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Assessment of nephroprotective potential of *Sida cordifolia Linn.* in experimental animals

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ABSTRACT

The present study was aimed to evaluate nephroprotective activity of an aqueous extract of root of Sida cordifolia Linn. (SCAE) against gentamicin and cisplatin induced experimental animal models. Nephrotoxicity was induced in wistar albino rats by intraperitoneal administration of gentamicin (100 mg/kg) and cisplatin (7 mg/kg). Effect of simultaneous administration of SCAE in different doses (200 & 400 mg/kg) by oral route was estimated using serum creatinine, urine creatinine, serum urea and blood urea nitrogen (BUN) level as renal markers. From the study, it was revealed that the aqueous extract of Sida cordifolia Linn. significantly ($P<0.001$) prevents renal damage by normalizing increased levels of renal markers. From the present study, it was concluded that aqueous extracts of Sida cordifolia Linn. possess potential nephroprotective activity in experimental animals.

Keywords: *Sida cordifolia Linn.* , Nephrotoxicity, Gentamicin, Cisplatin.

INTRODUCTION

Free radicals are highly reactive substances formed in the body as a result of metabolic processes. Many of these molecular species are oxygen (and sometimes nitrogen) centred free radicals and its non radical products [1]. The term “reactive oxygen species” (ROS) collectively denotes oxygen centred radicals (super oxide and hydroxyl radicals) as well as non-radical species derived from oxygen such as hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and hypochlorous (HOCl) acid. The increased production of ROS seems to accompany most forms of tissue injury. Free radicals can also react with DNA, proteins or lipids in the cell membrane and cause damage [2]. The involvement of ROS in aging and in many chronic diseases has been

considered. The defence provided by antioxidant systems is crucial for the survival of organisms. Detoxification of ROS in the cell is provided by both enzymatic and non-enzymatic systems which constitute the antioxidant defence systems. These antioxidants play a role in delaying, intercepting, or preventing oxidative reactions catalysed by free radicals [3]. Aminoglycosides have long been one of the common causes of drug induced nephrotoxicity. Gentamicin is a very effective antibiotic in treating gram-negative bacterial infection in both humans and animals. Gentamicin induced nephrotoxicity is a model of acute renal failure caused by oxidative stress generated through the induction of superoxide [4]. It has been demonstrated that gentamicin-induced nephrotoxicity is characterized by direct tubular necrosis, which is localised mainly in the proximal tubules. It is a complex phenomenon characterized by an increase in plasma creatinine and urea levels and severe proximal tubular necrosis, followed by deterioration and renal failure [5]. The toxicity of gentamicin is believed to be related to generation of reactive oxygen species (ROS) in kidney. Several reports have documented the pathogenesis of aminoglycoside induced renal tubular cell injury such as derangement of lysosomal, mitochondrial and plasma membrane structure. Furthermore, results of many studies have shown that the altered concentrations of various biochemical indicators of oxidative stress in kidney tissue are due to gentamicin. Because of the obvious mediation of ROS in gentamicin induced renal damage, several antioxidant agents have been used to block gentamicin induced nephrotoxicity [6, 7].

Sida cordifolia Linn. belonging to family Malvacea, commonly known as *Bala* (Hindi), Country mallow (English) is a small shrub found distributed throughout tropical and subtropical regions of India and Nepal. The plant has medicinal value like astringent, emollient, aphrodisiac, healing of wounds, diuretic and febrifuge [8] and is used in folk medicine for the treatment of inflammation of oral mucosa, blenorrhea, asthmatic bronchitis and nasal congestion [9]. Water infusion of whole plant of *Sida cordifolia Linn.* is reported to possess inhibitory activity in lipid peroxidation assay of rat brain homogenate and authors are of the opinion that main active principles were probably water soluble in nature [10]. Further to this, ethanolic extract of whole plant of *Sida cordifolia Linn.* is reported to possess significant *invitro* and *invivo* antioxidant activity in 2,2-Azinobis-3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) test and thiobarbituric acid (TBARS) assay respectively [11].

In view of the above findings, *Sida cordifolia Linn.* was selected to evaluate its nephroprotective potential in experimental animals. Since antioxidant rich herbs possess significant activity against various disease condition characterized by induced oxidative stress like atherosclerosis, hyperglycemia, nephrotoxicity etc.

The purpose of the current study was to investigate whether the administration of an aqueous extract of *Sida cordifolia Linn.*, has any protective effect against cisplatin and gentamicin induced nephrotoxicity in rats.

MATERIALS AND METHODS

2.1 Preparation of extract

The authenticated root was shade dried and powdered coarsely. Extraction was done according to standard procedures using analytical grade solvents. The powdered drug was extracted by the process of maceration. The extracts obtained were concentrated under reduced pressure and the percentage yield of aqueous extracts was calculated.

2.2 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±2 °c), humidity (55±5%) and 12h light and 12h dark cycles. The animals were fed with standard pellet diet and water *ad libitum*. The research protocol was approved by Institutional Animal Ethics Committee (IAEC).

2.3 Acute toxicity studies

Acute toxicity studies for aqueous extracts of *Sida cordifolia Linn.* was 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 24h for mortality [12].

2.4 Nephroprotective activity

2.4.1 Gentamicin induced nephrotoxicity

Healthy albino wistar rats were assigned to six different groups having six animals in each group. Control group, gentamicin treated group and gentamicin as well as test extract treated group. Each group received gentamicin 100 mg/kg/day intraperitonially for eight days except animals of normal control group. Animals of test extract treated group received test extract in different doses with gentamicin treatment. After dosing on 8^{th} day, individual animal was placed in separate metabolic cages for 24 hours for urine collection to determine urine output and urine creatinine content. At the end of the 24 hours blood samples were collected by retro orbital puncture under light ether anaesthesia. The serum was separated rapidly to assess blood urea nitrogen (BUN) and serum creatinine content for evaluation of nephroprotective activity [13, 14].

2.4.2 Cisplatin induced nephrotoxicity

The animals were divided into six experimental groups (n=6). Each group received the following treatment- Group I: Control group remain untreated; Group II: received Cisplatin i.p.(7mg/kg b.w.) for 5 alternate days; Group III to VI received test extract treatment in different doses for 10 days + Cisplatin i.p. (7mg/kg b.w.) for 5 alternate days. After dosing on 10^{th} day, individual animal was placed in separate metabolic cages for 24 hours for urine collection for determining urine output and urine creatinine content. At the end of the 24 hours, blood samples were collected by retro orbital puncture under light ether anaesthesia. The serum was separated rapidly to assess blood urea nitrogen (BUN) and serum creatinine content to evaluate nephroprotective activity [15, 16].

2.5 Statistical analysis

The data were expressed as mean ± SEM. Statistical differences between means were determined by one – way ANOVA followed by Tukey's post hoc test using graph pad instant software, San Digeo, U.S.A. Values of $P < 0.05$ were considered as significant.

RESULTS AND DISCUSSION

3.1 Acute toxicity studies

Acute oral toxicity study was carried out to find out safety of test extract (SCAE) under the study according to OECD Test guideline 425 (modified). Animals were observed for 14 days (post administration) with special attention for first 4 hours after administration. It was observed that all animals were only slightly sedated within first hour of administration, and were normal and active within two hours of post treatment. No other sign of toxicity was observed during total duration of observation, and all the animals survived 14 days post administration of test drugs.

From the above observation, it was concluded that test extract (SCAE) was safe up to 2000 mg/kg and LD_{50} was greater than 2000 mg/kg. D_1 (200 mg/kg) and D_2 (400 mg/kg) were selected, which represents $1/10^{th}$ and $1/20^{th}$ of 2000 mg/kg to evaluate nephroprotective activity in different drug induced nephrotoxicity in animal models.

3.2 Effect of gentamicin and SCAE on serum creatinine, serum urea and BUN

Urine creatinine, serum creatinine, serum urea and blood urea nitrogen (BUN) were found to be significantly (P<0.001) increased in rats treated with only gentamicin, whereas treatment with the aqueous extracts of root of *Sida cordifolia Linn.* reversed the effect of gentamicin indicating nephroprotective activity. (Table No. 1)

Effect of cisplatin and SCAE on serum creatinine, serum urea and BUN

Intraperitonially administration of cisplatin (7 mg/kg, b.w.) for 5 alternate days caused significant renal damage in all rats reflected by significant (P<0.001) increase in renal function markers like serum creatinine, serum urea, urine creatinine and blood urea nitrogen where as administration of SCAE in different doses prevented renal damage confirmed by significant (P<0.001) decreased level of renal biomarkers in SCAE treated animals. (Table 2)

DISCUSSION

Aminoglycoside antibiotics are well known to cause serious nephrotoxicity; therefore their clinical uses are limited. However, it is important to be aware of risk factors associated with incidence of their renal damages. The onset of deterioration of renal function induced by aminoglycosides for example gentamicin occurs after 5–7 days' of treatments between 80 and 150 mg/kg. In this study, gentamicin was injected intraperitonially at the dose of 100 mg/kg, for eight successive days, which is well known to cause significant nephrotoxicity in rats [17, 18]. The exact mechanism by which gentamicin-induced nephrotoxicity is unknown, however, several investigators reported that aminoglycoside antibiotics are a class of drug capable of causing the formation of ROS which can be directly involved in gentamicin-induced damage, end product of lipid peroxidation in tissues, results in a decrease in polyunsaturated fatty acid content, which serves as substrate for free radicals. The interaction between cationic drugs such as aminoglycosides, with the anionic phospholipid is considered the first step for the development of gentamicin toxicity [19, 20].

Table 1: Effect of gentamicin and SCAE on serum creatinine, serum urea and BUN

Groups	Serum Creatinine (mg/dl)	Serum Urea (mg/dl)	Urine Creatinine (mg/dl)	BUN (mg/dl)	SOD	CAT
Normal	0.87 ± 0.08	47.54 ± 2.62	89.57 ± 2.53	28.35 ± 2.15	6.1 ± 0.36	18.33 ± 1.01
Gentamicin (P_1)	4.76 ± 0.31^{c}	157.18 ± 3.48^{c}	231.68 ± 4.41^{c}	86.54 ± 2.91^{c}	3.71 ± 0.55^{b}	12.47 ± 0.68^{c}
SCAE 200 (P_2)	2.44 ± 0.32^{c}	136.09 ± 3.05^{c}	183.14 ± 3.32^{c}	61.46 ± 3.30^{c}	4.41 ± 0.48^{ns}	14.64 ± 0.68^{ns}
SCAE 400 (P_2)	1.86 ± 0.10^{c}	84.72 ± 3.04^{c}	113.95 ± 2.16^{c}	37.15 ± 2.53^{c}	5.24 ± 0.39^{ns}	17.2 ± 0.52^{b}

Values are expressed as Mean ±SEM, n = 6, P Values: ^{a} <0.05; ^{b} <0.01, ^{c} <0.001; ns Non significant, P_1: Compared to Normal control; P_2: Compared to rats receiving gentamicin alone

Cisplatin has been shown to cause nephrotoxicity in patients as well as in a variety of animal species. The rat model of cisplatin-induced nephrotoxicity is considered to be sensitive and reproducible system. Cisplatin-induced nephrotoxicity in rats was established by elevated serum urea, elevated serum creatinine, reduced creatinine clearance, increased kidney relative weight, increased urinary excretion of sodium and marked elevation of urinary GST activity. The effects of cisplatin were similar to those previously described. The underlying mechanisms of cisplatin-

induced nephrotoxicity are still not fully understood. It has been suggested that binding of cisplatin to the renal base transport system and the following peroxidation of membrane lipids may account for its nephrotoxicity. There is evidence suggesting that cisplatin exerts its nephrotoxic effects by the generation of free radicals [21, 22, 23, 24].

Table 2: Effect of cisplatin and SCAE on serum creatinine, serum urea and BUN

Groups	Serum Creatinine (mg/dl)	Serum Urea (mg/dl)	Urine Creatinine (mg/dl)	BUN (mg/dl)	SOD	CAT
Normal	0.72 ± 0.04	44.68 ± 2.81	81.6 ± 3.36	24.94 ± 2.67	4.79 ± 0.23	16.54 ± 0.74
Cisplatin (P_1)	5.04 ± 0.39 ^{c}	153.36 ± 3.92 ^{c}	229.88 ± 3.67 ^{c}	80.45 ± 3.15 ^{c}	3.47 ± 0.28 ^{a}	9.69 ± 0.56 ^{c}
SCAE 200 (P_2)	2.76 ± 0.38 ^{c}	137.95 ± 3.44 ^{a}	188.89 ± 2.36 ^{c}	48.29 ± 2.15 ^{c}	3.98 ± 0.37 ^{ns}	12.32 ± 0.61 ^{ns}
SCAE 400 (P_2)	1.98 ± 0.17 ^{c}	75.32 ± 2.42 ^{c}	111.88 ± 4.23 ^{c}	34.5 ± 2.52 ^{c}	4.92 ± 0.27 ^{a}	14.9 ± 0.76 ^{c}

Values are expressed as Mean ±SEM, n = 6, P Values : ^{a} <0.05; ^{b} <0.01, ^{c} <0.001; ns Non significant, P_1: Compared to Normal control; P_2: Compared to rats receiving cisplatin alone

In the present study, decrease in renal markers like serum creatinine, urine creatinine, serum urea and BUN in rats was observed especially in simultaneous SCAE treatment compared to gentamicin and cisplatin treated group (Table 1 & 2). In brief, the present study indicated that SCAE can provide marked protective effect against gentamicin and cisplatin induced nephrotoxicity especially when simultaneously administered with gentamicin and cisplatin. Our findings support that the simultaneous use of SCAE may therefore be more effective for clinical purposes with antioxidant properties. However, further investigations are essential to elucidate exact mechanism of protection and potential usefulness of SCAE as a protective agent.

CONCLUSION

The present study revealed that aqueous extracts of *Sida cordifolia Linn.* reversed the nephrotoxicity induced by gentamicin and cisplatin in experimental animals. This indicates that *Sida cordifolia Linn.* can be used as an adjuvant with gentamicin and cisplatin to get the therapeutic benefit of these drugs without chances of its prominent side effect, nephrotoxicity. *Sida cordifolia Linn.* might have exhibited nephroprotective activity by virtue of its antioxidant potential.

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