Acta Poloniae Pharmaceutica – Drug Research, Vol. 70 No. 3 pp. 533–538, 2013 ISSN 0001-6837
Polish Pharmaceutical Society

CITRULLUS COLOCYNTHIS FAILED TO COMBAT AGAINST RENAL DERANGEMENTS, IN SPITE OF ITS STRONG ANTIOXIDANT PROPERTIES

NAVEED ULLAH[1]*, MIR AZAM KHAN[1], AFZAL HAQ ASIF[2], TAOUS KHAN[3] and WAQAR AHMAD[1]

[1] Department of Pharmacy, University of Malakand, Chakdara, Pakistan
[2] Department of Pharmacology, Frontier Medical College, Abbottabad, Pakistan
[3] Department of Pharmacy, COMSATS Institute of Information Technology, Abbottabad, Pakistan

Abstract: Gentamicin is a potent antibiotic, effective against Gram negative bacteria. The most common adverse effect of gentamicin is nephrotoxicity. Present study was aimed to explore the protective potentials of *Citrullus colocynthis* against gentamicin induced nephrotoxicity due to its strong antioxidant properties. Toxic doses of gentamicin (80 mg/kg/day, *i.m.*) were administered alone and as co-therapy with the extract of *C. colocynthis* (25 mg/kg/day, *p.o.*). Physiological, biochemical and histological examinations were performed to compare the experimental and toxic groups (n = 6) with control group animals. Co-therapy of *C. colocynthis* with gentamicin protected changes in the body weight, blood urea nitrogen, creatinine clearance, proteins and lactate dehydrogenase excretions. However, a significant rise in serum creatinine and serum uric acid with fall in serum calcium and serum potassium was observed, which were significantly different from control group animals. Necrotic and ruptured tubules were also found abundantly. This study revealed that co-theapy of *C. colocynthis* with gentamicin for twenty one days, failed to protect renal injury associated by gentamicin in spite of its strong antioxidant properties.

Keywords: *Citrullus colocynthis*, gentamicin, *in vivo*, renal injury

Gentamicin has broad spectrum antibacterial activities, obtained from *Streptomyces*. It has strong pharmacological and antibacterial properties (1). The drug has dose dependent bactericidal activities with low probability of bacterial resistance (2). In spite of these properties, the drug has been used with fright due to its nephrotoxic properties. It has been reported that there will be no need for the development of new drugs against Gram negative bacteria if gentamicin was proved safe in high doses (3). Therefore, *Citrullus colocynthis* was aimed to explore its renal-protective properties. It belongs to family Cucurbitaceae; a perennial creeping herb with climbing stem, bearing spherical mottled green fruits, used for the treatment of fever, constipation, intestinal parasites, dropsy, visceral and cerebral congestions (4). It had also been reported to possess hypoglycemic (5) and antibacterial properties (6). Fruit of *C. colocynthis* have been presented to have beneficial effect in the improvement of glycemic profile without the toxic side effects in type II diabetic patients (7). The most important thing is the presence of large amount of phenols and flavonoids with strong antioxidant properties (8).

Retention of gentamicin in the proximal tubules produce oxygen associated metabolites with free radicals, causes renal damage (9, 10). The present *in vivo* study was therefore designed to assess the ability of *C. colocynthis* to protect against gentamicin induced renal damage in rabbit due to its strong antioxidant properties, by both kidney functions and histologic examination.

EXPERIMENTAL

Drugs and extraction of plant

Refobacin® injections (Gentamicin, Merck Private Ltd.) were purchased from Pharma-net, Abbottabad, Pakistan. Sufficient quantity of *C. colocynthis* fruit was collected from different areas of Khyberpukhtoonkhwa, Pakistan in the month of November 2010. After authentication by Umar Farooq, Professor of Botany, GPC-1 Abbottabad, Pakistan, the voucher specimen number 1019 has

* Corresponding author: e-mail: naveedjia@yahoo.com; naveedullahpharmacist@gmail.com; Mobile: 0092-345-5910522; Fax: +92-945763491

been stored in the same institution. Plant material after grinding was extracted with ethanol and evaporated under reduced pressure (11).

Protocol

24 Male rabbits (1-1.5 kg) were acclimated in animal house of FMC Abbottabad after the approval of study from University of Malakand Research Committee. All the animals were maintained on the same diet (raddish and cauliflower leaves). Four groups of animals (Table 1) were arranged in sucha way that each group contained six rabbits. Gentamicin, normosaline solution and extracts were administered according to dosage schedule given in Table 1.

Estimation of blood urea nitrogen (BUN), serum creatinine and serum uric acid

Berthelot's indophenol assay was used for estimation of BUN and Jaffe reaction was followed for the measurement of serum creatinine (12) by Power-lab (Merck, Germany) with the help of reagents (Pro-Dia, UAE). Readymade Reagents (Egypt) were used for the estimation of serum uric acid with the help of Power-lab (Merck, Germany). Further creatinine clearance was calculated by formula:

$$\text{Creatinine clearance} = \frac{\text{Urinary creatinine concentration}}{\text{Serum creatinine concentration}} \times \text{Urinary volume}$$

Estimation of serum electrolytes

Cresolphthalein complexone method was used for the estimation of serum calcium (13), by using reagents from Randox Laboratories (UK), while serum sodium and serum potassium were measured with the help of flame photometer (Model 410, UK) (13).

Estimation of urinary lactate dehydrogenase (LDH) and alkaline phosphatase (ALP)

Urinary excretion of LDH and ALP was estimated by reagents from Dia-sys GmbH, (Germany) following German Society of Clinical Chemistry (14) procedure.

Estimation of urinary protein excretion and urinalysis

Urinary estimation of protein was accomplished using reagents from Dia-sys GmbH (Germany) (15). Urinary examination was performed by light microscope (Leitz, Germany) and multiple reagent strips (URS-10, USA) for assessing further parameters.

Renal histology

After isolation, kidneys were fixed in formalin and dehydrated with alcohol. Clearing was done with xylene solution. A number of sections embedded in paraffin wax were cut down and examined with the help of microscope after staining with dyes (hematoxylin and eosin).

Statistical analysis

Results were analyzed by one way analysis of variance (ANOVA, Dunnett test) and $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

The present work was aimed to explore the reno-protective effects of *C. colocynthis* against gentamicin induced renal toxicity. As human susceptibility to aminoglycosides is less than that of animals, these drugs have the same toxic effects in both human and animals yet in remedial doses (16). It has been reported that efficient toxic side effects are observed only if gentamicin is given in higher doses (17, 18), however, one animal of group G and group GCco had seizures on last morning of the sacrifice day. Animals treated with daily dose of 30-60 mg/kg of gentamicin had necrotic proximal tubules with increased serum creatinine level and polyuria

Table 1. Daily dosage schedule for experimental period of twenty one days.

No.	Animals group	Daily dose received
1	C	0.9% saline solution 2 mL/kg
2	G	Gentamicin 80 mg/kg
3	GCco	Gentamicin 80 mg/kg/day & *C. colocynthis* 25 mg/kg
4	Cco	*C. colocynthis* 25 mg/kg

Table 2. Kidney functioning parameters on last day of study period.

Group	Weight loss % age	BUN (mg/dL)	Serum creatinine (mg/dL)	Creatinine clearance (mL/min)	Uric acid (mg/dL)
C	0.15 ± 0.91***	14.14 ± 1.12***	0.80 ± 0.1***	4.99 ± 1.1***	1.51 ± 0.02***
G	10.795 ± 1.09	54.18 ± 2.60	4.02 ± 0.14	0.76 ± 0.09	2.34 ± 0.12
GCco	7.22 ± 1.33*	47.18 ± 2.66*	3.89 ± 0.13	3.20 ± 0.25*	2.37 ± 0.20
Cco	0.45 ± 0.86***	14.81 ± 0.30***	0.89 ± 0.03***	3.03 ± 0.14	1.48 ± 0.10***

The mean ± SEM (n = 6), (*) significant, (**) very significant and (***) extremely significant.

Table 3. Serum electrolytes and urinary protein excretion.

Group	Serum. sodium (mEq/L)	Serum potassium (mEq/L)	Serum calcium (mg/dL)	Urea protein (mg/dL)
C	140.17 ± 1.01	5.10 ± 0.24***	9.72 ± 0.25***	1.81 ± 0.22***
G	137.67 ± 1.09	3.43 ± 0.17	7.68 ± 0.21	3.86 ± 0.32
GCco	138.83 ± 1.33	3.65 ± 0.22	8.41 ± 0.6	2.82 ± 0.34*
Cco	139.33 ± 0.99	5.40 ± 0.37***	10.0 ± 0.17***	1.93 ± 0.26***

The mean ± SEM (n = 6), (*) significant, (**) very significant and (***) extremely significant.

(17). Therefore, in the present work, 80 mg/kg/day of drug was used to generate significant renal damage. In the present study all of the animals survived throughout the experimental period.

Gentamicin has been reported to cause nephrotoxicity induced by rise of BUN and serum creatinine with significant fall in creatinine clearance (18, 19). The same results were observed in the present work. In the mid of experimental period, BUN raised significantly in group G to 37.78 ± 2.14 mg/dL when compared with group C (13.75 ± 1.04 mg/dL) and further increased in group G on last day to 54.18 ± 2.6 mg/dL, statistically different from group C (14.14 ± 1.12 mg/dL). BUN in group GCco (47.18 ± 2.66 mg/dL) and in group Cco (14.81 ± 0.30 mg/dL) were significantly different from group G animals (54.18 ± 2.60 mg/dL) as seen in Table 2. Serum creatinine was elevated in group G animals to 1.96 ± 0.14 mg/dL when compared with group C animals (0.71 ± 0.10 mg/dL) in mid of the study period and further increased on last day as shown in Table 2. Group GCco exhibited no significant rise in serum creatinine on the last day of the study period when compared with group G animals, whereas group Cco was significantly different in comparison with group G animals (0.89 ± 0.03 mg/dL *vs*. group G 4.02 ± 0.14 mg/dL). A significant fall in creatinine clearance of group G animals was observed (2.08 ± 0.25 mL/min when compared with group C animals (5.08 ± 0.82 mL/min in the mid of the study period, which further decreased on last day to 0.76 ± 0.09 mL/min when compared with group G animals (4.99 ± 1.16 mL/min). Creatinine clearance of group GCco was significantly different from group G animals (3.20 ± 0.25 mL/min) while group Cco was noted to be similar to that of group G as given in Table 2. Significant rise in serum uric acid was observed in group G and GCco animals when compared with group C animals (Table 2). Group C and Cco animals were found extremely different from group G animals.

The rise of serum creatinine and functional derangements have been described with a number of different mechanisms by different researchers. Further, they reported that serum creatinine and tubular necrosis are inter-related and the blocking of tubules with necrotic materials and leaking of filtrate is responsible for the rise of serum creatinine (20).

Gentamicin therapy has been presented with an increase in urinary sodium excretion and a decrease in the re-absorption of potassium (21), In the current study serum potassium was found to be decreased in both group G and GCco animals ($p < 0.0001$) significantly different from group C animals, as previously reported for gentamicin treated animals (22-

Table 4. Measurement of urinary volume and enzymes excretion.

Group	Urinary volume (mL)		Urinary LDH (U/L)		Urinary ALP (U/L)	
	Day 11	Day 21	Day 11	Day 21	Day 11	Day 21
C	200 ± 9.1	217 ± 19.7**	91.33 ± 1.8***	88.17 ± 2.2**	14.2 ± 0.9	14.1 ± 1.2
G	168 ± 11.9	126 ± 9.0	143.17 ± 3.5	103.17 ± 4.2	11.9 ± 1.1	12.4 ± 0.6
GCco	160 ± 8.6	136 ± 7.2	92.17 ± 1.3***	92 ± 1.3	12.9 ± 0.7	12.6 ± 0.7
Cco	196 ± 16.3	186 ± 14.6	88 ± 1.6***	89 ± 1.2*	11.03 ± 0.7	13.2 ± 0.7

The mean ± SEM (n = 6), (*) significant, (**) very significant and (***) extremely significant.

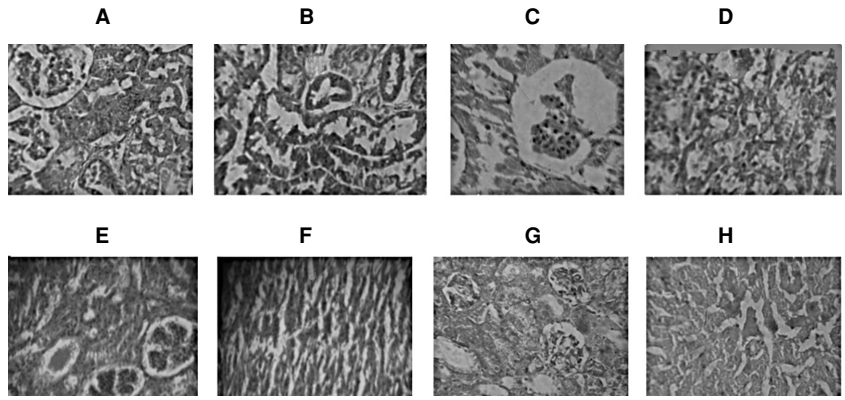

Figure 1. Renal histopathological examination: photomicrographs of group C animals: (**A**) cortex showing normal glomerulus, (**B**) medulla presenting normal tubules. Photomicrograph of group G animals: (**C**) cortex presenting necrosis, ruptured tubules, glomerular atropy and hydropic changes, (**D**) medulla showing dilated collecting tubules. Photomicrographs of group GCco animals: (**E**) cortex presenting necrotic glomeruli with hyaline filled tubules, (**F**) medulla presenting hyaline and granular casts with some ruptured tubules. Photomicrographs of group Cco animals: (**G**) cortex presenting no common abnormality, (**H**) medulla showing some hydropic changes

25). Gentamicin has been presented with hypokalemia (18), which may be due to sodium potassium ATPase depression (26). Group G animals lost their body weight throughout the study period, providing conditions of depressing nitrogen balance, which confirmed other report of considerable loss of potassium (22) like in group GCco animals, significantly different from group C animals.

Serum calcium decreased significantly in group G animals to 7.68 ± 0.21 mg/dL when compared with group C animals (9.72 ± 0.25 mg/dL) as reported formerly for gentamicin (24). However, in opposition, no change in serum calcium was observed by toxic doses of gentamicin (22). Serum calcium of group C and Cco animals were found significantly different from group G animals. However, no significant difference was noted in the serum calcium of group G and GCco animals, as given in Table 3. In nephrotoxicity, the resistance has been developed to parathyroid hormone what might be responsible for the generation of hypocalcemia (24). Further, in the present work no significant change in serum sodium was found in group G and other treated animals. However, it has been reported that the excretion of calcium, magnesium, sodium and potassium has increased in animals treated with toxic doses of gentamicin (18).

A significant increase in urinary protein excretion was observed on last day of study period in group G animals when compared with group C animals (Table 3). Further, mild increase in urinary protein excretion was also observed in group GCco animals (2.82 ± 0.34 mg/dL), but was still significantly different from group G animals (3.86 ± 0.32 mg/dL) providing a statement of reno-protective effects of plant extract. Urinary volume was observed to be decreased in all selected groups excluding group C (Table 4). To confirm nephro-

protective effects of certain drugs, tubular brush border enzyme has been estimated by different researchers (27), as like in the present study, where we used to measure urinary excretion of enzymes i.e., ALP and LDH. ALP was found to be unchanged throughout the study period (Table 4). However, urinary LDH excretion was increased in the mid of experimental period and was different and found extremely significant between group G animals and other treated animals including group C (Table 4). This rise of LDH excretion in group G was decreased by last day of study period but was still significantly different from group C animals. However, no significant difference was observed between group G (103.17 ± 4.28 U/L) and group GCco animals (92 ± 1.37 U/L) on the last day of study period. Significant amounts of red blood cells, leukocytes and cast cells were detected in group G animals, significantly different from group C and group Cco animals. However, significant amount of red blood cells and cast cells were also identified in the urine of group G animals. Significant amount of bilirubin was detected by reagent strip in group G animals, different from other groups. However, glucose and ketones were not detected in any group including group C and G. Urinary pH was recorded as basic throughout.

Group G animals were found to have necrosis of most of glomeruli with a number of ruptured tubules. Hydropic changes were also observed significant with loss of cellular pattern as shown in Figure 1C and 1D, significantly different from group C animals, which have normal glomerular appearance and no necrosis and hydropic changes (Fig, 1A and 1B). Hyaline and granular casts in the tubules of group G and GCco were observed, what may develop cellular degeneration leading to leakage of proteins. The blockage associated with cast cells causes fall in the rate of glomerular filtration directed to kidney damage (20). Group GCco animals were also observed with glomerular necrosis with loss of cellular pattern. Casts were also been observed (Fig. 1E and 1F). Regenerating tubular cells were observed stating that both regeneration and necrosis are present; as reported previously for gentamicin treated animals (18, 24). However, group Cco animals were found with such abnormalities like group C animals, but few casts and hydropic changes were observed as shown in Figure 1G and 1H. Some vacuoles in the proximal tubular cells were also observed; may be due to pinocytotic activity, developed by amino-acids and salts. The mechanism is being unknown but have no relation with cellular damage (28).

CONCLUSION

It can be concluded from current study that daily dose of 25 mg/kg of *C. colocynthis* for a period of twenty one days failed to protect renal functional and histological changes associated with gentamicin, despite of its strong antioxidant properties.

Competing interests

No competing interests are declared.

Acknowledgment

We acknowledge Higher Education Commission, Govt. of Pakistan for their financial support to the research project through Ph.D. fellowships.

REFERENCES

1. Jawetz H.: in Basic and Clinical Pharmacology, Katzung B.G. Ed., p. 645, Appleton and Lang, New York 1992.
2. Chow J.W., Fine M.J., Shlaes D.M., Quinn J.P., Hooper D.C., Johnson M.P., Ramphal R. et al.: Ann. Intern. Med. 115, 585 (1991).
3. Gilbert D.N., Bennett W.M.: Polyaspartic acid for the prevention of aminoglycoside nephrotoxicity, Prospectus 1993.
4. Anonymous: Hamdard Pharmacopoeia of Eastern Medicine, 2nd Impression, p. 373, Hamdard National. Foundation, Pakistan 1970.
5. Abdel-Hassan I.A., Abdel-Barry J.A., Tariq M.S.: J. Ethnopharmacol. 71, 325 (2000).
6. Memon U., Brohi A.H., Ahmed S.W., Azhar I., Bano H.: Pak. J. Pharm. Sci. 16, 1 (2003).
7. Huseini H.F., Darvishzadeh F., Heshmat R., Jafariazar Z., Raza M., Larijani B.: Phytother. Res. 23, 1186 (2009).
8. Kumar S., Kumar D., Manjusha., Saroha K., Singh N., Vashishta B.: Acta Pharm. 58, 215 (2008).
9. Fantone J.C., Ward P.A.: Am. J. Pathol. 107, 395 (1982).
10. Ueda N., Walker P., Goligorsky MS., Stein J.H., Shah S.V.: in Acute renal failure: New concepts and therapeutic strategies, p. 25, Churchill Livingstone, New York 1995.
11. Sayyah M., Khodaparast A., Yazdi A., Sardari S.: DARU 4, 301 (2011).
12. Smith S.T.: in Clinical chemistry, Principles, procedures, correlations, Bishop M.L., Duben-Von Laufen J.H., Fody E.P. Eds., p. 411, JB Lippincott Co., Philadelphia 1985.

13. Blosser N.: in Clinical chemistry, Principles, procedures, orrelations, Bishop M.L., Duben-Von Laufen J.L., Fody E.P. Eds., p. 263, JB Lippincott Co., Philadelphia 1985.
14. Deutsche G.K.C.: Z. Klin. Chem. Klin. Biochem. 10, 182 (1972).
15. Johnson A.M., Rohlfs E.M., Silverman L.M.: in Tietz Textbook of Clinical Chemistry, Burtis C.A., Ashwood E.R. Eds., p. 477, WB Saunders, Philadelphia 1999.
16. Kosek J.C., Mazze R.I., Cousin M.J.: Lab. Invest. 30, 48 (1974).
17. Tulkens P.M.: Toxicol. Lett. 46, 107 (1989).
18. Bennett W.M., Elzinga L.W., Porter G.A.: in The Kidney, Brenner B.M., Rector F.C. Eds., p. 1430, WB Saunders, Philadelphia 1991.
19. Moghaddam A.H., Javaheri M., Nabavi S.F., Mahdavi M.R., Nabavi S.M., Ebrahimzadeh M.A.: Eur. Rev. Med. Pharmacol. Sci. 14, 1011 (2010).
20. Solez K.: in International Review of Experimental Pathology, p. 321, Academic Press, New York 1983.
21. Derakhshanfar A., Bidadkosh A., Kazeminia S.: Iranian J. Vet. Res. 8, 231 (2007).
22. Brinker K.R., Bulger R.E., Dobyan D.C., Stacey T.R., Southern P.M., Henrich W.L., Cronin R.E.: J. Lab. Clin. Med. 98, 292 (1981).
23. Thompson J.R., Simmonsen R., Spindler M.A., Southern P.M., Cronin R.E.: Am. J. Kidney Dis. 15, 583 (1990).
24. Cronin R.E., Bulger R.E., Southern P., Henrich W.L.: J. Lab. Clin. Med. 95, 463 (1980).
25. Asif A.H., Rasool S.T., Khan T.M.: J. Clinic. Res. Bioeth. 3, 124 (2012).
26. Cronin R.E., Nix K.L., Ferquson E.R., Southern P.M., Henrich W.L.: Am. J. Physiol. 242, 477 (1982).
27. Furuta N., Nakada J.: Nippon Hinyokika Gakkai Zasshi 84, 1197 (1993).
28. Gilbert D.N., Wood C.A., Kohlhepp S.J., Kohnen P.W., Houghton D.C., Finkbeiner H.C., Lindsley J., Bennett W.M.: J. Infect. Dis. 159, 945 (1989).

Received: 23. 01. 2013