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NEPHROPROTECTIVE ACTIVITY OF CROCUS SATIVUS EXTRACT AGAINST GENTAMICIN AND/OR CEFTAZIDIME - INDUCED NEPHROTOXICITY IN RATS

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ABSTRACT

Gentamicin and Ceftazidime are commonly used antibiotics, but their use is limited by potential nephrotoxicity. In the present study, the nephroprotective activity of *Crocus sativus* was evaluated against Gentamicin and/or Ceftazidime-induced renal toxicity. Ethanolic extract of stigmas of *Crocus sativus* was administered (i.p.) once daily to albino rats 30 min. before administration of Gentamicin or Ceftazidime (i.m.) alone and in combination for 10 days. Nephrotoxicity was assessed by estimation of biochemical parameters, 24 hrs urine output, urinalysis, Erythrocyte Sedimentation Rate (ESR), body and kidney weights; and kidney histopathology were evaluated. Extract alone had no significant effect. In gentamicin-treated rats, body weights and urine output were significantly lower than control rats; along with marked proteinuria, significant increase in blood urea, serum creatinine, ESR and kidney weights with changes in serum electrolytes. This nephrotoxicity was confirmed by histopathology. Ceftazidime induced similar but lesser damage. Their combination induced toxicity greater than individual drugs. These changes were prevented by the extract of *Crocus sativus* - a promising nephroprotective agent.

KEYWORDS: **Gentamicin, Ceftazidime, Nephrotoxic, *Crocus sativus*, Nephroprotective**

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INTRODUCTION

Saffron, the dried stigmas of *Crocus sativus* (*C. sativus*) Linn. (Fam: Iridacea) is the popular spice used around the world. Saffron extracts appear to have anti-tumour[1], radical scavenger[2], anticonvulsant[3], antinociceptive[4], anti-inflammatory effects[4] and favorable effects on learning and memory[5, 6]. It has been shown to prevent renal toxicity induced by Cisplatin[7, 8]. Cephalosporins with or without aminoglycosides are considered to be drugs of choice for proven or suspected gram-negative microbial infections especially caused by *Klebsiella, Enterobactor, Proteus, Pseudomonas, Providentia, Serratia and Haemophilus* species. However, approximately 8 - 26% of patients who receive aminoglycosides for more than several days will develop renal function impairment that is almost reversible and about 10-15% of patients develop acute renal failure. Cephalosporins are not as toxic to the kidney as aminoglycosides however; they increase aminoglycoside-induced nephrotoxicity. Thus, renal toxicity is the dose- limiting factor for these drugs. Until now, little progress with success has been made to minimize the nephrotoxic potential of these drugs besides dose adjustments in patients with impaired renal function. The present study is undertaken to explore the renoprotective effect of *C. sativus* in rats against nephrotoxicity caused by an aminoglycoside viz. Gentamicin and a cephalosporin viz. Ceftazidime.

MATERIALS AND METHODS

I) Fresh *C. sativus* stigmas brought from Kashmir were authenticated by Regional Research Laboratory, (CSIR), Jammu-Tawi. *C. sativus* stigmas (64 g) were extracted with 95% of ethanol. Thin layer chromatography was done for confirmation of the main constituents in the extract. Column chromatography was performed using different solvents - petroleum benzene, chloroform, methanol and water successively to obtain four main constituents.

II) The water fraction was separated by ascending paper chromatography. Active ingredient of water fraction was confirmed by thin layer chromatography using different reagents, which revealed a single spot. Isolated active ingredient was subjected to infrared spectroscopy to confirm the functional groups. These functional groups match with those of crocin[9].This ingredient termed as "*C. sativus* extract" (CSE) has been used for studying renoprotective action.

III) The protocol was approved by the Institutional Ethics Committee and CPCSEA guidelines were followed. Female albino rats weighing 150–250 g were divided into 8 groups; each of 6 animals (Table 1). They were given food and water ad libitum.

Table 1
Groups and Treatment Schedule

No.	Groups	Dose (mg/kg) once daily for 10 days	Route
1	Control – Distilled water	2 ml	IP
2	"*C. sativus* extract" (CSE)	50	IP
3	Gentamicin	40	IM
4	Ceftazidime	500	IM
5	Gentamicin and Ceftazidime combination	40 and 500	IM
6	CSE * + Gentamicin	40	IM
7	CSE * + Ceftazidime	500	IM
8	CSE * + Gentamicin and Ceftazidime combination	40 and 500	IM

**** Pretreatment (Dose 50 mg/kg IP once daily for 10 days) 30 min. before study drugs***

The following parameters were studied.

a. General behavior, body weight, mortality; if any.
b. 24 hrs urine output and urinalysis - pH, proteins and microscopy
c. Biochemical parameters:- Blood urea, Serum creatinine, Serum electrolytes
d. Erythrocyte sedimentation rate (ESR).
e. Kidney weight.
f. Histopathology: Macroscopic and microscopic examination of kidney.

Among these parameters, general behavior and body weight were observed every day, urine analysis and urine output were assessed on 1st and 10th day of the study and biochemical parameters at the end of study. After 10 days of treatment, animals were sacrificed under ether anesthesia. Blood was withdrawn for estimations; kidneys were excised, weighed and examined histopathologically. Histopathologist was unaware of treatment assignments. Histopathological changes were assigned scores[10].

Score 0 - Normal architecture.

Score 1 - PCT dilatation, focal granulovacuolar epithelial cell degeneration and granular debris in tubular lumens (<1% of total tubular population).

Score 2 - Epithelial necrosis and desquamation involving < half of cortical tubules.

Score 3 - Epithelial desquamation and necrosis involving more than half of proximal tubules **Score 4** - Complete or almost complete tubular necrosis.

The numerical data were expressed as mean ± standard error of Mean (S.E.M.) and analyzed by unpaired Student's t test. $P<0.05$ was taken as criterion for significance.

RESULTS AND OBSERVATIONS

None of the rats exhibited any abnormal responses, stereotypes and bizarre behavior. Moreover, no mortality was observed among the animals of any group. Rats treated with CSE alone did not show any significant change in body weight whereas a significant decrease in body weight was observed in Gentamicin (Day 1 - 215.00 ± 11.2 g., Day 10 - 199.2 ± 10.7 g, $P<0.05$), Ceftazidime (Day 1 - 218.30 ± 13.5 g, Day 10 -206.07 ± 14.3 g, $P<0.05$) and combination treated group (Day 1- 196.70 ± 9.20 g, Day 10 - 180.00 g ± 8.60 $P<0.01$) as compared to their pre-treatment body weight. However, body weight did not differ in all these three groups when pre-treated with CSE. CSE treated rats did not show any significant change in ESR at the end of 2 hrs where as Gentamicin (2.60 ± 0.14 mm), Gentamicin and Ceftazidime combination treated groups (2.74 ± 0.17 mm) showed a significant ($P<0.05$) increase in ESR whereas Ceftazidime treated groups did not show any significant change as compared to control group (2.03 ± 0.27 mm). However, all these three treatment groups did not show any significant change in ESR after pre-treatment with CSE.

CSE alone treated rats did not show any significant change whereas Gentamicin and/or Ceftazidime treated group showed a significant decrease in urine output as compared to baseline. However, in all these three groups after pretreatment with CSE, urine output did not decrease significantly. Proteins were not observed in urine samples of control as well as CSE groups of rats, whereas Gentamicin and/or Ceftazidime, treated rats showed increased excretion of proteins (+++). However, proteins were totally absent when these rats received pretreatment with CSE. The pH of urine of control group as well as CSE groups of rats was 7– 8, whereas urinary pH of rats treated with Gentamicin, Ceftazidime and combination was 5 – 6, 6 – 7, and 4–5 respectively. Microscopic examination of urine from drug treated groups did not differ significantly from control group rats. CSE treated rats did not show any significant change in serum potassium and sodium levels where as Gentamicin and/or Ceftazidime treated groups showed a significant increase in serum potassium and a significant decrease in serum sodium levels, as compared to control group. However, these groups did not show any significant change in serum potassium and sodium levels after pre-treatment with CSE (Table No. 2).

Table 2
Effect of C. sativus extract on Serum Electrolytes levels in Gentamicin and/or Ceftazidime treated rats (n=6)

Groups	Control	*C. sativus*	Genta-micin	Cefta-zidime	Gentamicin + Ceftazidime	Pretreatment with *C. sativus* 50 mg/kg		
						Genta-micin	Cefta-zidime	Gentamicin + Ceftazidime
Dose (mg/kg)		**50**	**40**	**500**	**40 + 500**	**40**	**500**	**40 + 500**
Serum Potassium (meq/L)	3.53 ± 0.26	4.03 ± 0.15	4.90* ± 0.36	4.53* ± 0.34	4.69* ± 0.30	4.58 ± 0.43	4.07 ± 0.21	4.12 ± 0.26
Serum Sodium (meq/L)	139.50 ± 0.68	136.42 ± 1.40	134.17** ± 1.40	134.33* ± 2.20	133.75* ± 2.48	135.42 ± 2.15	137.83 ± 0.56	136.75 ± 2.47

Figures are Mean ± SEM
**** P < 0.05, ** P < 0.01 compared to Control***

CSE treated rats did not show any significant change in blood urea and serum creatinine levels where as Gentamicin and/or Ceftazidime treated group showed a significant increase in blood urea and serum creatinine levels, as compared to control group. However, these groups did not show any significant change in blood urea and serum creatinine levels after pretreatment with CSE (Table 3).

Table 3
Effect of C. sativus extract on Biochemical parameters in Gentamicin and/or Ceftazidime treated rats (n=6)

Groups	Control	*C. sativus*	Genta-Micin	Cefta-zidime	Gentamicin + Ceftazidime	Pretreatment with *C. sativus* 50mg/kg		
						Genta-micin	Cefta-zidime	Gentamicin + Ceftazidime
Dose (mg/kg)		**50**	**40**	**500**	**40 + 500**	**40**	**500**	**40 + 500**
BUN (mg/dl)	34.67 ± 4.87	35.16 ± 5.05	90.67* ±23.50	89.99* ± 17.60	108.67** ± 20.86	36.00 ± 6.24	36.16 ± 3.93	35.50 ± 2.34
Serum Creatinine (mg/dl)	1.38 ± 0.19	1.53 ± 0.18	2.20* ± 0.28	2.17* ± 0.28	2.27** ± 0.18	1.48 ±0.19	1.53 ± 0.16	1.57 ± 0.16

Figures are Mean ± SEM
**** P <0.05, ** P <0.01 compared to control***

CSE treated rats did not show any significant change in kidney weights. Gentamicin (Rt. 1.25 ± 0.06 g , Lt. 1.22 ± 0.05 g, P<0.05), Ceftazidime (Rt.1.22 ± 0.06 g, Lt. 1.17 ± 0.08 g, P<0.05) and combination (Rt.1.30 ± 0.01 g, Lt.1.27 ± 0.04 g, P<0.01) treated groups of rats showed a significant increase in kidney weights as compared to control groups of rats (Rt.1.03 ± 0.05 g, Lt. 1.02 ± 0.04 g). However, kidney weights did not show any significant change in all these three groups pretreated with CSE. Macroscopic examination of the kidneys was unremarkable among the different groups of rats. Microscopically, kidneys from a control group of rats showed normal structure. Histological picture of kidneys of CSE treated rats was not significantly different from control. The kidneys of rats treated with Gentamicin showed dilatation of proximal tubules, congestion and areas of focal and granular debris. With Ceftazidime treatment, there was also dilatation of proximal convoluted tubules, congestion and infiltration of inflammatory cells such as neutrophils and occasional macrophages, eosinophils and basophils. The kidneys of rats treated with Gentamicin and Ceftazidime combination showed congestion, desquamation, interstitial nephritis with infiltration of neutrophils and very prominent bilobed eosininophils. Pre-treatment with CSE

extract has prevented the histological changes induced by Gentamicin and/or Ceftazidime. These histomorphological changes in kidneys were graded and shown in Table 4.

Table 4
Grading of Histopathological examination of rat Kidneys treated with C. sativus extract, Gentamicin and/or Ceftazidime

Groups	Control	*C.sativus*	Genta-micin	Cefta-Zidime	Gentamicin + Ceftazidime	Pretreatment with *C. sativus* 50 mg/kg		
						Genta-micin	Cefta-zidime	Gentamicin + Ceftazidime
Dose (mg/kg)		**50**	**40**	**500**	**40 + 500**	**40**	**500**	**40 + 500**
Score 0	+++ (6)	+++ (6)	(0)	(0)	(0)	+++ (6)	+++ (6)	+++ (6)
Score 1	(0)	(0)	+++ (2)	+++ (4)	++ (2)	(0)	(0)	(0)
Score 2	(0)	(0)	++ (2)	++ (2)	++ (1)	(0)	(0)	(0)
Score 3	(0)	(0)	+ (1)	(0)	++ (2)	(0)	(0)	(0)
Score 4	(0)	(0)	++ (1)	(0)	+++ (1)	(0)	(0)	(0)

Each Group: Total 6 animals.
Figure in brackets indicates no. of animals.

Histology of Kidney of CSE treated rats did not differ from the control rats. Rats treated with Gentamicin had scored 1-4. Rats treated with Ceftazidime had a score of 1-2. In rats treated with Gentamicin and Ceftazidime combination, more intense changes were observed (Score 1-4). Pretreatment with CSE resulted in almost normal kidney architecture (Score 0) in rats treated with Gentamicin, Ceftazidime alone and their combination.

DISCUSSION

Aminoglycosides are one of the commonest causes of drug-induced nephrotoxicity. Aminoglycosides accumulate in proximal tubular cells and typically provoke acute renal failure after 7-10 days of exposure. To date, there are no specific therapies for established acute renal failure due to nephrotoxicity. The incidence of nephrotoxic acute renal failure can be reduced by tailoring the dosage of potential nephrotoxins to body size and glomerular filtration rate[11]. In the present study, renoprotective effect of water soluble fraction of ethanolic extract of stigmas of *C. sativus* was examined against Gentamicin and/or Ceftazidime–induced nephrotoxicity models in rats. In control rats, body weight increased insignificantly from 198.30 to 230.3 g, 24 hrs urine output decreased from 9.9 to7.5 ml. but this was not statistically significant. Urinalysis did not reveal proteins and pH of urine was 7-8. ESR was 2.03 mm at the end of 2 hrs. Serum potassium and sodium levels were 3.53 and 139.50 mq/L respectively. Blood urea and serum creatinine levels were 34.67 mg and 1.38 mg/100 ml respectively. The right and left kidney weights were 1.03 and 1.02 g respectively. Microscopic examination of kidneys revealed normal renal architecture.

Animals treated with Gentamicin (40 mg/kg i.m.) for 10 days showed a significant decrease in 24 hrs urine output and increased excretion of proteins in urine. Renal function insufficiency is known to decrease excretion of electrolytes. This was reflected by a significant increase in serum potassium and significant decrease in sodium as compared to control rats. However, body weights of these rats were decreased significantly. These rats also had a significant rise in ESR. Gentamicin treated rats also had a significant increase in blood urea and serum creatinine. This biochemical evidence is also suggestive of renal function insufficiency. Besides significant increase in kidney weights, histopathological examination of kidneys also exhibited changes suggestive of significant renal damage. Gentamicin (40 mg) was also shown to be nephrotoxic[12,13].

Gentamicin-induced nephrotoxicity results from intracellular accumulation and retention of Gentamicin[14,15]. The biochemical events leading to tubular cell damage and glomerular dysfunction are poorly understood but they may involve perturbations of structures of cellular membranes. Similar observations were also seen in rats treated

with Ceftazidime (500 mg/kg i.m.) for 10 days. However, histopathological examination of kidneys of Ceftazidime treated rats demonstrated less intense damage than Gentamicin treated rats. Cephaloridine, a nephrotoxic cephalosporin antibiotic was shown to change thiol status in the renal cortex before development of significant morphological changes[16].

Concurrent administration of aminoglycosides and cephalosporins are known to cause nephrotoxicity synergistically. This was evident in rats treated with both Gentamicin (40 mg/kg i.m.) and Ceftazidime (500 mg/kg. i.m.) alone and combination (40 + 500 mg/kg i.m. respectively), as shown by more significant ($P<0.01$) changes in all the renal parameters. The synergistic nephrotoxicity is evident in histopathological evaluation also. CSE treated rats did not show significant changes in 24 hrs urine output, urinalysis and blood urea and serum creatinine, serum electrolytes and ESR compared to control. Body weights and kidney weights of rats were also similar to control rats. Microscopic evaluation of kidney also did not differ from control rats. Thus, CSE (50 mg/kg) per se does not have any significant effect on kidneys or kidney function. In Gentamicin and Ceftazidime treated rats, pretreatment with CSE has prevented the changes which would have otherwise been produced by Gentamicin and Ceftazidime in all the parameters suggestive of renal tubular damage. The attainment of near normality in histological picture of kidneys with CSE pretreatment further elucidates its renoprotective effect. Results were similar when rats were treated with combination of Gentamicin and Ceftazidime. These findings suggest that CSE has significantly neutralized the toxic effects of Gentamicin and/or Ceftazidime on renal tubules.

Thus, this study substantiates the renoprotective potential of water fraction of ethanolic extract of CSE. CSE (50 mg/kg) when administered i.p. for 5 days with 3 mg/kg of cisplatin, significantly reduced serum blood urea, serum creatinine as well as cisplatin-induced rise in serum total lipids. It also protected from cisplatin-induced falls in leucocyte counts, haemoglobin levels and osmotic fragility of erythrocytes and also prevented the increase in haematocrit[7]. The exact mechanism by which CSE exerts its protective effects against Cisplatin, Gentamicin and Ceftazidime-induced nephrotoxicity are not yet known. The possible mechanisms may include inhibition of binding of gentamicin to renal brush border[17], decrease in tubular cell lipoperoxidation in response to toxic injury[10,18].

CONCLUSION

The hydroalcoholic extract of *C. sativus* appears to be a promising nephroprotective agent, given simultaneously with Gentamicin and/or Ceftazidime. However, further studies should be carried out to explore the renoprotective effect of CSE after establishment of nephrotoxicity and in various doses.

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