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Nephroprotective Effect of Fresh Leaves Extracts of *Sida Cordifolia* Linn in Gentamicin Induced Nephrotoxicity in Rats

Bhatia Lovkesh^{1*}, Bhatia Vivek², Grover Manav³

¹College of Pharmacy, Pt. BDSUH Rohatak, Haryana, India

²NIMS Institute of Pharmacy, NIMS University, Jaipur, Rajasthan, India

³Macloied Pharmaceutical Ltd., Baddi, H.P., India

ABSTRACT

The present study was aimed at evaluating the ethanolic and aqueous extracts of leaves of *Sida cordifolia* Linn. for nephroprotective effect in gentamicin-induced nephrotoxicity in rats. Nephrotoxicity was induced in Wistar rats by intraperitoneal administration of gentamicin 100 mg/kg/d for eight days. Effect of concurrent administration of ethanolic and aqueous extracts of leaves of *Sida cordifolia* Linn. at dose of 200 and 400 mg/kg b.w. respectively was given by oral route. Serum creatinine, serum urea, urine creatinine and blood urea nitrogen (BUN) were determined on day 9. Histopathological study of kidney was also done. Both the extracts produced significant ($P < 0.001$) nephroprotective activity in Gentamicin induced nephrotoxicity models as evident by decrease in serum creatinine, serum urea, urine creatinine and BUN levels in extract treated groups which was elevated by gentamicin, which was further confirmed by histopathological study. Gentamicin induced glomerular congestion, blood vessel congestion, and epithelial desquamation, accumulation of inflammatory cells and necrosis of the kidney cells were found to be reduced in the groups receiving extracts of *Sida cordifolia* Linn. along with gentamicin.

KEYWORDS: Gentamicin, *Sida cordifolia* Linn., Nephroprotective.

***Corresponding Author**

Lovkesh Bhatia
College of Pharmacy, Pt. BDSUH
Rohatak, Haryana
E Mail- lbcognosy@gmail.com

INTRODUCTION

Aminoglycosides have long been one of the common causes of drug induced nephrotoxicity. Gentamicin induced nephrotoxicity is a model of acute renal failure caused by oxidative stress generated through the induction of superoxide¹. It has been demonstrated that gentamicin-induced nephrotoxicity is characterized by direct tubular necrosis, which is localised mainly in the proximal tubules. It is complex phenomenon characterized by an increase in plasma creatinine and urea levels and severe proximal tubular necrosis, followed by deterioration and renal failure². The toxicity of gentamicin is believed to relate to generation of reactive oxygen species (ROS) in kidney. Because of the obvious mediation of ROS in gentamicin induced renal damage several antioxidant agents have been used to block gentamicin nephrotoxicity^{3,4,5}

Sida cordifolia Linn. belonging to family Malvaceae is one of the popular herb in folklore use and a part of several *rasayana* formulations. *Rasayana* are clinical speciality of ayurveda, consumption of which is associated with prevention of diseases, counteracting ageing process – by means of optimizing homeostasis. Such preparations are invariably either with a proven / potential antioxidant activity. *Sida cordifolia* Linn. herb chosen for the current study is a part of *rasayana*. Phytochemical constitutes of *Sida cordifolia* includes a phytosterol – ecdysterone and other minor constitutes that include alkaloids, carboxylated tryptamines namely S-(+)-N₂- methyltryptophan methylester and hypaphorine, quinazolines namely vasicinone, vasicine and vasicinol β – phenethylamine, ephedrine and pseudoephedrine, and an acylsterylglycoside – sitoindoside from roots of *Sida cordifolia*.⁶ Phytochemical analysis of leaves of *Sida cordifolia* have demonstrated the presence of sympathomimetic amines, ephedrine and pseudoephedrine – potential vasoconstrictor, vasicinone and vasicine (bronchodilator alkaloids), flavonoids and saponins.⁷⁻⁹

The present study was carried out to determine the effect of ethanolic and aqueous extracts of root of *Sida cordifolia* Linn. for nephroprotective activity.

MATERIAL AND METHODS

PLANT MATERIAL

The fresh leaves of *Sida cordifolia* Linn. was procured and authenticated by Head of Department, Botany, Rajasthan University, Jaipur, Rajasthan. A voucher specimen of same has been deposited.

PREPARATION OF THE ROOT EXTRACT

The authenticated leaves were shade dried and powdered coarsely. The powdered drug was defatted by extracting with pet-ether (60-80⁰ C). Coarse powder of the root (500gm) was soxhlet extracted with 90% ethanol. The aqueous extract was prepared by the process of maceration. The extracts obtained were concentrated under reduced pressure to yield ethanolic (8.2%) and the aqueous extracts (6.4 %).

ANIMALS

The healthy Wistar albino rats of either sex weighing between 150-200 g were taken for the study. They were housed under controlled conditions of temperature (23±2⁰c), humidity (55±5%) and 12h light and 12h dark cycles. The animals were fed with standard pellet diet and water *ad libitum*. Approval of the Institutional Animals Ethics Committee was taken (IAECPB/Ref. 10/11)

ACUTE TOXICITY STUDIES

Acute toxicity studies for aqueous and ethanolic extracts of *Sida cordifolia* Linn. were conducted as per OECD guidelines 423 using albino Wistar rats. Each animal was administered the aqueous solution of the extract by oral route. The animals were observed for any changes continuously for the first 2h and up to 24 h for mortality.

GENTAMICIN INDUCED NEPHROTOXICITY IN RATS

Albino rats (150-180 gm) of either sex were used for the study. Animals were divided into six groups, each containing six animals. The study was carried out for nine days and treatment was given for eight days. Group I served as control group and received distilled water p.o. for eight days. Group II served as gentamicin group. The gentamicin treated group received 100 mg/kg/day gentamicin by the intraperitoneal (i.p.) route. Group III and IV received 200 and 400 mg/kg b.w. of aqueous extract of *Sida cordifolia* Linn. (SCA) respectively. Group V and VI received 200 and 400 mg/kg b.w. of ethanolic extract of *Sida cordifolia* Linn. (SCE) respectively.

Animals of groups III to VI were administered 100 mg/kg b.w. of gentamicin i.p. along with extracts p.o. for 8 days. After dosing on the day 8, individual rats were placed in separate metabolic cages for 24h for urine collection to determine urine creatinine content. Blood samples were collected via retro-orbital puncture at the end of these 24h, the serum was rapidly separated and processed for determination of serum creatinine, serum urea, blood urea nitrogen (BUN), using of Span Diagnostic

kits. Body weight of animal was also recorded. Rats were sacrificed and both kidneys were isolated from each rat. The kidneys were processed for histopathological examination^{5,10}.

RESULT AND DISCUSSION

There was no change in normal behavioral pattern of animals and no sign and symptoms of toxicity were observed during the first 2h and no mortality was observed till 24h. Extracts were safe up to a maximum dose of 2000 mg/ kg b.w. The biological evaluation was carried out at doses of 200 and 400 mg/kg b.w by oral route.

Urine creatinine, serum creatinine, serum urea and blood urea nitrogen were found to be significantly ($P<0.001$) increased in rats treated with only gentamicin, whereas treatment with the aqueous and ethanolic extracts of root of *Sida cordifolia* Linn. reversed the effect of gentamicin indicating nephroprotective activity. [Table No. 2, Fig1,2,3].

Group	Treatment	Serum creatinine (mg/dl)	Serum urea (mg/dl)	Urine creatinine (mg/dl)	BUN (mg/dl)	Body weight (%change)
I	Control	0.65±0.73	47.17±0.15	89.71±3.54	12.21±1.3	2.23±0.27
II	Gentamicin	4.21±0.13*** ^a	169.6±1.73*** ^a	206.8±5.56*** ^a	89.2±1.83*** ^a	-17.4±1.32*** ^a
III	SCA 200	1.57±0.26** ^a	107.9±1.07*** ^a	145.8±4.32*** ^a ,*** ^b	65.21±1.54*** ^a	-10.93±1.13*** ^a ,* ^b
IV	SCA 400	0.93±0.08*** ^b	89.34±1.09*** ^b	108.8±2.61*** ^b	40.13±0.34*** ^b	-7.41±1.79*** ^a ,*** ^b
V	SCE 200	0.77±0.7*** ^b	78.75±1.08*** ^b	163.5±5.55*** ^a ,*** ^b	24.31±1.75*** ^b	-8.8±0.16*** ^a ,* ^b
VI	SCE 400	0.70±0.15*** ^b	63.47±1.14*** ^b	114.8±3.63*** ^b	35.37±1.12*** ^b	-3.92±0.39*** ^a ,*** ^b

n=6, Values are expressed as mean±S.D., SCA and SCE 200 and 400 indicate *Bauhinia variegata* aqueous and ethanolic extracts at 200 and 400mg/kg b.w. respectively. * $p<0.05$, ** $P<0.01$, *** $P<0.001$, a- indicates comparison with control group and, b-indicates comparison with gentamicin treated group.

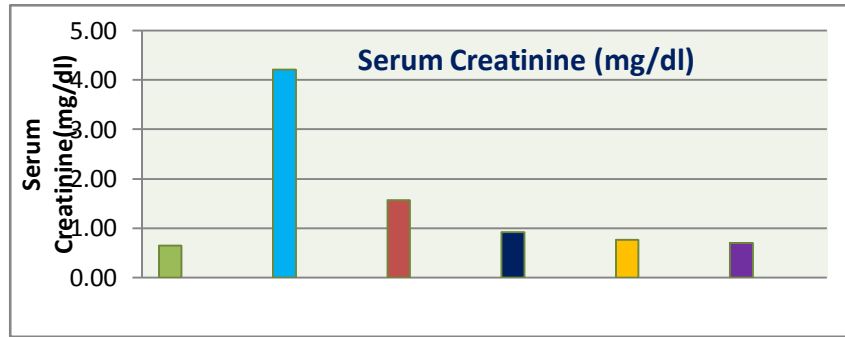


Fig.1 Effect of extracts of *Sida cordifolia* on serum creatinine levels in Gentamicin induced and all extract treated rats.

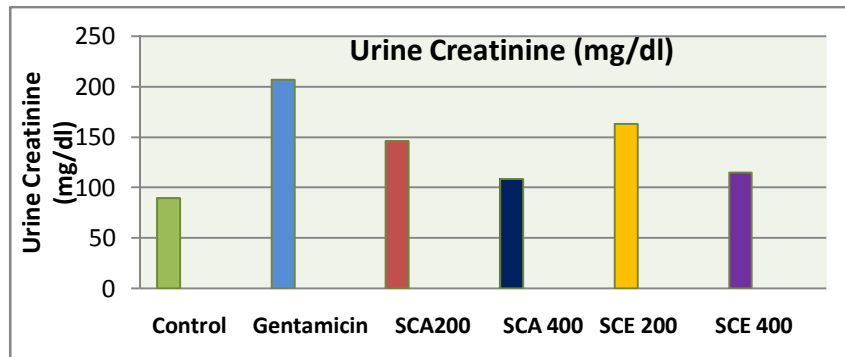


Fig.2 Effect of extracts of *Sida cordifolia* on urine creatinine levels in Gentamicin induced and all extract treated rats.

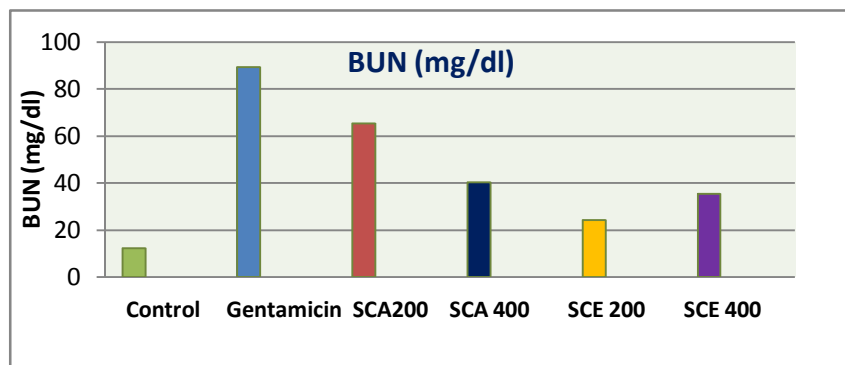


Fig.3 Effect of extracts of *Sida cordifolia* on Blood urea nitrogen levels in Gentamicin induced and all extract treated rats.

There is a simultaneous significant ($P < 0.001$) decrease in the gentamicin-induced nephrotoxicity when the antioxidant defense system is effective. The increased production of ROS in gentamicin-induced nephrotoxicity may be a result of inactivation of antioxidant enzymes such as SOD and GSH-Px. A relationship between nephrotoxicity and oxidative stress has been confirmed by many investigations. The impairment in kidney functions is accompanied by increase in serum creatinine and urea level and kidney tissue MDA levels that indicates lipid peroxidation. It is one of the essential compounds for maintaining cell integrity participation in the cell metabolism^{11,12}. The significant and progressive weight loss in gentamicin treated rats may possibly be due to the injury renal tubules and the subsequent loss of the tubular cells to reabsorb water, leading to dehydration and loss of body weight.

Phenolic compounds present in medicinal plants have been reported to possess powerful antioxidants activity. Root bark of *Sida cordifolia* Linn is reported to contain polyphenolics, steroids, saponins and triterpenes. *Sida cordifolia* has been reported to contain quercetin, rutin, apigenin and apigenin 7-O-glucoside. Flavonoids and quercetin in particular are potent antioxidants and are known to modulate the activities of various enzyme systems due to their interaction with various biomolecules^{13,14}. They show antiatherogenic and anticarcinogenic activities by blocking LDL oxidation and inhibition of processes of bioactivation of carcinogens. Quercetin decreases the lipid peroxide formation, restoration of glutathione status and the activities of antioxidant enzymes during gentamicin-induced nephrotoxicity¹⁵. Extract showed dose depended protective effect. *Sida cordifolia* might have exhibited nephroprotective activity by the virtue of its antioxidant activity.

HISTOPATHOLOGICAL EXAMINATION

Control showed normal glomerular and tubular histology whereas gentamicin peritubular and blood vessel congestion and result presence of inflammatory cells in kidney section from the gentamicin-treated group. Mononuclear cell infiltrated mainly in the sub-capsular region and hyaline changes, vacuolization and necrosis in the proximal tubular epithelial cells was seen in gentamicin group. Gentamicin group also showed interstitial edema in tubular cells. Concurrent treatment with the extracts was found to reduce such changes in kidney histology induced by gentamicin [Fig: 4]

Treatment with extracts could prevent cell damage such as tubular vacuolization, glomerular congestion and interstitial edema. According the pathological result it can be stated that extracts of *Sida cordifolia* Linn. had protective effect against degenerative injury caused by gentamicin.

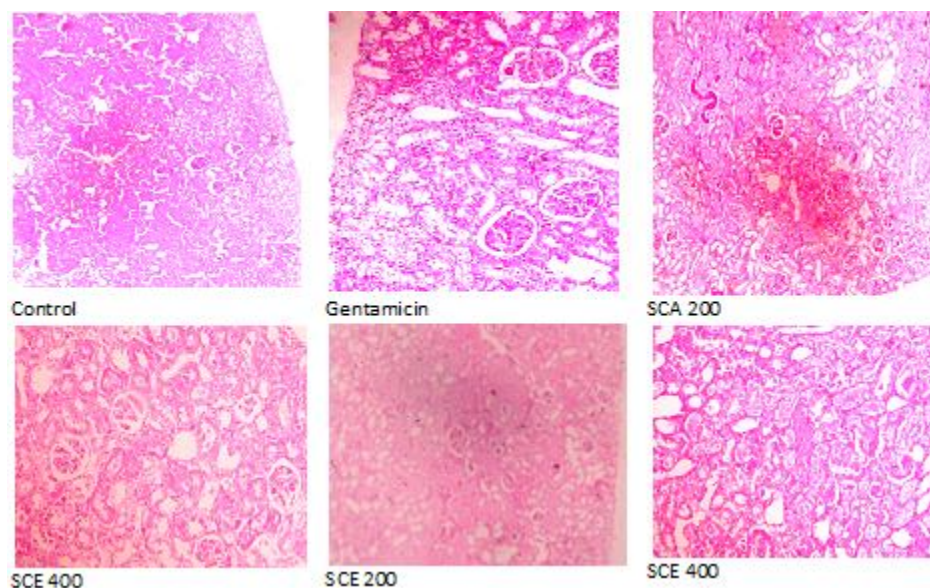


Fig.4 Histopathological view of renal section from different groups stained with hematoxylin and eosin

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