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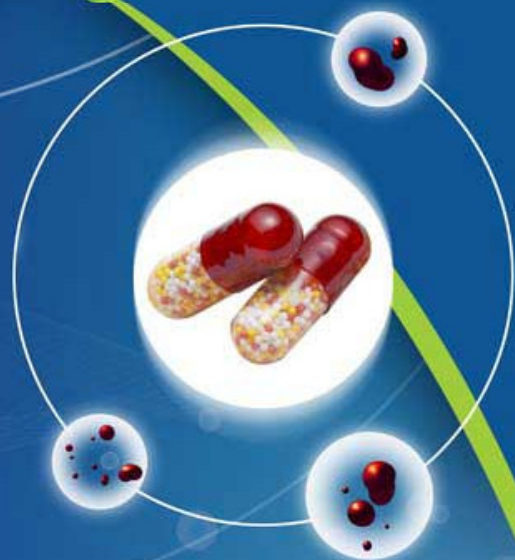
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──── **Research** Article ────

POSSIBLE ROLE OF SODIUM CROMOGLYCATE, A MAST CELL STABILIZER IN HALTING GENTAMICIN NEPHROTOXICITY IN RATS

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ABSTRACT

Gentamicin is commonly used in treating life threatening gram-negative bacterial infections, butmajor limitation is its nephrotoxic effect. Free radical generation and reduction in antioxidantdefense mechanisms are mainly considered to be involved in gentamicin induced nephrotoxicity,but the exact mechanism still remains unclear. Mast cells are infiltrated in the inflamed kidneyand their degranulation releases various inflammatory mediators during various renal diseases.We have investigated the possible involvement of mast cell stabilizer sodium cromoglycate [SCG] in halting gentamicin-nephrotoxicity in rats. Administration of gentamicin [GM] 100mg/kg i.p. for 10 days in rats induced nephrotoxicity, characterized by significantly [P<0.05]increased serum creatinine, blood urea nitrogen, urinary protein, renal oxidative stress and mastcell density, decreased creatinine clearance and histopathological alterations. Co-administrationof SCG [24mg/kg/day] attenuated significantly [P<0.05], all these biochemical parameters,reduced mast cell density and prevented the histological alteration such as intracellular edema,glomerulus narrowing and necrosis in epithelial cells of the proximal tubules. It is concluded thatmast cells have a detrimental role in gentamicin-induced nephrotoxicity and SCG stabilizes mastcells, reduces the release of various proinflammatory cytokines and thus, prevents the toxiceffects of GM as seen in both, the biochemical and histopathological parameters.

Key words: Gentamicin, Nephrotoxicity, Mast cells, Sodium cromoglycate

INTRODUCTION

Gentamicin is extensively used in the clinical practice for the treatment of life threatening gramnegative bacterial infection but the major limitation to its usefulness is limited by the development of nephrotoxicity. Prevalence rate of gentamicin induced nephrotoxicity is approximately 15-35% [1-3]. Oxidative stress by Superoxide, peroxinitrite anions and hydrogen peroxide are main Pathophysiological feature reported to cause nephrotoxicity [2], increased intracellular calcium level [3],

and reduction of glomerular filtration rate [GFR], acute and chronic renal failure have also been reported to be involved in gentamicin induced nephrotoxicity [4]. Despite many hypotheses tested in animal models, the exact mechanism of Gentamicin induced Nephrotoxicity still remains unclear [5]. Mast cells are bone marrow–derived hematopoietic cells that share phenotypic characteristics with monocytes/macrophages [6] and are involved in patho-physiological function such as tissue injury and repair, allergic inflammation and host defences. They synthesize and secrete a variety of mediators, activating and modulating the functions of nearby cells and initiating complex physiological changes. Mast cells participate

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in manily inflammatory kidney diseases, particularly those associated with fibrosis [7, 8]. Mast cells are constitutively expressed in small numbers in normal kidney [9], but renal injury correlated positively with interstital mast cell accumulation and degranulation [10-12]. Mast cells can be degranulated in response to mechanical trauma, increased temperature, chemical agents, anaphylactic toxins, calcium and basic compounds, in addition to hypersensitivity conditions [13]. Sodium cromoglycate [Cromolyn] is a mast cells stabilizer that inhibits mast cell activation and degranulation by averting transmembrane influx of calcium ions [14]and thus the release of inflammatory mediators. It has been used as preventive in conditions like asthma and inflammatory conditionsof the eye. Recently mast cell stabilizers have been reported to show beneficial effects in streptozotacin [STZ] induced nephropathy[15, 16] and cisplatin induced kidney injury [17] but their role in gentamicin nephrotoxicity has not been studied so far. Therefore, present study has been designed to investigate the possible role of mast cell stabilizer in preventing gentamicin nephrotoxicity in rats.

MATERIAL AND METHODS

Animals

Twenty four adult wistar-albino rats of either sex weighing approximately 180- 220 g were used in this experimental study. Rats were provided by the animal house of ISF College of Pharmacy, Moga, Punjab. The rats housed in plastic cages in a temperature-controlled room were exposed to normal day and light cycle and were fed on standard chow diet and water *ad libitum*.

Experimental protocol

The experimental protocol used in the present study was approved by the Institutional Animal Ethical Committee of ISF College of pharmacy, Moga. Rats were divided randomly into four equal groups including six animals each.

Group I (Normal control) (NC): Rats were maintained on standard food and water regime and no treatment was given.

Group II (Gentamicin control)(GMC): Rats were administered Gentamicin (100 mg/kg, i.p.) daily for 10 days.

Group III (Sodium cromoglycate treated gentamicin nephrotoxicity group)(SCG + GM): Rats were administered Sodium cromoglycate (24mg/kg/day i.p.)[18]for 10 days along with gentamicin from 1st day.

Group IV (Lisinopril treated gentamicin nephrotoxicity group) (LIS + SCG): Rats were administered Lisinopril (1mg/kg/day p.o.)[19] for 10 days along with gentamicin from 1st day.

Sample collection and biochemical assays:

After 24 hour from the last dose, urine samples were collected for urinary protein and urinary creatinine determination in cylinder containing sodium azide 0.1% to minimize bacterial growth. Blood samples were collected through retro-orbital sinus for determination of serum creatinine and blood urea nitrogen (BUN).

Renal function test

Serum creatinine concentration and urinary creatinine (for creatinine clearance estimation) was estimated by alkaline picrate method [20]using commercially available kit (Coral clinical system, Goa, India). Proteinuria was estimated by pyrogallol red method [21]using the commercially available kit (Coral clinical system, Goa, India). BUN was estimated by Berthelot method [22]using the commerciallyavailable kit (Coral clinical system, Goa, India).

Renal weight and kidney collagen content

It was determined as an index of glomerular, mesengial expansion and renal fibrosis. Rats were sacrificed by cervical dislocation; kidney was excised by opening abdominal cavity. Kidney weight / body weight [KW/BW] % was calculated according to formula [23, 24]. The renal cortical tissue was used for the determination of kidney collagen content. Total collagen content of kidney was determined by analysis of hydroxyproline content. Hydroxyproline was determined

colorimetrically of renal cortical tissue using a modified method of Jamal et al, [25].

Oxidative stress

Renal oxidative stress was assessed in term of renal thio-barbituric acid reactive substance (TBARS) and reduced glutathione (GSH). Renal TBARS, an index of lipid peroxidation, was estimated according to the method described by Ohkawa et al., [26]. GSH level was estimated by Ellman [27].

Histopathological study

One kidney from each rat was fixed in 10% formalin solution. Changes in glomeruli were assessed morphologically and histologically as described by Tomohiro et al. [28], in 3 μm thickness sections and stained with hematoxylin and eosin to assess the pathological changes that occured in the glomeruli using light microscope. Mast cell density was quantified by counting number of toludine blue positive mast cells per field using 0.1% toludine blue stain as described previously [11].

Chemicals

Gentamicin injection were of (Nicolas piramal ltd, Mumbai), SCG was bought from Yarro Chem Products, Mumbai. Lisinopril bought from Sigma Aldrich Ltd, USA. All other chemicals used in present study were of analytical grade.

Statistical Analysis

All values were expressed as mean ± S.D. Various biochemical parameters i.e. blood urea nitrogen, serum creatinine, urinary proteins, renal hypertrophy were statistically analyzed using one way ANOVA followed by Tukey's multiple comparison test. The p value < 0.05 wasconsidered to be statistically significant.

RESULTS

Effect of Sodium Cromoglycate (SCG) and lisinopril(LIS) treatment on renal function

Effects of gentamicin (100 mg/kg/day, i.p. for 10 days) on group II renal function were observed. There were significantly higher (p<0.05) level of serum creatinine, blood urea nitrogen (BUN) and protein in urine while creatinine clearance levels was significantly less [p<0.05] in group II in comparison to normal control. Treatment with SCG (24 mg/kg/day i.p. for 10 days)or lisinopril with gentamicin administration provided a marked protective effect with significantly (P<0.05) decreased serum creatinine, BUN, protein in urine and significantly increased creatinine clearance level [Table1].

Table I: Variables of Biochemical parameters tested

Groups	Serum reatinine [mg/dl]	BUN[mg/dl]	Creatinine Clearance [ml/min.]	Protein in urine [mg/24hr]	Renal collagen content [mg/g of renal cortex]
Normal Control	0.87 ±0.06	18.87 ± 1.69	0.38 ± 0.93	4.89 ± 0.94	2.48 ± 0.40
Gentamicin Control	1.88 ± 0.39[a]	62.63 ± 3.85[a]	0.11 ± 0.03[a]	21.89 ± 3.24[a]	4.29 ±0.42[a]
SCG treated Nephrotoxic rats	1.1 ± 0.27[b]	43.48 ± 2.8[b]	0.26 ± 0.07[b]	12.52 ± 2[b]	3.09 ± 0.06[b]
Lisinopril treated Nephrotoxic rats	1.14 ± 0.26[b]	45.84 ± 6.36[b]	0.27 ± 0.11[b]	13.03 ± 1.79[b]	3.40 ± 0.08[b]

All values expressed as mean ± SD [a]P<0.05 GMC vs. NC; [b]P<0.05 SCG+GM, LIS+GM vs. GMC.

Effect of SCG and lisinopril on KW/BW and collagen content

Gentamicin administered rats showed significant (P<0.05) increased KW/BW %, and total renal cortical collagen content. SCG treatment significantly reduced (p < 0.05) KW/BW % [Fig.1] and renal cortical collagen content in nephrotoxic rats [Table 1]. Effect of lisinopril on KW/BW was not significant.
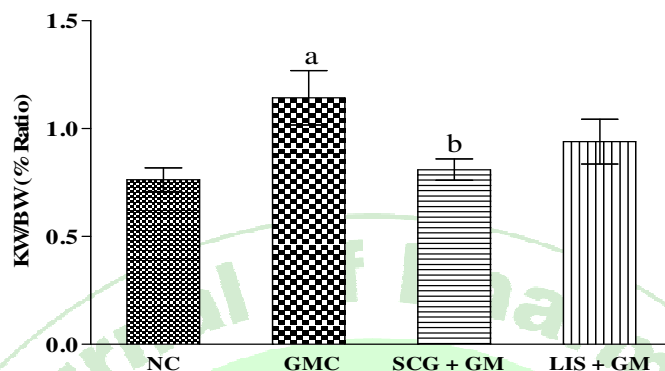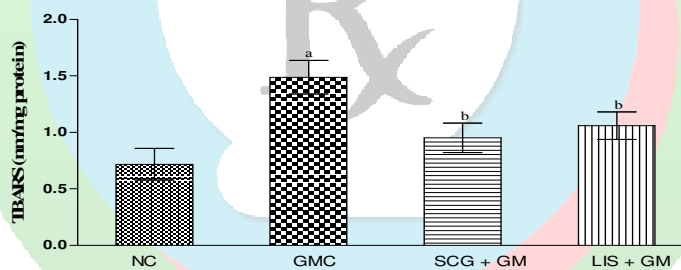

Fig.1: Effect of various pharmacological interventionson KW/BW%.

All values are expressed as mean ± S.D. [a]P<0.05= gentamicin control (GMC) vs. normal control (NC); [b]P<0.05 = sodium cromoglycate + gentamicin (SCG+GM)vs. gentamicin control
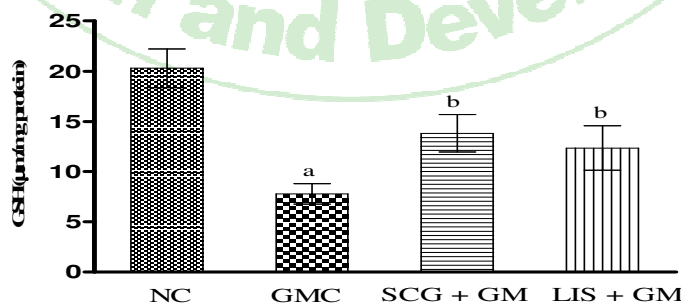
Effect of SCG and lisinopril on oxidative stress

Administration of gentamicin significantly increased (p < 0.05) TBARS level and decreased (p <0.05) GSH level in kidney; both are index of increased oxidative stress. Treatment with SCG or lisinopril in gentamicin administered rats showed significantly decreased (p < 0.05) oxidative stress [Fig. 2 and 3].

Fig.2. Effect of various pharmacological interventionsonrenal TBARS.

All values are expressed as mean ± S.D. [a]p<0.05 GMC vs. NC; [b]p<0.05 SCG+GM and LIS+GM vs. GMC.

Fig.3. Effect of various pharmacological interventionsonrenal GSH level.

All values are expressed as mean ± S.D. [a]p<0.05 GMC vs. NC; [b]p<0.05 SCG+GM and LIS+GM vs. GMC.

Effect of SCG and lisinopril on histology of kidney

Microscopic examination of renal tissues stained with H&E revealed that there were normal renal glomeruli surrounded by capsule and normal proximal, distal and convoluted tubules in normal control group [Fig.4A]. However, degeneration, desquamation, intracellular edema, glomerulus narrowing and necrosis were observed in epithelial cells of the proximal tubules in rats of GM control group. Degenerated and desquamated epithelial cells were in lumens of tubules [Fig.4B]. Large number of inflammatory cells infiltrated in the form of mononuclear cells and intertubular hemorrhage in the renal sections of this group. Glomerulus congestion and swelling were also observed in the basement membrane whereas there was mild lesions inglomerulus and tubules of the renal tissues of rats treated with GM + SCG when compared withthe GM-treated group [Fig. 4C]. Moreover, treatment with lisinopril (1 mg/kg p.o., 10 days)markedly protected the gentamicin-induced nephrotoxicity kidney from renal pathological changes [Fig. 4D].
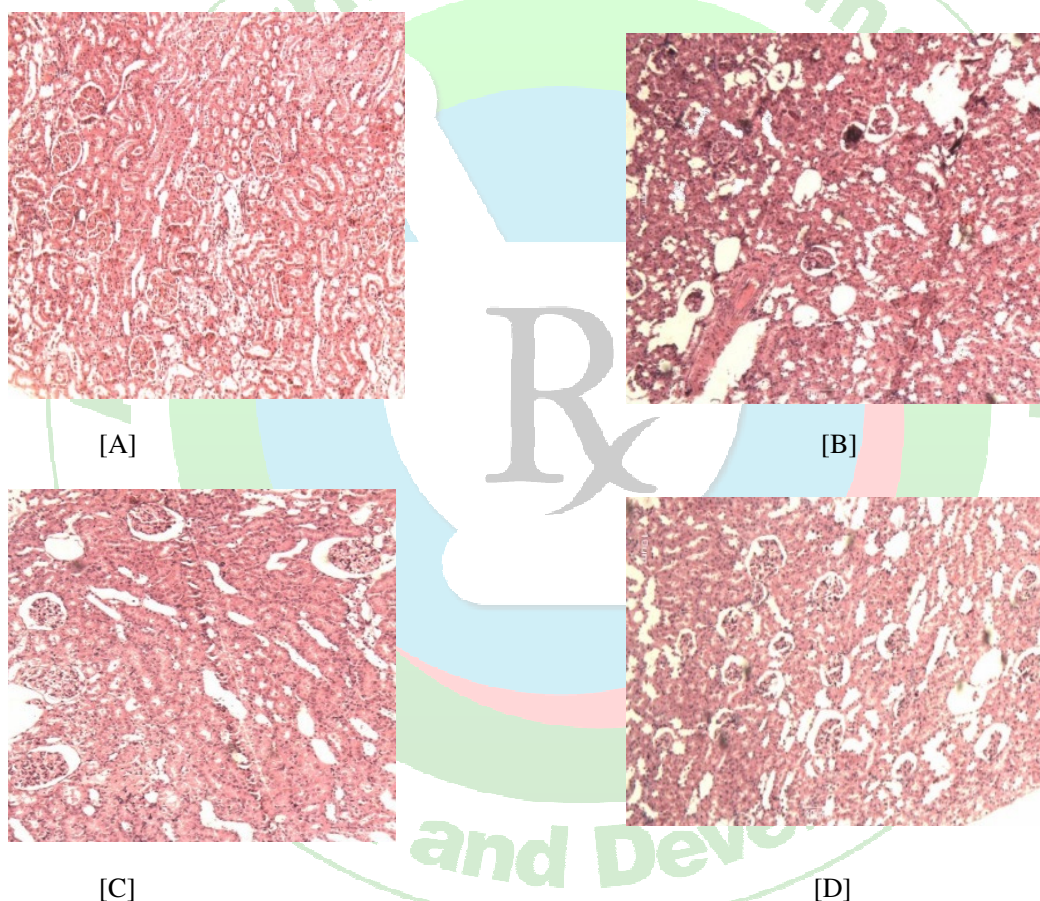
Fig.4. Histopathological view of renal sections stained with hematoxylin and eosin [10X][A]:NC; [B]: GMC; [C]: GM + SCG;[D]: GM+ LIS

Effect of SCG and lisinopril on renal mast cells density

Gentamicin administered rats showed significantly increased (p< 0.05) renal mast cells density in comparison to normal control rats. Treatment with SCG significantly decreased (p < 0.05) mast cells density in renal tissue. However treatment with lisinopril did not affect the elevated renal mast cell density in gentamicin treated rats [Fig.5].
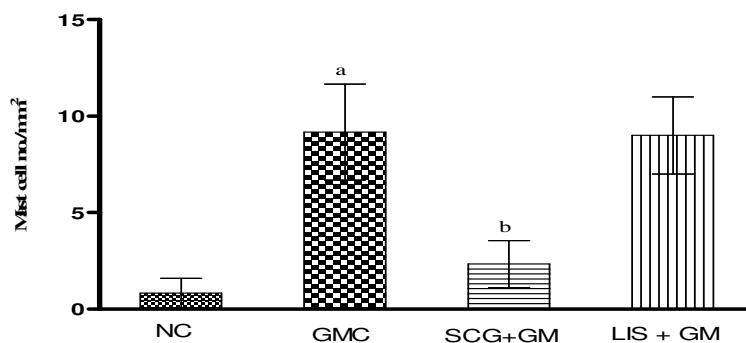
Fig.5.Effect of various pharmacological interventions on Mast Cell Density level.

Data are expressed as Mean ± S.D., [a]$p<0.05$ GMC vs. NC; [b]$p<0.05$ SCG+GM vs. GMC.

DISCUSSION

The present study explored the possible role of sodium cromoglycate, a mast cell stabilizer in halting the gentamicin nephrotoxicity in rats. Nephrotoxicity of gentamicin is associated with acute and chronic renal failure [4,29]. Various mechanism of Gentamicin nephrotoxicity reported are renal free radical generation, reduction in antioxidant defense mechanisms, acute tubular necrosis and glomerular congestion , resulting in diminished glomerular filtration rate and renal dysfunction [2, 4]. However, treatment option for managing gentamicin nephrotoxicity is limited due to incomplete understanding of the major pathogenesis of this disease.

Increased serum creatinine, blood urea nitrogen, proteinurea have been documented reliable index of gentamicin nephrotoxicity [2]. In the present study significantl (P<0.05) elevated levels of all these parameters and decreased level of creatinine clearance have been documented as an index of renal dysfunction associated with gentamicin treatment.

The increase in lipid peroxidation and decrease in the reduced form of glutathione have been associated with an index of oxidative stress [30]. In the present study, the lipid peroxidation in the kidney assessed in terms of measuring TBARS has been noted to increase significantly (P<0.05), which was accompanied with consequent reduction in GSH.

Increase in number of renal mast cells is associated with an induction of fibrosis and accumulation of extracellular matrix protein in the kidney of patients with diabetic nephropathy [31]. Mast cells are involved in renal deterioration by inducing tubulointerstitial injury in patients with glomerulonephritis [32]. Their density has been shownto increase in the renal interstitium of diabetic kidney and the increase in mast cell density iswell-correlated with the relative interstitial volume, serum creatinine and urea in patients with diabetic nephropathy [33].

Histological screening in nephropathy patients have shown significant increases of mast cells ranging from 3-fold in benign glomerulopathy, to 20-fold in IgA nephropathy, to 25-fold in cases of chronic glomerulonephritis, analgesic nephropathy, to 60-fold in diabetic nephropathy. While in some cases, influx was accompanied by an increased expression of stem cell factor [SCF], the growth and differentiation factor for mast cells [34, 35]. In our study, we have observed 8-10 fold increased mast cell density in rats administered gentamicin 100mg/kg for 10 days.

Mast cells are the chief source of chymase, which is released during degranulation of mast cells [36], and is responsible for production of angiotensin II, activation of TGF-β and alteration in lipid metabolism which leads to glomerulosclerosis and tubulointerstitial fibrosis as reported in patients with diabetic nephropathy [37]. Degranulation of mast cells also release tryptase, histamine, heparin,

leukotriene, cytokines, [38], TNF-α[39], minor amount of renin [40]and many other proinflammatory mediators. These are involved in increased production of extracellular matrix proteins [41]and, upregulation of TGF- β1 gene expression [42]. The over expression of TNF-α is implicated in the development of renal lesions in patients with diabetic nephropathy [43]and in addition was shown to induce renal hypertrophy in rats with diabetic nephropathy [44]. Furthermore, it has been suggested that released histamine significantly increase intracellular accumulation of gentamicin [45, 46]and gentamicin accumulation is the cause of nephrotoxicity.

It is interesting to note that gentamicin in low dose (4mg/kg) for 1-2 weeks reduced glomerular renin secretion from normal kidney [47]. At 100mg/kg/day gentamicin reduced ACE secretion from kidney [48]. However, mast cells have been found to secrete renin [40] and are the chief source of chymase [36] both of which stimulate generation of angiotensin- II that stimulates TGF-β production and suppresses matrix degradation favouring increased extracellular matrix deposition leading to apoptosis and fibrosis. This supports our contention to stabilize mast cells that will halt gentamicin induced nephrotoxicity.

SCG is a mast cell stabilizer which inhibits the release of pre-formed and newly synthesized chemical mediators from mast cells involved in allergic and inflammatory responses[17, 49].Moreover sodium cromoglycate reduces the increase in number of residential mast cells [50, 18].This contention is strongly supported by the results obtained in the present study that treatment with sodium cromoglycate markedly reduced the gentamicin-induced increase in mast cell density and their degranulation in the kidney and thus halted the progression of gentamicinnephrotoxicity.

The reactive oxygen species [ROS] are known to activate mast cells through intracellular Ca2+ mobilization [51]. TGF-β, Ang-II and TNF-α released from the degranulated mast cells may further augment the ROS production [52, 53] in the kidney, this increased

production of ROS in Gentamicin-induced nephrotoxicity causes inactivation of antioxidant enzymes such as SOD and GSH [30, 54], and deteriorate the structure and function of kidney. Thus, mast cell stabilization may reduce the renal oxidative stress in gentamicin induced nephrotoxicity in rats. This contention is supported by the results obtained in the present study that treatment with sodium cromoglycate decreased the renal TBARS levels and increased the GSH levels. Taken together, it may be suggested that the resident renal mast cells degranulation-mediated release ofvarious mediators such as TGF-β, chymase, tryptase, renin, histamine and TNF-α may play a pathogenic role in the development of gentamicin-induced nephrotoxicity.

The gentamicin administered rats showed increased KW/BW ratio and increased total renal collegen content. The increase in KW/BW may be attributed to the presence of inflammatory edema and decreased body weight. Increased collagen content is associated with progressive interstitial fibrosis and extracellular matrix accumulation in renal injury [31]. This was markedly prevented by treatment with sodium cromoglycate, suggesting the additional beneficial effect of sodium cromoglycate in halting the development of gentamicin nephrotoxicity. In this study histopathological analyses showed that there was degeneration, desquamation, intracellular edema, glomerulus narrowing and necrosis in epithelial cells of the proximal tubules. These results confirm that kidney is very sensitive to gentamicin toxicity. These results were also observed by others in their study [55]. The renoprotective effect of lisinopril, an ACE inhibitor has been well reportedin basic and clinical studies [56, 57]. Therefore it has been employed as a standard drug in the present study to compare the renoprotective potential of SCG. In the present study, treatment with lisinopril prevented the development of gentamicin nephrotoxicity without affecting the density of resident mast cells.Our findings are in agreement to earlierfindings that lisinopril has no effect on the mast cells density in pericardium [19, 58].

On the basis of the above discussion, it may be concluded that gentamicin-induced nephrotoxicity is associated with an increase in resident renal mast cells density and consequent degranulation of mast cells with augmented renal oxidative stress. So SCG, being a mast cell stabilizer, may have halted the development of gentamicin-induced nephrotoxicity possibly by preventing the degranulation of resident renal mast cells, reducing the renal mast cell density and protecting kidney from oxidative stress. Most**[>**50%] of the SCG is excreted from kidney in urine unmetabolized[59].

In this study, it is first time observed that mast cells stabilization prevent gentamicin nephrotoxicity. However, further work is required to find out suitable orally effective mast cell stabilizer as SCG is poorly absorbed orally[59] and used by inhalation.

ACKNOWLEDGEMENTS

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