Young and Friedman, 2001). Similar to above observations, albumin levels were improved with the extract treatment indicating reversal of renal damage caused by gentamicin. Uric acid is water insoluble and end product of purine nucleotide metabolism. The increased amount of uric acid associated with renal calculi. Enhanced tubular pressure and intra renal pressure due to crystal deposition causes reduce in renal blood flow and enhance in renal vascular resistance. Reduced renal blood flow and enhanced tubular pressure cause decline in GFR and can result in acute renal failure (Conger, 1990). The reduced levels of uric acid with methanol extract treatment in gentamicin-induced

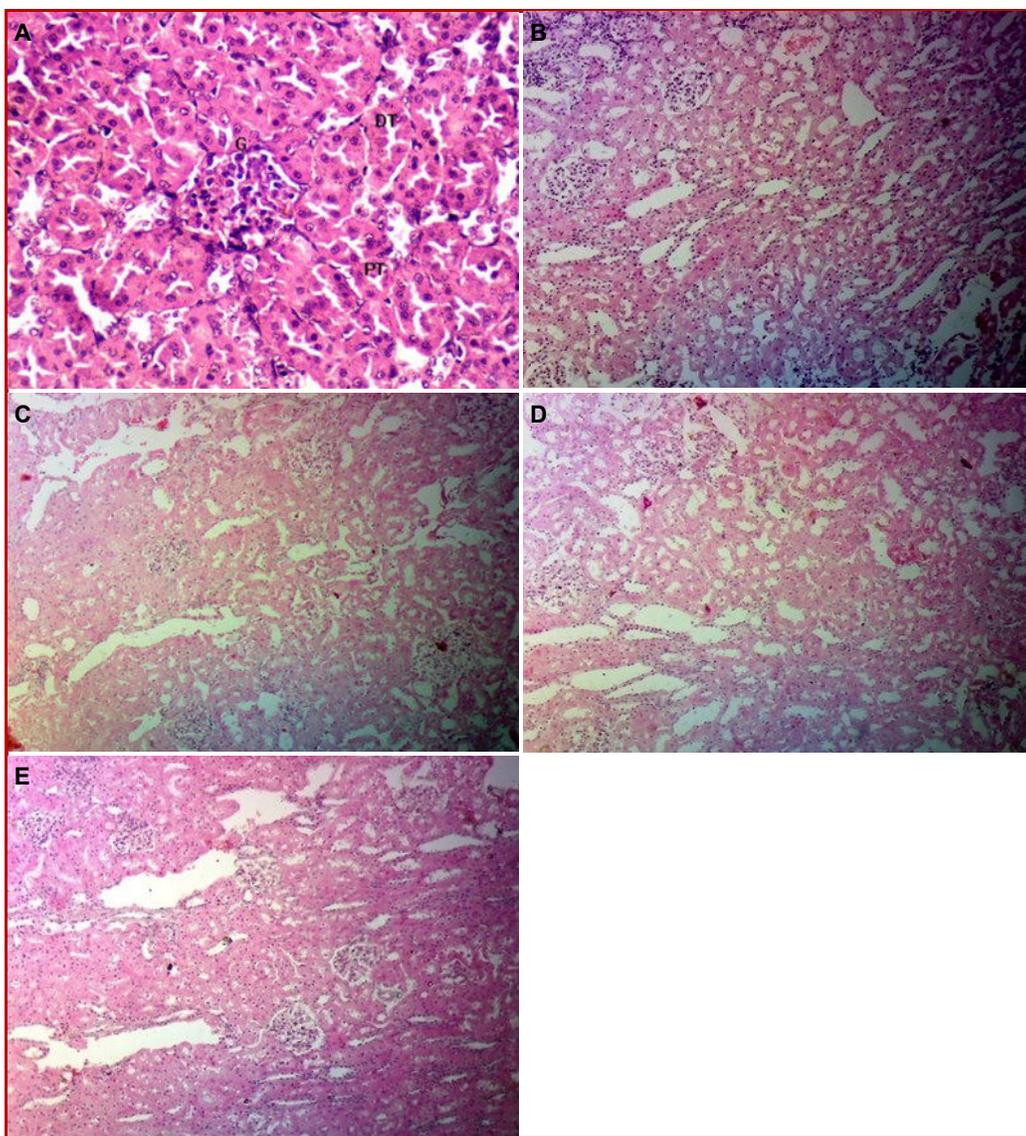


Figure 1: Histopathological analysis of rabbit's kidney. A) control group; B) Disease control; C) Standard; D) Methanol extract, 500 mg/kg (nephroprotective study); E) Methanol extract, 500 mg/kg (nephrocurative study)

toxicity in the present study also supports the other parameters in the study which are indicative of nephroprotective and curative effect of methanolic extract of *C. nocturnum*.

Conclusion

Biochemical, histopathological analyses supported nephroprotective and nephrocurative effects of methanolic extract of *C. nocturnum* leaves.

Ethical Issue

Care and handling of animals followed internationally accepted procedures according to the Institute for Laboratory

Animal Research's Guide for the Care and Use of Laboratory Animals.

Conflict of Interest

All authors have completed the ICMJE uniform disclosure form and declare no support from any organization for the submitted work.

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Author Info

Uzma Saleem (Principal contact)

e-mail: uzma95@gmail.com