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Research Article

PROTECTIVE EFFECT OF HIBISCUS SABDARIFFA ON GENTAMICIN-INDUCED NEPHROTOXICITY

G.K.A. Adepoju*[1], M.N. Ekor[2] and A. Oladepo[3]

[1]Faculty of Basic Medical Sciences, Ajayi Crowther University, Oyo, Nigeria.
[2]Department of Pharmacology, Redeemers' University, Ogun State, Nigeria.
[3]Department of Clinical Pharmacy and Biophärmacy, Faculty of Pharmacy, Olabisi Onabanjo University, Sagamu, Ogun State, Nigeria.

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***Corresponding author:**
***G.K.A. Adepoju**
Faculty of Basic Medical Sciences, Ajayi Crowther University, Oyo, Nigeria. iconpharm@gmail.com

ABSTRACT

This study aimed at investigating the ability of *Hibiscus sabdarifa* (HS) to protect against gentamicin-induced nephro-toxicity, Gentamicin is widely used in the treatment of many gram-negative infections because of its availability and affordability; its nephrotoxic effect, however, represents a serious clinical problem limiting its use at the usually recommended doses. Gentamicin was administered to Wistar rats at a dose or 100 mg/kg body weight per day subcutaneously for five (5) consecutive days to induce acute nephro-toxicity. Blood samples were collected and analyzed for blood urea, serum creatinine, urinary sodium and potassium as well as oxidative enzymes. Results showed that gentamicin induced acute renal failure as indicated by the significant elevation in plasma urea and creatinine concentrations. The methanolic extract of HS adequately protected against the gentamicin-nephrotoxicity. The study provides a leeway to increase the therapeutic indices of the aminogyoosides. The availability and affordability of HS in developing countries will serve as a public health strategy to protect against the nephrotoxic effect of this drug and can be used as an adjunct to gentamicin therapy in these cnuntries where malnutrition has been implicated as a risk factor to gentamicin-induced nephrotoxicity.

KEYWORDS: Nephrotoxicity, *Hbiscus sabdariffa*, infections, aminoglycosides, therapeutic index, creatinine.

INTRODUCTION

The kidney, an organ for homeostatic control, is made up of a large number of functional units called nephrons. The total number of nephrons varies between species and within any one species as a function of age. The main functions of the kidneys consist of excretion of waste products such as urea. creatinine and in the course of this, homeostatic control.[1]

Nephro-toxic injury can lead to acute renal failure in which the kidneys suddenly lose their ability to function, or chronic renal failure in which the kidney function slowly deteriorates. Both of these nephropathies can be attributed to the use of antibiotics (antibiotic nephropathy) and non-steroidal anti-inflammatory drugs (analgesic nephropathy). Antibiotics implicated in nephro-toxicity include the aminoglycosides and amphotericin B; methiathin, sulphonamides etc provoke allergic reactions that destroy the kidney's functional units.[2] While the determinants of cell damage still remain undefined, more knowledge concerning the mechanism causing the impairment of the renal functions is available.[2,3] This mechanism includes activities of the Renin-Angiotensin System (RAS) and the ensuing local vaso-constriction appears to be primarily responsible for the decrease in glomerular filtration.[1,4] This explains the aggravating effect of the NSAIDs on aminoglycoside nephrotoxity since they inhibit the production of the vasodilatory prostaglandin E (PGE) An increase in proximal intra-tubular free flow pressure of single nephrons, most likely related to nephritic obstruction has also been observed.[5,6] while hyposmotic polyuria, characteristic of the aminglocoside toxicity, has been shown to result from the decrease fluid re-absorption by the proximal tubules secondary to an impaired solute re-absorption.[7,8] Manifestations of renal pathology include ion-wasting phenomenon of haematuria, proteinuria, glomerulonephritis, renal tubular syndromes, nephrogenic diabetes insipidus and hypertension.

The aminoglycosides have long been one of the commonest causes of drug-induced nephro-toxicity.[9,10,11,12] This, in addition to ototoxicity, is the main limitation to their therapeutic uses. This is manifested as a fall in creatinine clearance. Gentamicin, an important member of this group. is an important agent for the treatment of many gram-negative infections because of the long and wide experience with its low cost (hence affordability). Its nephrotoxic effect however represents a serious clinical problem limiting its use at the usually prescribed doses. The specificity of gentamicin for renal toxicity is apparently related to its preferential accumulation in the renal proximal convoluted tubules (30-100 times) more than in serum.[13]

As stated earlier, the wide use of aminoglycosides and their implication in drug-induced nephrotoxicily raise a challenge of which goal is to reduce or protect against the major side effect. This has attracted much effort and attention over time.[14]

Many different chemical agents were used to prevent nephrotoxicity in both animal models and human subjects.[16] Walker and Shah.[9] have reported the use of hydroxyl radical scavengers. The amino acids polyasparagines and polyaspartic acid have been demonstrated to possess nephro-protective effect against gentamicin-induced nephro-toxicity.[17,18] Lipoic acid has also been shown to inhibit gentamicin-induced nephro-toxicity in rats. The protective effect of probucol against this gentamicin-induced nephotoxicity in rats has also been demonstrated.[17,19] just as that of cephalothin.[20,21]

Among the main approaches to achieving this protection have been with the use of anti-oxidants. These include caffeic acid phenethyl ester (CAPE).[12] Dimethyl Sulfoxide, (DMSO).[11] and zinc induced metallothionein synthesis.[22] Hibiscus sabdariffa (HS), commonly known as Red Sorrele, Roselle, Indian sorrel, Guinea Sorrelm, Zobo (Hausa), etc is a potent naturally occurring free-radical scavenger which has flavonoids contained in the flower are the pigments that give the plant the reddish-purple colours.[23,24] The biological activities of the flavonoids, in general, have been detailed.[25,26] The calyx (the fleshy edible sepal) surrounding the seed boll In the flower is bright red and acid and can be used as preservatives, jelly and as juice.[27,28] Its ability to also relax spasm, decrease gastro-intestinal molility and the anti-ulcer activity have also been detailed.[29,30,31]

The plant's constituents have been extensively identified.[32,33,34] *HS* has been used extensively in different parts of the world for medicinal and nutritional purposes.[35,36] The juice is consumed as Zobo (Hausa) flavoured or not and served hot or cold.[30] It has been shown to have a broad range of therapeutic effects.[30,37] While various authors have identified the toxicological profile of the plant.[35,38,39] Other authors.[40,41,42,43] had also shown the beneficial effects of the consumption of the plant.

The hepato-proteclive effect of the plant against some chemically induced hepato-toxicity have been identified.[22,40,44] In fact, where the plant has been shown to be toxic, the doses applied ranged from 1-5 g/kg body weight which was far exceedingly higher than the protective doses tested in this research (100 mg-400 mg/kg body weight).[45,46]

Therefore, this study is to examine whether the plant (HS) will protect against gentamicin-induced nephrotoxicity.

MATERIALS AND METHODS

Chemicals: 1-chloro-2,4-dinitrobenzene (CONB) (Sigma USA), thio-barbituric acid, metaphosphoric phosphate buffer and carbonate buffer were purchased from MRS Scientific Ltd UK. Adrenalin from Aldrich Sigma, Gentamicin (Lek Slovenia). Glutathione and bovine albumin were obtained from Randox Laboratories UK. Other chemicals including tri-chloroacetic acid dipotassium chromate, hydrogen peroxide, acetic acid, formalin solution (10%), Eliman's reagent and Ethylenediamine tetra acetic acid (EDTA) were all of analytical grade.

Plant material: Dried calyx of Hibiscus sabdaniffa was collected in Sagamu, Ogun State, South-West of Nigeria. The specimen was validated at the Forestry Research Institute of Nigeria (FRIN), Nigeria. The leaves were washed with water, shade -dried and then oven- dried at 40^{o}C and pulverized. The constituent was extracted with absolute methanol using cold method of extraction. This was evaporated to obtain the solid extract, which was preserved in the refrigerator until ready for use. The dose of the extract was administered on a kg body weight basis.

Animals and experimental design: Adult male and female albino rat of the Wistar Strain were obtained from the Department of Biochemistry, University of Ibadan, Nigeria and kept under good conditions for 21 days for acclimatization before the start of the experiment during which they were provided with standard rat pellets and water ad libidum. The animals were randomly assigned into five (5) groups of six (6) rats in each group and treated as follows.

Group 1: Normal saline (control group) 1 mg/kg body weight

Group2: Gentamicin Inj, only (100 mg/kg)

Group 3: Gentamicin Inj. (100 mg/kg) + extract (200 mg/kg).

Group 4: Gentamicin Inj. (100 mg/kg) + extract (400 mg/kg)

Group 5: Extract Only (400 mg/kg)

The gentamicin was administered at a dose of 100 mg/kg/day subcutaneously every 24 h for 5 consecutive days. Groups 3 and 4 were pre-treated with the extract.

The rats were killed by stunning and blood samples collected by cardio – puncture to determine the serum activities of GST, CAT, GSH, the serum albumin and total protein levels. The kidneys were immediately removed, a small portion removed and rinsed in ice-cold saline for preparation for cytosolic fraction for enzyme assays.

Preparation of sample for enzyme assays: The rats were sacrificed and blood was collected from the rats by cardiac puncture. Renal function was evaluated by quantifying the levels of urea serum chloride, bicarbonate, cholesterol and creatinine. The levels were determined by using standard laboratory methods. Serum albumin urea, creatinine, Na, K ions and total protein levels were determined using standard laboratory methods of Kingsley and Frankel,[47] Bartel et al.[48] Segal,[49] Schales and Schales,[50] Gluthathione–s-transferases (GST) activity was estimated following the procedure of Bartel, Bohmer & Heier,[48] Habig et al,[51] reduced gluthathione (GSH) by the procedure of Beutler et al.[52] and Catalase (CAT) activity was assessed according to the method of Singha.[53] based on the induction of the disappearance of H_20_2 by CAT. Lipid peroxidation was determined by the method of Misra and Fridorich.[54]

Data Analysis: The data were organized to express the mean + SEM (standard error or the mean) and were analyzed by the student's F-test statistics and chi-square by comparing control with the other treated groups. All the statistical result were expressed at the 95% confidence limits (i.e $p \leq 0.05$).

RESULTS AND DISCUSSION

In this study, the effect of the methanolic extract of *Hibiscus sabdariffa* (HS) on gentamicin-induced nephrotixicity was investigated to increase the search for agents with antioxidant and nephro-protective properties that would protect against this side effect of aminoglycosides in order to improve their therapeutic indices.

Results show that rats treated with 100 mg/kg/day dose of gentamicin injection subcutaneously for five days developed acute renal failure as indicated by the significant elevation in plasma urea and creatinine concentrations when compared with the untreated (control) group ($p < 0.05$) (Tables 1,2,7).

Table 1 shows that reduction of plasma urea concentrations was observed at a dose of 400 mg/kg of the extract. However, pre-treatment with the extract at 200mg/kg could not protect against gentamicin-induced high plasma urea levels. There is, therefore, no statistically significant difference in the effect of the extract at 200 mg/kg (c) and the gentamicin-only treated group (b) (F_{cal} = 0.002, F_{table} =5.12). Hence the protective effect of the extract is dose- dependent. Hence, a statistically significant difference was observed in the nephro-protective effects of the two concentrations of the extract (F_{cal} = 14.5, F_{table} = 5.12). The effects of the extract at 400 mg/kg in the gentamicin-treated rats is statistically non–significant when compared with the control (X^2_{cal} = 0.07, X^2_{table}= 3.84: F_{cal} = 1.94; F_{table} = 5.32). Hence the extract is effective. Treatment with the extract alone (400 mg/kg), on the other hand, significantly reduce the plasma urea concentration. This is statistically significant when compared with the control (F_{cal} =8.98; F_{table} = 5.12; X^2_{cal} =0.53, X^2_{table} = 3.84). Hence the extract is effective in reducing urea levels whether gentamicin –induced or not. The extract can, therefore, be used both prophylactically and therapeutically.

Table 2 presents the effect of the methanolic extract of HS on creatinine plasma level of gentamicin-treated rats.

Comparing the control group (a) with the gentamicin-only treated rats shows that a statistically significant difference exists (F_{cal} = 62.5; F_{table} = 5.12). Hence gentamicin actually increases plasma creatnine levels hence, nephrotoxic.

As observed with the effect in Table 1, there is a statistically significant increase in creatinine plasma levels in the gentamicin treated rats (b,c,d) when compared with the control (a) due to the nephrotoxic effect effect of gentamicin on the renal function. However, a significant difference exists in the effect of the extract on the gentamicin treated rats (c,d) (F_{cal} = 13.0; F_{table}= 5.12). The extract at 200mg/kg would not protect against the gentamicin-induced increase in creatinine levels but offered protection at 400 mg/kg. Therefore, the protective effect is does-related. In fact, there is no statistically significant difference between the effect of the extract at 200 mg/kg and the group treated with gentamicin alone (without the extract). (F_{cal} = 25.12, F_{table} = 0.23).

Comparing the effect of the extract alone at 400mg/kg with the control (a,e) shows no statistically significant difference on the plasma creatinine levels (X^2_{cal} = 0.15, X^2_{table} = 3.84). Hence, the extract is effective and does not in itself increase serum creatinine levels. Thus, the extract can be used as a prophylactic to protect against increased plasma creatinine levels. But a statistically significant differenceexists in the effects of the extract alone at 400mg/kg and the gentamicin=only treated rats group (b,d) (F_{cal} = 39.9, F_{table} = 4.96). Hence, the extract could protect against increase plasma creatinine levels especially gentamicnin –induced.

There is no statistically significant difference between the effect of the extract at 400 mg/kg in the gentamicin-treated rats and the control (a,d) (X^2_{cal} = 0.002, X^2_{tab} = 3.84; F_{cal} = 3.07, F_{table} = 5.12). Hence, the extract is effective in protecting against gentamicin-induced nephrotoxicity.

Table 1: Effect of methanolic extract of *Hibiscus sabdariffa* on urea level in the plasma of Gentamicin-treated rats.

		Conc. of urea)mg/100ml						
Treatment	Dose (mg/kg)	1	2	3	4	5	6	Mean±S.E
Control (a)	-	50	42	67	33	75	-	53.4±7.8
Gentamicin (b)	100	117	158	283	267	350	158	221 ± 32.4
Gentamicin + Extract (c)	100 200	283	150	350	108	225	-	2322 ± 43.8
Gentamicin + Extract (d)	100 400	67	75	58	50	67	100	69.5±7.0
Extract (e)	400	25	33	17	33	33		29.0±18.3

Table 2: Effect of methanolic extract of *Hibiscus sabdariffa* on Creatline level in the plasma of Gentamicin-treated rats.

		Conc. of urea)mg/100ml						
Treatment	Dose (mg/kg)	1	2	3	4	5	6	Means±S.E
Control (a)	-	1.2	0.8	1.4	0.8	1.6	-	1.2±0.2
Gentamicin (b)	100	3.0	2.8	4.0	3.6	4.2	3.2	3.4±0.2
Gentamicin + Extract (c)	100 200	4.4	2.4	3.6	2.0	3.8	-	3.2 ±0.5
Gentamicin + Extract (d)	100 400	1.6	1.8	1.2	1.2	1.4	2.4	1.6±0.2
Extract (e)	400	0.4	0.8	0.6	0.2	1.0	0.8	0.6±1.2

Table 3: Effect of methanolic extract of *Hibiscus sabdariffa* on GSH level in the plasma of Gentamicin-treated rats.

GSH concentration						
Treatment Group	1	2	3	4	5	Means±S.E
Control (normal saline) (a)	30	22	14	34	64	32.8±8.5
Gentamicin 100 mg/kg (b)	34	4	24	30	31	24.6±5.4
Gentamicin 100 mg/kg Extract 200 mg/kg (c)	34	130	130	149	124	113.0±20.3
Gentamicin 100 mg/kg+Extract 400 mg/kg (d)	38	16	16	22	22	23.0±26.6
Extract only 400 mg/kg (e)	114	110	114	24	-	90.5±22.2

The extract at 400 mg/kg alone or in the gentamicin-treated rats reduced induced Increases in plasma creatinine levels. However, a statistically significant difference exists in the effects of the extract alone and in the gentamicin- treated rats (F_{cal}= 18.92, F_{table} 4.96).

Table 3 shows the effect of the methanolic extract of HS on glutathione (GSH) levels of gentamicin -treated rats. An association between gentamicin nephrotoxicity and oxidative stress has been confirmed in many experimented models (Mari-Paule and Tulkens, 1999). Oxidative stress has been implicated in the pathogenesis of a variety of clinical disorders. The extract at 200 mg/kg increases the levels of GSHmore significantly than at 400 mg/kg of the extract ingentamicin- treated rats. (X^2_{cal}= 22.23). This is an irony! Whereas the extract at 400 mg/kg increased the GSH levels in non-gentamicin-treated rats, it can also be observed that the extract at 200 mg/kg in gentamicin-treated rats and its 400 mg/kg in non-gentamicin treated rats significantly increased the GSH levels than in the other groups. The two doses are equally effective as there is no statistically significant difference in their activity (X^2_{cal}=0.45; X^2_{table} 3.84). Hence, it is cost-effective for therapy and can also be used prophylactically.

Table 4 shows the methanolic extract of HS on the level of activity of glutathione S-transferase (GST) in gentamicin-treated rats. There is no statistically significant difference in the effect of the 200mg/kg and 400mg/kg of the HS extract in the gentamicin-treated rats (X^2_{cal} = 0.85). There is no statistically significant difference between the levels of GST in the 400mg/kg extract only and the control. (X^2_{cal} = 0.18). The extract protects against gentamicin-induced oxidative damage to the kidneys.

Table 5 shows the result of the extract on the lipid peroxidation level of gentamicin-treated rats. The result shows that gentamicin-induced nephrotoxicity was associated with increased lipid peroxidation though not statistically significant when the HS extract alone was compared with the control (X^2_{cal}=0.38). There is, also, no statistically significant difference between the effects of the 200 mg/kg and the 400 mg/kg of the extract in gentamicin-treated rats (X^2_{cal} 0.73).

Table 4: Etract of methanolic extract of Hibiscus sabdanffa on the levels of activity Glutathione S-Transferase (GST) Gentamicin-treated rats.

GSH concentration						
Treatment Group	1	2	3	4	5	Means±S.E
Control (normal saline) (a)	0.031	0.038	0.042	0.068	0.069	0.05 ± 0.00079
Gentamicin 100 mg/kg (b)	0.019	0.006	0.046	0.077	0.093	0.06±0.01
Gentamicin 100 mg/kg Extract 200 mg/kg (c)	0.011	0.184	0.162	0.133	0.129	0.124±0.03
Gentamicin 100 mg/kg+Extract 400 mg/kg (d)	0.319	0.035	0.027	0.042	0.029	0.05±0.003
Extract only 400 mg/kg (e)	0.041	0.037	0.029	0.032		0.035+0.0000008

Table 5: Extract of methanolic extract of Hibiscus sabdanffa on the Lipid peroxidation Levels of Gentamicin-treated rats.

GSH concentration						
Treatment Group	1	2	3	4	5	Means±S.E
Control (normal saline) (a)	31.8	48.1	59.3	54.5	-	48.4 ± 6.0
Gentamicin 100 mg/kg (b)	48.8	50.3	-	-	-	49.5±0.8
Gentamicin 100 mg/kg Extract l200 mg/kg (c)	40.9	33.2	27.1	25.24	-	31.6±3.5
Gentamicin 100 mg/kg+Extract 400 mg/kg (d)	32.7	24.8	57.5	44.1	16.7	35.2±7.2
Extract only 400 mg/kg (e)	39.1	95.81	70.81	87.3	-	71.2±14.4

Table 6: Effect of methanolic extract of Hibiscus sabdanffa on Catalase activity in Gentamicin-treated rats.

Catalase Activity						
Treatment Group	1	2	3	4	5	Means±S.E
Control (normal saline) (a)	1.76	1.25	0.67	2.60		1.57±0.4
Gentamicin 100 mg/kg (b)	1.29	2.46	1.74	-	-	1.50±0.8
Gentamicin 100 mg/kg Extract l200 mg/kg (c)	2.34	21.11	11.12	25.13	-	1.49±5.1
Gentamicin 100 mg/kg+Extract 400 mg/kg (d)	13.63	3.22	73.97	-	-	30.3±2.2
Extract only 400 mg/kg (e)	0.79	10.46	6.28	-	-	5.64±2.8

Table 7: Effect of Gentamicin and Hibiscus sabdanffa on renal parameters.

Parameter	Control	Hibiscus Sabdaritta	Gentamicin	Gentamicin + HS 200 mg/kg	Gestemicin+ HS 400 mg/kg
Urea (mg/100 ml)	53.4±7.8	29.0±18.3	221±32.4	232.2±43.8	69.5±7.0
Creatinine (mg/100ml)	1.2±0.2	0.6±1.2	3.4±0.2	3.2±0.5	1.6±0.2
Glutathione-S-transference s	0.05±001	0.035±0.00001	0.06±01	0.124±0.03	0.05±0.003
Lipid peroxidation	48.4±6.0	71.2±14.4	49.5±0.8	31.6±3.5	35.2±7.2
Calalase	1.57±0.4	5.64±2.8	1.50±0.8	14.9±5.1	30.3±22.0
GSH (reduced Gluthathione)	32.8±8.5	90.5±22.2	24.6±5.4	113.0±20.	23.0±26.0
Sodium lon level (meg/L)	13.6±1.7	142±2	142±1.4	143±3.0	140±1.4
Potassium ion level (meg/L)	27.8±3.2	24.8±1.7	19.4±1.7	27.8±2.3	23.5±2.2
Total protein (serum)	8.54±0.8	7.50±0.3	8.48±0.2	8.10±0.4	7.50±0.3
Total protein (kidney)	0.9±0.2	1.05±0.3	0.9±0.2	0.8±0.3	1.05±0.3

Hence the effect is not dose-related suggesting that the cytoprotective effect of this extract may be effective at such reduced doses though not statistically significant in all the groups.

Table 6 shows the effect of the extract on the catalase activity in the gentamicin-treated rats. Though the activity of the antioxidant enzyme is not significantly affected by gentamicin treatment, the activity was however increased by the extract in a dose-dependent manner as a statistically significant difference is observed between the two doses. (X^2_{cal}= 4.51). Thus the dose at 400ng/mg/kg increases CAT activity than the 200 mg/kg in the gentamicin-treated rats. Aside this, the extract alone does not exert any statistically significant effect on the CAT activity compared with the same dose in the gentamicin treated rats (X^2_{cal} =0.97). When the extract alone was compared with the control, no statistically significant difference was observed (X^2_{cal}=0,18). This increased activity of the CAT by the extract increases the antioxidant

enzymatic defence and possibly contribute to the mechanism of protection against gentamicin-induced nephrotoxicity.

CONCLUSION

It has been shown that the study of aminoglycoside nephrotoxicity has clearly identified several critical mechanisms; the knowledge of which allows clinical strategies for the safer use of these drugs. The main objective is to increase the therapeutic indices of this group of drugs. The co-administration of polyaspartic acid and desferroxamine still deserves preclinical and clinical development.

This is a great limitation particularly in developing countries because of the great health burden of the governments and individuals and, more so, when malnutrition has been demonstrated as a risk factor to gentamicin-induced hephrotoxicty as a result of the association between malnutrition and infection diseases.[55]

However, natural sources, like the *Hibiscus sabdariffa* that is usually taken as drink in these countries, will help as a public health strategy to protect against the nephrotoxic effect of this group of drugs commonly used and abused there. From the findings of this study, it offers a great potential; it is affordable, available and easy to prepare. It is can, therefore, be used as an adjunct to gentamicin therapy.

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