Evaluation of the effect of *Boerhavia diffusa* on gentamicin-induced nephrotoxicity in rats

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ABSTRACT

Background: Ayurvedic literature claims that *Boerhavia diffusa* possesses rejuvenative properties especially related to the urinary system. **Objective:** To evaluate effect of aqueous extract of root of *Boerhavia diffusa* in gentamicin-induced nephrotoxicity in rats. **Materials and Methods:** Study was conducted in two parts, using 40 rats in each part. Rats were equally divided into five groups for each part. Group 1: Normal control, Group 2: Disease control and Groups 3, 4, and 5: α-lipoic acid (ALA) and 200 and 400 mg/kg of *B. diffusa*, respectively. All groups, except Group 1, concomitantly received gentamicin 150 mg/kg/day for 10 days. Parameters measured in part I were blood urea nitrogen (BUN), serum creatinine, kidney malondialdehyde (MDA), and glutathione (GSH) levels, kidney injury on histopathology; in part II, paraaminohippurate (PAH) clearance. **Statistical Analysis:** Mean ± SD of body weight, creatinine, BUN, MDA, GSH and PAH clearance were compared using parametric tests. Median histopathology scores were compared using Kruskal–Wallis test. '*P*' value of < 0.05 was considered significant. **Results:** High dose of gentamicin caused significant elevation in BUN, serum creatinine and kidney MDA, fall in kidney GSH and histopathological damage in disease control group as compared with normal control ($P < 0.05$). Treatment with *B. diffusa* prevented changes in above parameters, comparable to ALA. Effects of both doses of *B. diffusa* were significantly better than disease control ($P < 0.05$). *B. diffusa* did not show significant improvement in PAH clearance, which was reduced due to gentamicin damage. **Conclusion:** *B. diffusa* exerted protection against structural and functional damage induced by gentamicin possibly due to its antioxidant properties.

Key words: α-lipoic acid, acute kidney injury, aqueous extract, *Boerhaavia diffusa* roots, paraaminohippurate clearance

INTRODUCTION

Kidney, being a major organ of excretion, is inevitably exposed to high concentrations of both endogenous (rhabdomyolysis and hemolysis) and exogenous (radiocontrast agents and chemotherapeutic agents) toxins. Intrinsic renal damage due to cytotoxins is a common cause of acute kidney injury (AKI). AKI is characterized by the sudden impairment of kidney function resulting in the retention of nitrogenous and other waste products normally cleared by the kidneys. It complicates 5-7% of acute care hospital admissions and up to 30% of admissions to the intensive care unit.[1]

Aminoglycosides are often used in combination with beta-lactam antibiotics and have a rapid bactericidal effect, and are available at an affordable cost and have less incidence of resistance, making them a drug of choice for treatment of several life-threatening infections.[2,3] Among all aminoglycosides that are used for systemic infections, gentamicin is the most nephrotoxic. At least 10-25% of patients receiving therapeutic doses of gentamicin are at an increased risk of developing AKI.[1,3] Gentamicin-induced renal damage is linked with marked increase in lipid peroxidation, nitrotyrosine formation, and protein oxidation in the renal cortex.[4] This renal impairment can be quantified in terms of a rise in nitrogenous waste products in the blood (blood urea nitrogen [BUN] and serum creatinine), kidney levels of glutathione (GSH)

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as well as products of lipid peroxidation, that is, malondialdehyde (MDA) and histopathology grading of the extent of injury, using experimental animals.

Researchers have evaluated different approaches like atrial natriuretic peptide, low dose dopamine, endothelin antagonists, loop diuretics, prostaglandin analogues, sodium bicarbonate, and α-lipoic acid (ALA) to manage AKI.[1,4-6] However, the current treatment of AKI is still empirical. Therapeutic agents are used indiscriminately without considering the underlying etiology of AKI. Though these agents have shown favorable results in several experimental models of ischemic or nephrotoxic AKI, they have either failed to show consistent benefit or proved ineffective when used therapeutically.[1,5]

Owing to the limitations of these agents of modern medicine, researchers are exploring the traditional system of medicine for compounds that are already being used by ayurvedic physicians for treating patients having impaired renal function.[4] *Boerhaavia diffusa* L. is herbaceous plant of the family Nyctaginaceae. It is known as *punarnava* in Sanskrit, which means that it 'renews the body'.[7] As per the Ayurvedic literature, it is claimed to be rejuvenative to the urinary system.[8] Ayurvedic text also mentions that *B. diffusa* improves the function of impaired kidneys and in edematous conditions, it helps the normal kidneys expel the excess fluid out of the body very effectively.[7,9] Various experimental studies have also illustrated its diuretic and possible nephroprotective effects against acetaminophen-induced renal damage.[10,11] However, the exact mechanism of diuresis and nephroprotective potential has not been evaluated. Taking leads from the available literature, we planned an experimental study to evaluate the effect of *B. diffusa* on another model of nephrotoxicity, namely, gentamicin-induced nephrotoxicity in rats and to explore the possible mechanisms involved in reversing the renal damage.

MATERIALS AND METHODS

The experimental study was approved by the Institutional animal ethics committee prior to its commencement. The project approval number was AEC/09/2011. The study was conducted in accordance with the guidelines laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

Experimental animals
Eighty male Wistar rats, weighing 180-250 g, bred in the Central Animal House of our institute were used for the study. They were housed under standard laboratory conditions, with a temperature of about 22 ± 3°C and relative humidity of 30-70%. They were kept in a group of four in polypropylene cages with husk paddy as the bedding with stainless steel top grill having facilities for providing food and water. They had free access to drinking water, which was provided in polypropylene bottles with stainless steel sipper tubes, and commercial rat feed in the form of pellets. Twelve hourly light and dark cycles were maintained.

Study drugs
Gentamicin was purchased as a vial of Genticyn, containing 80 mg/2 ml of gentamicin sulfate. ALA was procured from Sigma Aldrich, USA. The aqueous extract of *Boerhaavia diffusa (rakta punarnava)* was provided by Shree Dhootpapeshwar Ayurveda Research Foundation, Mumbai, along with its certificate of analysis. Its yield was 5%. *B. diffusa* was used in two doses: 200 mg/kg, which is extrapolated from the dose advocated for humans in therapeutic practice of Ayurveda, and 400 mg/kg, which corresponds to a dose that has shown a nephroprotective effect in other models of nephrotoxicity.[7,11,12] ALA and *B. diffusa* were suspended in 0.5% carboxymethylcellulose (CMC), which was procured from Sigma Aldrich, USA.

Experimental design
The study was carried out in two parts. For part I, 40 rats were used. The body weight, BUN, and serum creatinine of the animals was measured on day 0. They were then randomly divided in five groups, each group consisting of eight rats. The groups were as follows: Group 1 (Normal control) received distilled water injections i.p. and 0.5% CMC orally, daily for 10 days. Group 2 (Disease control) received gentamicin 150 mg/kg i.p. and 0.5% CMC orally, daily for 10 days. Group 3 (Positive control) received gentamicin 150 mg/kg i.p. and 25 mg/kg ALA orally, daily for 10 days. Groups 4 and 5 served as our test groups. Both groups received gentamicin 150 mg/kg i.p., in addition, they received 200 mg/kg and 400 mg/kg *B. diffusa*, respectively, orally, daily for 10 days. For part II, a separate set of 40 rats was used. The animals were divided into groups and treated similarly for part II. Identification of animals was done with cage number and individual marking on tail.

The parameters assessed on the 11th day were as follows:

Part I
Body weight was measured on day 11. Two milliliters of blood was collected by retro-orbital puncture of the rats for estimation of biochemical parameters (BUN and serum creatinine). The rats were then sacrificed by administration of ketamine 100 mg/kg by the intraperitoneal route. Laparotomy was performed and both the kidneys were carefully dissected. The right kidney was washed gently in ice-cold phosphate buffered saline, blotted dry, weighed,

and then divided in two parts, such that two-third part was used for estimation of kidney MDA levels and one-third part used for the estimation of kidney GSH levels. The left kidney was immediately immersed in 10% neutral buffered formalin and processed further for the preparation of histopathological sections.

1. Blood urea nitrogen: BUN was estimated on a semi-autoanalyzer (ERBACHEM-5) using kits manufactured by Transasia Biomedicals[13]
2. Serum creatinine: Serum creatinine was estimated on a semi-autoanalyzer (ERBACHEM-5) using kits manufactured by Transasia Biomedicals, using alkaline picric acid method (Jaffe's reaction)[14]
3. Kidney MDA level: It was assessed using thiobarbituric acid reagent, which combines with MDA, a product of lipid peroxidation, to form a stable colored compound whose optical density is read at 532 nm on a spectrophotometer (ERBACHEM).[15] The MDA levels were calculated using a standard working curve of MDA (2-40 nmol/ml) and expressed as nmoles/g of kidney
4. Kidney GSH level: It was assessed using 5-5'dithiobis-(2-nitrobenzoic acid) DTNB reagent, which is catalyzed by GSH to form a colored compound, whose optical density is read at 3 min at 412 nm on a spectrophotometer (ERBACHEM).[16] The GSH levels were calculated using a standard working curve of GSH (1-40 μg/ml) and expressed as microgram/gram of kidney
5. Histopathological examination: The histopathological sections were stained with hematoxylin and eosin and were examined at 10× and 40× magnification by a pathologist who remained blinded to the experimental groups. Following lesions were graded in the renal cortex: Desquamation and necrosis of tubules which may be focal, partial or complete and the extent of cortex involved. Grades ranged from '0' representing normal renal architecture and '4' representing total or near total proximal tubular necrosis.[17]

Part II
Estimation of paraaminohippurate clearance
The clearance of p-aminohippuric acid (PAH) was used for estimating the flow of plasma through the kidney. The single injection clearance method of p-aminohippuric acid (30 mg/kg) without urine collection was followed to assess the effective renal plasma flow. Successive blood samples were collected by retro-orbital puncture of rats, in heparinized tubes just prior to administration of PAH and then after 30 and 60 min following PAH injection.[18-20] The estimation of PAH clearance was based on the principle that p-amino compounds develop yellow color with acidic solution of p-dimethylaminobenzaldehyde (Ehrlich's reagent), which can be measured at 465 nm. The intensity

of the color developed is directly proportional to the PAH present in the plasma.[21]

Statistical analysis
Results were expressed as Mean ± Standard Deviation (SD). The data were tested for normality (Kolmogorov–Smirnov test). For comparison of parametric data within the same group (body weight, serum creatinine, BUN), Student's paired *t*-test was used. For comparison of parametric data between multiple groups (body weight, serum creatinine, BUN, kidney MDA, and GSH levels as well as PAH clearance), one way analysis of variance (ANOVA) was used; if significance was detected by ANOVA, a *post-hoc* Tukey's test was applied. If the data did not pass the normality test, it was considered to be nonparametric. For comparison of nonparametric data within the same group, Wilcoxon matched-pairs signed-ranks test was used. For comparison of nonparametric data between multiple groups, Kruskal–Wallis test was used; if significance was detected, a *post-hoc* Dunn's test was applied. Histopathology scores were expressed as median and Kruskal–Wallis test was used to compare the median scores between multiple groups, if significance was detected, a *post-hoc* Dunn's multiple comparisons test was applied. A 'P' value of < 0.05 was considered as significant for all parameters. Analysis was done using software GraphPad InStat (version 3.06).

RESULTS

The results of the study are presented part-wise:

Part I
Effect on the body weight
Table 1 shows the body weight of the rats in various groups, at baseline (i.e. day 0) and day 11. The body weight of all rats in all groups was comparable on day 0.

The rats in group 1 showed a significant weight gain over 11 days as compared with day 0 ($P < 0.05$). However, the rats in group 2 did not show a significant change in

Table 1: Effect of study drugs on body weight in a rat model of gentamicin-induced nephrotoxicity

Groups	Body weight (g)	
	Day 0	Day 11
1 (normal control)	177.50±21.21	190.00±28.79*
2 (disease control)	197.14±17.99	198.57±25.45
3 (positive control)	172.86±29.84	181.00±29.08***
4 (test group: *B. diffusa* 200 mg/kg)	190.83±37.20	200.50±36.35**
5 (test group: *B. diffusa* 400 mg/kg)	196.00±34.29	203.50±34.49**

n=8 per group; Values indicate mean±SD, *P<0.05, **P<0.01, ***P<0.001 using paired *t*-test (as compared with respective day 0)

body weight from the baseline. Pretreatment of groups 3, 4, and 5 with ALA, *B. diffusa* 200 and 400 mg/kg, respectively, produced a significant increase in the body weight over 11 days. However, the body weight of all rats between all groups did not statistically differ from each other on day 11.

Effect on BUN and serum creatinine

As shown in Table 2, the BUN and serum creatinine levels of all the groups were comparable at baseline (day 0). Group 2 showed a significant rise in these levels over 11 days ($P < 0.05$), while the levels of groups 3, 4, and 5 did not change significantly. When the 11th day BUN and serum creatinine levels of all groups were compared, we found that group 2 showed significantly higher levels as compared with the normal control ($P < 0.05$ for BUN and $P < 0.01$ for serum creatinine). Groups 3 and 4 showed significantly lower levels as compared with the disease control ($P < 0.05$). Group 5 also showed a fall in BUN and serum creatinine values, but the fall in serum creatinine was not significant as compared with the disease control.

Effect on renal MDA and GSH levels

Figures 1 and 2 represent the kidney MDA and GSH levels, respectively, in the rat kidneys on day 11.

Kidney MDA levels in rats from group 2 were significantly higher ($P < 0.01$) than group 1. The kidney MDA levels of rats in groups 3, 4, and 5 were significantly lower as compared with group 2 ($P < 0.01$). Kidney GSH levels in rats from group 2 were significantly lower ($P < 0.001$) than group 1. The kidney GSH levels of rats in groups 3, 4, and 5 were significantly higher as compared with group 2 ($P < 0.001$).

Effect on renal histopathology

The histopathological sections of kidneys are shown in Figure 3.

Animals in group 1 showed normal renal morphology. Animals in group 2 showed evidence of tubular necrosis and desquamation involving more than half of the proximal tubules (median grade: 3). Histopathological grade of rats in groups 3 and 4 differed significantly from the disease control ($P < 0.05$). Group 3 showed prominent tubular epithelial necrosis and desquamation but it involved less than half of cortical tubules (median grade: 2). Group 4 showed desquamation of tubular epithelial cells in small foci (median grade: 1). Animals in group 5 had a median histopathology grade of 2, which was not significantly different from the disease control ($P > 0.05$).

Part II

Effect on paraaminohippurate clearance

Figure 4 depicts the results of PAH clearance, which was estimated on day 11.

The PAH clearance was significantly low in group 2 as compared with group 1. Although the PAH clearance in

Table 2: Effect of study drugs on BUN and serum creatinine in a rat model of gentamicin-induced nephrotoxicity

Groups	BUN (mg/dl)		Serum creatinine (mg/dl)	
	Day 0	Day 11	Day 0	Day 11
1 (normal control)	19.09±5.45	20.86±5.35	0.68±0.32	0.60±0.22
2 (disease control)	17.13±2.86	54.76±17.83*#	0.69±0.30	1.81±0.93S #
3 (positive control)	17.56±5.50	23.38±6.83d	0.54±0.10	0.70±0.16d
4 (test group: *B. diffusa* 200 mg/kg)	20.12±4.69	22.89±10.10d	0.58±0.09	0.72±0.39d
5 (test group: *B. diffusa* 400 mg/kg)	21.10±4.62	26.69±21.58dd	0.60±0.27	0.98±0.43

n=8 per group; values indicate mean±SD, *P<0.01, using paired *t*-test (as compared with respective day 0), SP<0.05, using Wilcoxon matched-pairs signed-ranks test (as compared with respective day 0), #P<0.01, using Kruskal–Wallis test, f/b post-hoc Dunn's test (as compared with normal control), dP<0.05, ddP<0.01 using Kruskal-Wallis test, f/b *post-hoc* Dunn's test (as compared with disease control). BUN=Blood urea nitrogen
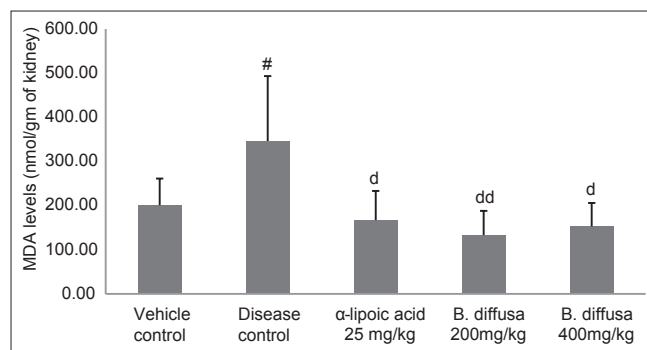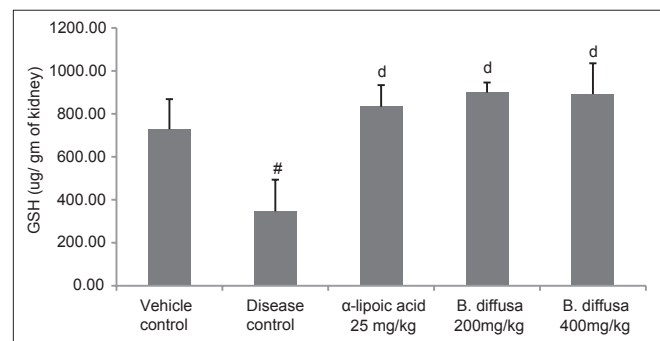

Figure 1: Effect of study drugs on kidney MDA levels of rats with gentamicin-induced nephrotoxicity. *n* = 8 per group, Bars indicate mean. Error bars represent SD. #*P* < 0.01, using one-way ANOVA f/b *post-hoc* Tukey's test (as compared with normal control), d*P* < 0.01, dd*P* < 0.001 using one-way ANOVA f/b *post-hoc* Tukey's test (as compared with disease control)

Figure 2: Effect of study drugs on kidney GSH levels of rats with gentamicin-induced nephrotoxicity. *N* = 8 per group, Bars indicate mean. Error bars represent SD. #*P* < 0.001, using one-way ANOVA f/b *post-hoc* Tukey's test (as compared with normal control), d*P* < 0.001, using one-way ANOVA f/b *post-hoc* Tukey's test (as compared with disease control)
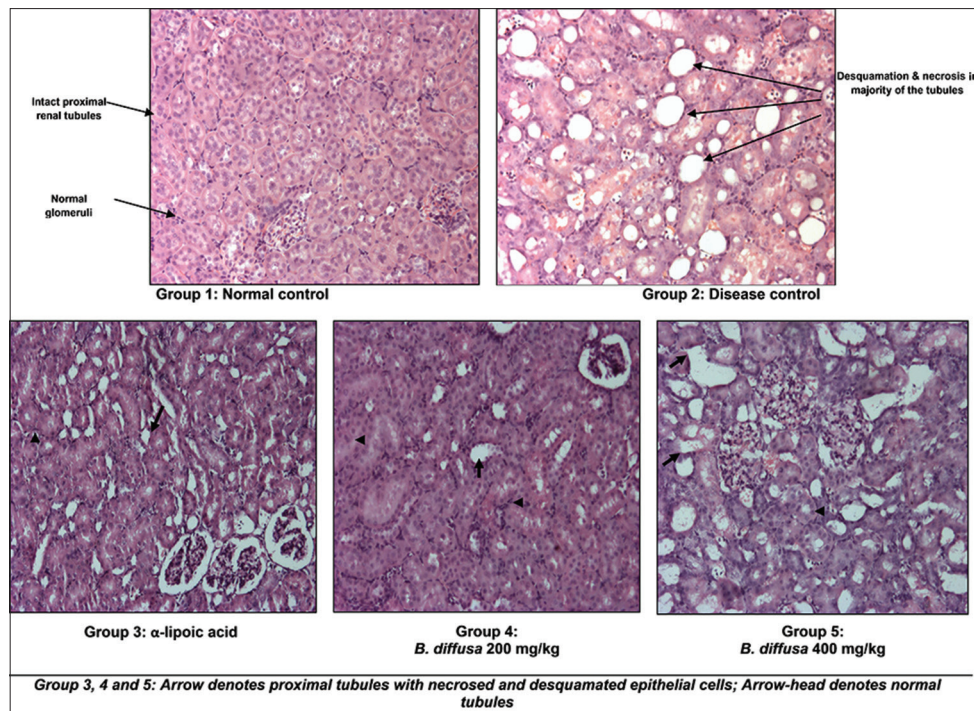
Figure 3: Cross section of cortex of kidney (H and E, ×10)

groups 3, 4, and 5 was higher than group 2, there was no significant difference when compared with group 2.

DISCUSSION

Approximately 8-26% of patients receiving an aminoglycoside for several days develop mild renal impairment.[3] Nephrotoxicity of aminoglycosides is a concern in all clinical settings, but takes special relevance among patients who are critically ill, elderly, hypovolemic, suffering from diabetes or cardiovascular diseases, taking concurrent medications that are nephrotoxic, have preexisting renal disease or septic shock or are exposed to high concentration of culprit drug due prolonged administration or errors in dosing, etc.[2,3,22] Prevention of drug-induced nephrotoxicity is therefore, an unmet therapeutic need that will lead to a significant improvement in the pharmacotoxicological profile and enhance the clinical utility of many drugs including aminoglycosides.

Animal models of drug-induced renal injury have been pivotal in understanding the mechanisms of nephrotoxicity and may help to develop effective therapy for the optimal and cause-specific management of renal failure in the clinical set-up. Gentamicin, cisplatin, nonsteroidal antiinflammatory drugs, ifosfamide-induced acute renal failure mimics the renal failure that occurs as a result of administration of respective drugs in the clinical set-up.[23] Gentamicin nephrotoxicity is one of the common causes of drug-induced AKI in hospitalized patients.[3,23] Data
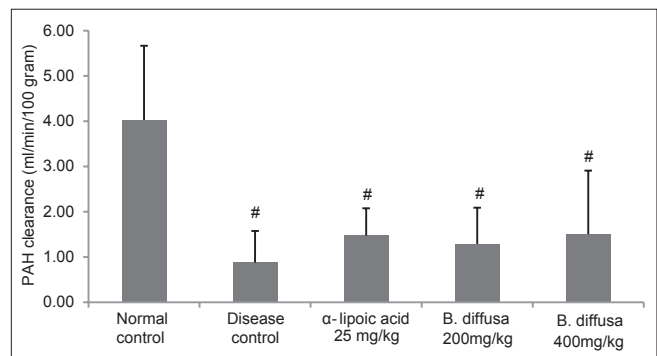

Figure 4: Effect of study drugs on PAH clearance in rats with gentamicin-induced nephrotoxicity. $N = 8$ per group, Bars indicate mean. Error bars represent SD. [#]$P < 0.001$, using one-way ANOVA f/b *post-hoc* Tukey's test (as compared with normal control)

pertaining to mechanisms of aminoglycoside toxicity has been extensively studied in experimental models and also in patients receiving gentamicin.[2] Low multiples of human therapeutic doses to animals (e.g. gentamicin in a dose of 10-20 mg/kg of body weight for a laboratory rat) produce characteristic lysosomal changes in proximal tubular cells of the kidneys and signs of tubular dysfunction including focal necroses and apoptosis. In humans, these changes are evident at therapeutic doses and may progress to overt renal failure. However, animals show a minimal change in kidney function. Therefore, in order to produce extended cortical necrosis and overt renal dysfunction that resembles the clinical picture, high doses (40 mg/kg or more for gentamicin) are required to be administered to animals.[6] In our study, we administered gentamicin sulfate

in a dose of 150 mg/kg/day for 10 days. This dose of gentamicin was standardized in our institute for producing nephrotoxic changes and was found to produce consistent and reproducible nephrotoxicity.[24,25]

In part I, administration of gentamicin sulfate 150 mg/kg for 10 days induced AKI in the disease control, as evidenced by a significant increase in both BUN and serum creatinine. Researchers have reported similar findings of increase in serum creatinine and levels of nitrogenous waste products in the model of gentamicin-induced AKI.[5,23,26] Also, the disease control group failed to gain significant weight, whereas the normal control group showed a physiological increase in the body weight over 11 days, significant as compared with its baseline weight. This difference of weight gain could be attributed to the catabolic state occurring as a result of acute renal failure due to gentamicin.

Extensive research has been performed to elucidate the mechanisms involved in gentamicin-induced AKI and as per literature, oxidative stress is one of the mechanisms by which gentamicin produces impairment in renal function. Gentamicin directly increases the production of mitochondrial reactive oxygen species (ROS) like superoxide anions and hydroxyl radicals from the respiratory chain. These ROS may cause cellular damage and death by diverse mechanisms, such as inhibition of the electron transport chain and subsequent adenosine triphosphate (ATP) production, release of cytochrome c, apoptosis inducing factor, from the mitochondrial intermembrane space, cell cycle arrest due to DNA damage, lipid peroxidation, membrane destabilization, inhibition of Na/K ATPase pump, which leads to cellular swelling, loss of membrane integrity, and necrosis. Lipid peroxidation being one of the mechanisms of cell damage and death is attributable to increased production of MDA, which is a major product of lipid peroxidation.[27,28] Our disease control group showed a significant rise in the renal levels of MDA and a significant fall in the renal levels of GSH as compared with the normal control group after receiving gentamicin sulfate for 10 days, which corroborates with the mechanism of oxidative damage to kidneys caused by gentamicin. Also, in the disease control group, the histopathology of the kidneys demonstrated widespread proximal tubular desquamation and necrosis, with swollen epithelial cells and inflammatory infiltrate. The glomeruli, however, showed minimal damage. This is in agreement with the characteristic aminoglycoside-induced kidney injury, where aminoglycosides accumulate in and affect the proximal tubular cells, but minimally affect the glomeruli.[17]

The objective of the study was to evaluate the nephroprotective effect of the aqueous extract of *Boerhaavia diffusa* in a model of acute nephrotoxicity at two doses (200 and 400 mg/kg/day). The effects of *B. diffusa* were compared with the disease control as well as the positive control ALA. ALA was chosen as the positive control because it is considered to be a universal antioxidant. It is a 'metabolic antioxidant', that is, it is accepted by human cells as a substrate and is reduced to dihydrolipoic acid (DHLA). Unlike other antioxidants, DHLA is not destroyed by quenching free radicals, but rather can be recycled from lipoic acid (LA).[29] ALA was administered orally in the dose of 25 mg/kg/day.[24,26]

In our study, pretreatment with ALA maintained the serum creatinine and BUN levels comparable to normal control group, possibly owing to the renal protection against oxidative injury. This was further confirmed by a significant reduction in kidney MDA levels and near-normal GSH levels. Consistent with the maintenance of renal function, ALA also significantly limited the morphological kidney damage as evidenced by the improved histopathological grade (median grade: 2). Also, treatment with ALA preserved the physiological weight gain of the animals over 11 days, as the body weight on day 11 was significantly more as compared with their baseline body weight. These findings were found to be consistent with the studies that have evaluated ALA in the model of renal injury due to other causes. ALA in a dose of 25 mg/kg/day, when administered concurrently with gentamicin, had attenuated the biochemical alterations and improved the histopathological grade of gentamicin damaged kidneys.[24,26] The antioxidant property of ALA has also been similarly demonstrated in two other models: Renal injury due to cyclosporine A and due to ischemia-reperfusion, where ALA was found to normalize the renal function.[30,31]

In our study, rats receiving both doses of *B. diffusa*, that is, 200 and 400 mg/kg/day showed physiological weight gain over a period of 11 days. They showed a similar and significant reduction in the BUN and serum creatinine levels as compared with the disease control, which was also found to be comparable to ALA. These findings demonstrate the functional improvement in the kidney produced by *B. diffusa*. Also, both doses of *B. diffusa* significantly reduced the MDA levels and maintained the GSH levels in the treatment groups. The MDA and GSH values with the two doses of *B. diffusa* were found to be comparable to each other as well as to ALA and were near normal. This finding is supported by the study of Shisode *et al.,* who have demonstrated the antioxidant activity of aqueous, ethanolic, and chloroform extract of *B. diffusa in vitro* using assay of DPPH radical scavenging, antiproteolytic activity, polyphenol oxidase inhibition, hydroperoxide inhibition and ferric reducing power.[32]

Also, the aqueous extract of root of *B. diffusa* was found to be protective against acetaminophen (200 mg/kg/day for 14 days) induced kidney injury, an established model of drug-induced nephrotoxicity. In this study, pretreatment with *B. diffusa* in a dose of 200 and 400 mg/kg/day for 14 days improved the functioning of the kidneys, which was attributed to the antioxidant effect of *B. diffusa*.[11] Also, in our study, the kidneys of rats treated with 200 mg/kg/day of *B. diffusa* showed a significant improvement in histopathology (median grade: 1) as compared with the disease control. The renal cortex showed only focal desquamation, affecting <1/2 of tubules, with normal appearance of proximal tubular cells and decreased eosinophilia and inflammatory infiltrate. The glomeruli remained normal. However, although the higher dose of 400 mg/kg/day of *B. diffusa* showed improvement in histopathological grade (median grade 2), it was not found to be significant as compared with disease control. *B. diffusa* had shown a similar improvement in histopathological damage in acetaminophen treated kidneys. The protective effect of *B. diffusa* against acetaminophen-induced renal injury was dose-dependent.[11] However, our findings reflect that the aqueous extract of root of *B. diffusa* in a dose of 200 and 400 mg/kg/day provide protection to the same extent (as shown by functional, biochemical, and histopathological parameters) against acute tubular necrosis induced by gentamicin, therefore the lower dose of *B. diffusa*, that is, 200 mg/kg/day may be recommended for future studies.

As the other objective of our study was to explore the possible mechanism involved in improving renal function, it was of interest to us to measure the PAH clearance as a parameter, which reflects the blood flow to the kidney. As PAH clearance reflects the effective renal plasma flow, the improvement in PAH clearance reflects the improvement in renal blood flow.[33] In our study, disease control showed significantly less PAH clearance as compared with the normal control. These findings were consistent with the findings of Palav.[25] Gentamicin decreases GFR by two mechanisms – activation of the renin-angiotensin system followed by local vasoconstriction and necrotic obstruction of tubules leading to an increase in intratubular free-flow pressure in the proximal tubules.[6] Although improvement in PAH clearance was observed in groups receiving both doses of *B. diffusa* as well as ALA, it was not found to be statistically significant as compared with the disease control. Both *B. diffusa* and ALA may not have exerted direct effects on the renal vasoconstriction, but the improvement in PAH clearance could possibly be attributed to the alleviation of the necrotic obstruction of tubules and reduced intratubular pressure, which hinders the glomerular filtration. Unfortunately, there is no published literature available to support our findings

where PAH clearance has been estimated to measure the functional capacity of the kidney in a model of gentamicin-induced nephrotoxicity.

The science of Ayurveda does not describe the kidney disorders in terms of acute or chronic renal failure. However, it mentions disorders of renal function in terms of various symptoms and signs like dysurea *(Mutrakrucchra)*, suppression of urine *(Mutraghata)* and retention of urine *(Mutravrodha)*. *B. diffusa* belongs to the group of herbs that are commonly used for the treatment of these conditions. *B. diffusa* alone or in combination with other herbs has also been used to relieve edema *(shotha)* of several causes.[34] *B. diffusa* is a part of several polyherbal preparations *(e.g. Vidarighrita, Bhadravahaghrita)*, which are used to relieve symptoms presumably occurring due to renal dysfunction.[35]

Also, modern literature has shown that *B. diffusa* contains a large number of compounds such as flavonoids, alkaloids (punarnavine), steroids, triterpenoids, lipids, lignins, carbohydrates, proteins, and glycoproteins. Flavonoids, alkaloids, glycosides, and sterols have established antioxidant activity.[8] Any of these or their combination may be responsible for the positive effects seen in our study.

Taking into consideration the utility of *B. diffusa* in Ayurveda medicine and the experimental evidence generated so far, further studies are required to identify the active principles present in the aqueous extract of root of *B. diffusa* and evaluating the same for the nephroprotective effect.

Most studies, which evaluate the protective role of any agent against toxin induced–damage, administer the agent prophylactically or concurrently with the damaging agent. In this study, we administered *B. diffusa* concurrently with high dose of gentamicin. Gentamicin-induced nephrotoxicity is more commonly seen in association with certain risk factors like old age, hypovolumia, comorbidities like diabetes or cardiovascular diseases, concurrent therapy with medications that are nephrotoxic, presence of preexisting renal disease or septic shock, etc., Coadministration of a protective agent like *B. diffusa* and aminoglycoside may possibly ameliorate the renal damage in these groups of patients.

Further studies are therefore needed to explore the complete therapeutic potential of these agents. These studies may include assessment of other parameters to determine improvement in renal function such as urine analysis (microscopy and biochemistry for enzymuria, glucosuria, proteinuria, urine electrolytes, etc). Also, estimating the levels of other renal antioxidant enzymes,

such as catalase, superoxide dismutase, may provide additional information on the mechanisms involved in reinstating the antioxidant defenses of the kidney.

Lastly, it would be interesting to evaluate the possible role of *B. diffusa* in reversing the established acute renal injury due to aminoglycosides or other drugs having similar mechanisms of damage, that is, the therapeutic potential of these agents in treating drug-induced nephrotoxicity or shortening the duration of recovery from nephrotoxicity.

CONCLUSION

Our study established the nephroprotective potential with special relevance to the antioxidant mechanism of *B. diffusa*. As this plant drug is extensively used by Ayurvedic practitioners to alleviate symptoms of renal disorders, this experimental study should be followed by further experimental and clinical research to establish and exploit its protective role in drug-induced kidney injury.

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