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Guarana Seed Extract Prevents Nephrotoxicity Caused by Gentamicin Treatment in Mice

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Abstract

Gentamicin is a widely used aminoglycosidic antibiotics family member since its discovery in 1963. Like any other medications, gentamicin causes nephrotoxicity due to the oxidative stress caused by its pharmacodynamics. This study aims to examine the antioxidant power of the guarana seed extract in protecting renal tissue as a supplement. Forty male mice were divided into four groups (group one normally fed, the second group was treated with 300 mg/kg of guarana seed extract daily, group three was injected intraperitoneally with 100 mg/kg of gentamicin daily and the fourth group was co-treated with both 300 mg/kg of guarana seed extract and with 100 mg/kg of gentamicin daily) for two weeks. Serum levels of urea, creatinine, AST, ALT, IL-1B and IL-6 were significantly elevated in gentamicin treated group and that changes were not found in the guarana cotreated group. In gentamicin administered mice, a significant reduction was found in two antioxidants SOD and GPX accompanied with downregulation of Ho-1 and Nef2 while, that did not happen in the guarana seed extract cotreated group. Furthermore, both histopathology and immunohistochemistry slides show that the guarana seed extract prevents the degenerative and necrotic events in epithelial tubular tissues caused by gentamicin toxicity. In conclusion, our data suggest that gentamicin can damage renal tissues when given at 100 mg/kg/day and, however, the guarana seed extract may capable of preventing that event when cotreated with the gentamicin as a supplement.

Keywords: Gentamicin; Guarana; Nrf2; Ho-1; Nephrotoxicity; SOD; GPX

Introduction

Over the last century, antibiotics have been used to treat most bacterial infectious diseases and since that physicians are always trying to manage between the great benefits of this family of medications and their toxicity. Gentamicin is a widely used aminoglycosidic antibiotics family member since its discovery in 1963. It is an effective antibiotic against gram-negative bacteria and prescribed particularly for Pseudomonas aeruginosa infections [1]. On the other hand, like any other medications, Gentamicin has several side effects some are common such as stomach pain, vomiting or nausea and some are rare such as hair loss, ear ringing or dizziness [2]. Several studies have reported that gentamycin administration may induce hepatotoxicity, nephrotoxicity, neurotoxicity or ototoxicity and that was due to either high dosage or duration of the treatment course [3-6]. Some studies have reported that some vitamin supplementation may reduce the toxicity of exposure to gentamicin. A study published in 1999 have concluded that vitamin E supplement potentially reduces gentamicin toxicity in experimental rats [7]. Another one has reported that the co-supplementation of vitamin E and C prevented the nephrotoxicity of gentamicin in rats as well [8]. On the other hand, Garib et al. have demonstrated that vitamin D supplements failed to protect the renal toxicity caused by gentamicin administration in rats [9].

In another context, several studies have revealed that guarana (*Paullinia cupana*) supplementations reduce the oxidative stress in both humans and animals caused by exposure to different toxic materials such as Cadmium and sodium nitroprusside [10-12]. Historically, Brazilian Indians were the first society that discovers and consume guarana seeds [13]. The first medical report on the medicinal effects of guarana seeds drink was written by Johannes Bettendorf in the 17th century which mentioned that guarana seeds drink has a painkilling effect and is beneficial for fever and crumps [7,13]. In addition, Yonekura et al. have concluded that guarana active principles such as catechins reduce oxidative stress via either direct action or up-regulation of antioxidant enzymes [10]. Another study has revealed that guarana extract has an anti-inflammatory effect in animals exposed to methylmercury [14]. This study is an attempt to find out whether guarana seeds extract can be used to reduce the toxicity caused by gentamicin treatment in the experimental animals and to what extent that can be safe.

Methods and Materials

Materials

The guarana seed extract was purchased from Source Naturals, California, USA. Gentamicin injections were purchased from Jamjoom Pharma Corporate, Jeddah, Saudi Arabia. Serum kidney function tests kits (T. protein, Albumin, Urea and Creatinine) together with ALT, AST kits were purchased from Abcam plc, Cambridge, UK. Serum analysis kits of IL-1B, IL-6, GPX and SOD were purchased from Sigma-Aldrich Corp. St. Louis, MO, USA. Bax antibodies were purchased from Cell

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Signaling Technology, Inc. Danvers, MA, USA. A real-time qPCR kit was purchased from Qiagen, Germantown, USA.

Experimental design

Forty male mice weighed between 25-30 gram were divided into four groups of 10 each (group one normally fed as a control group, the second group was treated with 300 mg/kg of guarana seed extract daily, the third group was injected intraperitoneally with 100 mg/kg of gentamicin daily and the fourth group was co-treated with both 300 mg/kg of guarana seed extract as well as injected intraperitoneally with 100 mg/kg of gentamicin daily). The experiment lasted two weeks and on the final day, mice were exposed to isoflurane and slaughtered for serum extraction and renal tissue collection. The experimental protocol has been approved by the Scientific Research and Ethical Committee (SREC) of the University College of Turbah, Taif University.

Serum analysis

Serum levels of kidney function parameters were estimated using Colorimetric spectrophotometry technology. Serum levels of IL-1B, IL-6, GPX and SOD were measured by sandwich Elisa technology using a Bio-Rad xMark™ Microplate reader.

Gene expression analysis

Kidney tissue samples were collected in 1 ml of trizole reagent each and stored at -20°C. Specimens were homogenized, centrifuged at 4°C in chloroform solution and then supernatants were collected for total RNA extraction. To each specimen, equal volumes of Isopropanol were added and centrifuged at 12000xg. Precipitates (total RNAs) were collected and rinsed with 70% of ethanol and dissolved in DEPC water. The extracted total RNAs were mixed with oligo dT and incubated for 5 minutes at 70°C in a thermal cycler for cDNA forming. Samples then were mixed with (10mM of dNTPs, 100U of M-MulV the reverse transcriptase and 2 µL of 10x RT-buffer). The mixtures were incubated at 37°C for an hour in a thermal cycler followed by 10 minutes at 90°C to stop enzyme activity. Primers are listed in Table 1 of the candidate genes that were used for qRT-PCR analysis. PCR reactions had been run following the optimized protocol provided with the kit. PCR products were analysed using a Bio-Rad CFX Real-Time PCR Detection System.

Histopathological investigation

Kidney tissues had been collected from the mice of experimental groups and immediately fixed in 10% of buffered neutral formalin. Samples then were washed with tap water and dehydrated using 70% of alcohol then cleared in xylene. Samples were embedded, cast and sectioned into 5 microns sections in Paraffin wax. Samples then were stained following the routine staining technique (H and E stains) for microscopic investigation.

Immunohistochemical examination of Bax

For immunohistochemistry reaction kidney tissue slides were washed with xylene twice (three minutes each) for deparaffinization followed by multiple washes in gradient ethanol concentrations. Under cold tap water, samples were washed to remove the rest of the ethanol. Samples then were treated with 3% H_2O_2 for 10 min for peroxidase suppression and then heated in 10 mM citrate buffer at 121°C for 30 minutes for antigen retrieval. Samples were blocked in 5% normal serum for 15 minutes and incubated overnight with a rabbit polyclonal anti-Bax antibody (1:100; Abcam, Discovery Drive, Cambridge, CB2 0AX, UK) in Phosphate-Buffered Saline (PBS) at 4°C. Three washes with PBS were applied to each slide before incubated with a Rabbit polyclonal Secondary Antibody to Mouse IgG-H and L (HRP) for 15 minutes at room temperature. Finally, slides had been incubated with Diamino-Benzidine (DAB) and counterstained with hematoxylin for 10 seconds at room temperature before reading.

Statistical analysis

Data are presented in means ± standard error of means for 10 animals of each group. Statistical analysis was performed using Microsoft Excel 2010. P values <0.05 were considered as significant.

Results

Guarana seed extract prevents renal toxicity caused by gentamicin exposure

As shown in Table 2 gentamicin toxicity significantly elevated serum levels of both urea and creatinine to about three folds with p values ≤ 0.05 corresponding to the control group and that was accompanied by a striking elevation of serum levels of both ALT and AST enzymes which indicates a sort of renal injury. These clear changes were significantly prevented in mice co-treated with 300 mg/kg of guarana seed extract in which of p values of serum levels of urea and creatinine were ≤ 0.05 corresponding to the gentamicin treated group as shown in Table 2.

The antioxidant and anti-inflammatory role of guarana seed extract

Table 3 shows the data of two antioxidant enzymes Glutathione Peroxidase (GPX) and Superoxide Dismutase (SOD) as well as two proinflammatory mediators Il-1b and IL-6. It is clear that gentamicin toxicity significantly increases serum levels of both pro-inflammatory mediators Il-1b for about 100% and Il-6 for more than 300% as shown in Table 3. On the other side, levels of both antioxidant enzymes were reduced in mice exposed to gentamicin toxicity. However, guarana seed extract had significantly diminished these effects in the gentamicin + guarana seed extract co-treated group as demonstrated in Table 3.

Real-time PCR analysis of Ho-1, Nrf2 and NF-kβ

Figure 1 illustrates the quantitative analysis of the gene expression of Ho-1, Nrf2 and NF-kβ. There was a significant downregulation of both antioxidative genes Ho-1 and Nrf2 under the effect of gentamicin treatment. That reaction was accompanied by the upregulation of NF-kβ the inflammatory responsive gene. On the other side, the co-treatment of guarana seed extract with gentamicin significantly prevented these genetic expression responses as shown in Figure 1.

Results of histopathological examination

The kidney of control mice showed normal glomerular and tubular pictures with normal cortical and medullary architecture. No histological difference was observed in kidneys of guarana-

Gene	Forward	Reverse
Ho-1	5'-CGCCTCCAGAGTTTCCGCAT -3'	5'-GACGCTCCATCACCGGACTG-3'
Nrf2	5'-CGCCTGGGTTCAGTGACTCG -3'	5'- AGCACTGTGCCCTTGAGCTG-3'
NF-kB	5′-CACTGTCTGCCTCTCTCGTCT-3′	5′-AAGGATGTCTCCACACCACTG-3′

Table 1: Primer sequences for the studied genes.

Variables	Urea (mg/dl)	BUN (mg/dl)	Creatinine (mg/dl)	ALT (U/L)	AST (U/L)
Control	22.3 ± 0.69	22.5 ± 6.6	0.78 ± 0.02	16.3 ± 0.65	20.6 ± 2.1
Guarana	25.1 ± 1.3	17.6 ± 2.4	0.81 ± 0.006	19.1 ± 1.4	23.2 ± 2.5
Gentamycin	67 ± 4.6*	10.4 ± 0.32	2.1 ± 0.23*	65.2 ± 3*	85 ± 3
Guarana + Gentamycin	32.2 ± 3.4**	15.1 ± 1.5	0.8 ± 0.01**	26.8 ± 0.7**	28.7 ± 3.5

Values are means of ten mice ± SEM. * represents P values ≤ 0.05 corresponding to the control group. ** Indicates P values ≤ 0.05 corresponding to Gentamicin treated group.

Table 2: Serum levels of renal function tests of control and experimental groups.

Variables	IL-1B (pg/ml)	IL-6 (pg/ml)	GPX (U/L)	SOD (U/ml)
Control	155 ± 8.1	61 ± 6.3	190 ± 40.7	3.37 ± 0.16
Guarana	162 ± 34.7	71.5 ± 7.9	191 ± 36.3	2.9 ± 0.3
Gentamycin	308 ± 15.7 *	200.5 ± 16.2*	131.2 ± 9.6	1.86 ± 0.3 *
Guarana + Gentamycin	169 ± 15.5 **	105 ± 7.6 **	188.5 ± 21.1	3.2 ± 0.17 **

Values are means of ten mice ± SEM. * represents P values ≤ 0.05 corresponding to the control group. ** Indicates P values ≤ 0.05 corresponding to Gentamicin treated group.

Table 3: Serum levels of cytokines and antioxidant enzymes of control and experimental groups.

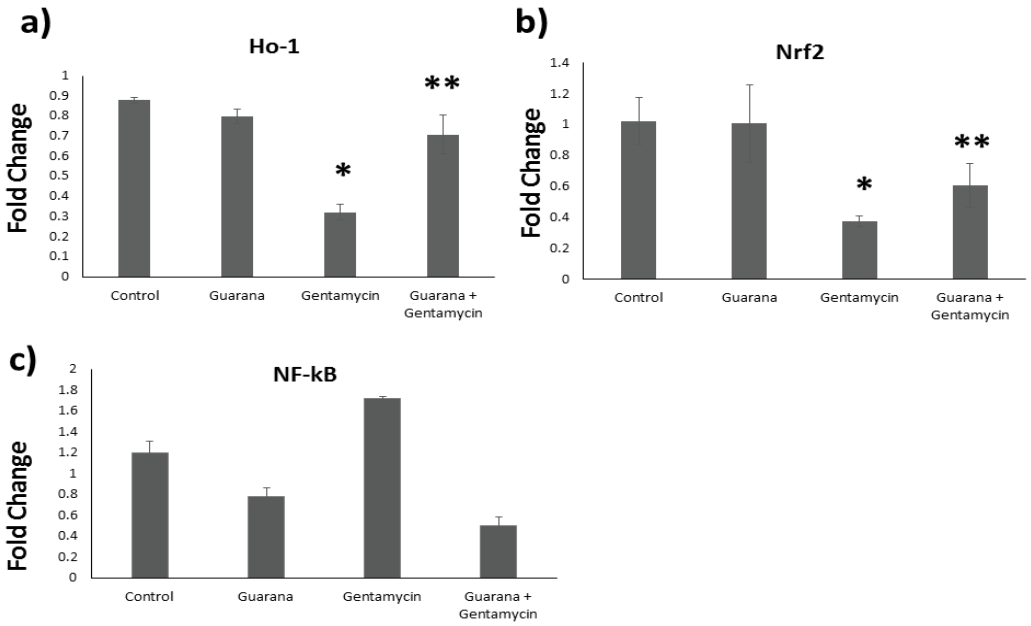

Figure 1: Real-time PCR analysis of Ho-1, Nrf2 and NF-kB genes. (a) Expression fold change of Ho-1 in control and experimental groups. (b) Expression fold change of Nrf2 in control and experimental groups. (c) Expression fold change of NF-kB in control and experimental groups. * indicated P values <0.05 corresponding to control, ** indicated P value <0.05 corresponding to Gentamicin treated group.

administered mice compared to the control group. The kidney of the gentamicin treated group showed numerous degenerative and necrotic changes represented by hydropic degeneration of tubular epithelium together with pyknosis of numerous nuclei of tubular cells with the condensation of its nuclear chromatin and cytoplasmic hypereosinophilia. Shrinkage of glomerular tufts was also detected. The slides of the group that co-treated with both gentamicin and guarana showed restoration of normal histology with moderate congestion of renal blood vessels (Figure 2).

Results of immunohistochemical expression of Bax

Both control and guarana-administered groups showed an absence of Bax expression in glomerular and tubular cells. On the other hand, the gentamicin-administered group showed prominent expression of Bax in tubular cells together with weak expression in glomerular tufts. While the gentamicin+ guarana seed extract co-treated group showed a clear reduction of Bax expression with faint immunostaining in tubular cells (Figure 3).

Discussion

In general, antibiotics are very useful medications against a wide spectrum of pathogenic bacteria but under consideration of management between their benefits and risks. Gentamicin is a member of this broad family its benefits in fighting bacteria often accompanied with some extent of toxicity. Different health centres in different

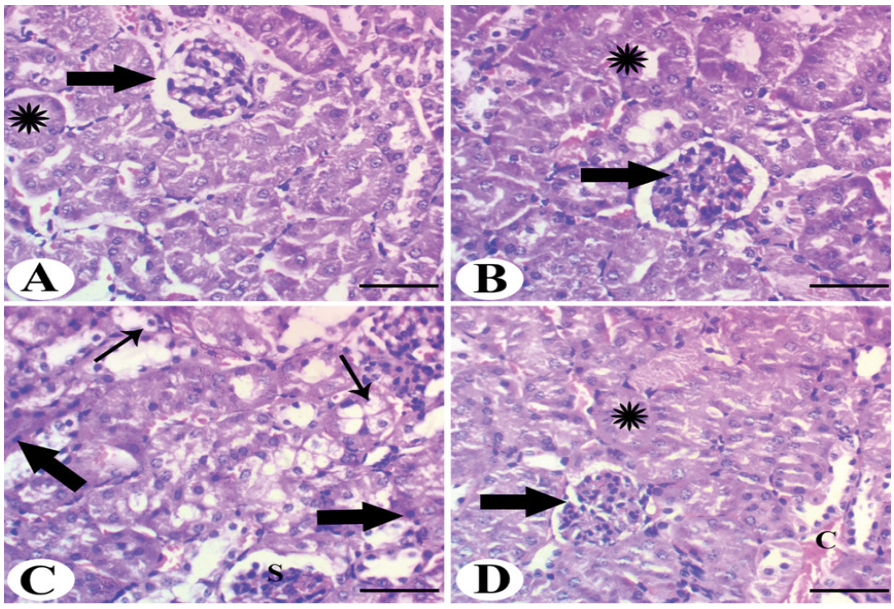

Figure 2: Results of histopathological examination. A and B. Kidneys of control and guarana-administered mice showed normal histology of glomeruli (arrows) and tubules (*). C. Kidney of gentamicin group showed hydropic degeneration (thin arrows), pyknotic nuclei with cytoplasmic hypereosinophilia (thick arrows) together with shrunk glomeruli (S). D. Kidney of gentamicin group treated with guarana showed restoration of normal architecture with congestion of renal vessels. Scale bar= 50 µm.

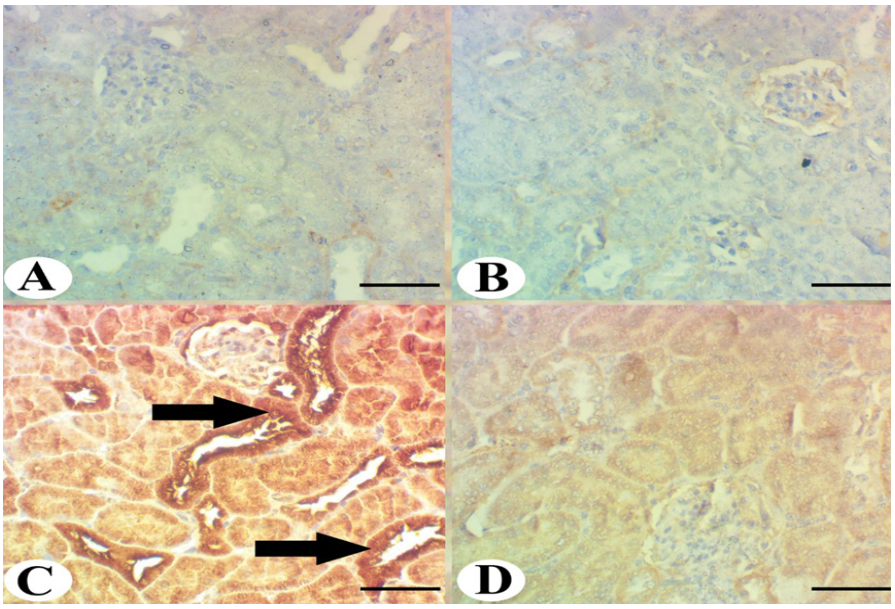

Figure 3: Results of Bax immunostaining. A and B. Kidneys of control and guarana-administered mice showed an absence of Bax expression. C. Kidney of gentamicin group showed extensive Bax immunostaining in renal tubules (arrows) with weak expression in glomerular tufts. D. Kidney of gentamicin group treated with guarana showed faint Bax immunostaining in tubular cells. Scale bar= 50 µm.

countries try to follow a certain protocol for using gentamicin with either children or adults to avoid any potential risks. Several medical reports have mentioned that gentamicin may cause nerve damage, ototoxicity, renal and hepatic injuries [15-18]. Gentamicin may cause tubular damage as a result of epithelial cytotoxicity that leads to tubular epithelial cell necrosis [19-21]. These renal severe effects were mainly due to the strong oxidative stress caused by hydrogen peroxide and superoxide production in epithelial tubular cells as a response to gentamicin pharmacodynamics [22-24]. On the other hand, current work is an attempt to find out whether or not guarana seed extract is useful as an antioxidant supplement to avoid the drawbacks of gentamicin treatment. This natural substance is rich in caffeine and

polyphenols, and this type of content may have antioxidant properties [25,26].

Clinically, serum levels of both urea and creatinine are used as renal function indicators and to assess the extent of kidney health or injury. Herein, we have estimated serum levels of these organic compounds and as presented in Table 2 gentamicin treatment at 100 mg/kg/day significantly increased serum urea to about 200% (three folds) and similar effect with serum creatinine levels as shown in Table 2 while in guarana seed cotreated group both urea and creatinine levels remain close to their levels of the control group. These changes were accompanied by a significant increase in both ALT and AST enzymes which means there was significant damage in renal tissue in those mice injected with gentamicin, however, the guarana seed extract preserved these enzymes serum levels as demonstrated in Table 2. In addition, the histopathology pictures clearly show that gentamicin caused degenerative and necrotic events in tubular epithelial cells which were not found in histopathology slides of mice that cotreated with the guarana seed extract as shown in Figure 2.

On the molecular scale, the expression quantity of two antioxidant genes was estimated using the real-time PCR technology (Heme oxygenase -1 (Ho-1) and Nuclear factor erythroid-2 (Nrf2)). Gentamicin treatment significantly downregulated both antioxidants and upregulated Nf-kβ gene as a response to the inflammatory condition caused by the oxidative stress increase as shown in Figure 1. In contrast, those gene expression changes were significantly prevented by the guarana seed extract Figure 1.

These data were confirmed via serum analysis of two pro-inflammatory cytokines IL-1B and IL-6 and two antioxidants GPX and SOD in which guarana seed extract prevented the significant reduction of both antioxidants caused by gentamicin toxicity that in turn increased the pro-inflammatory cytokines which were reduced by the guarana seed extract cotreatment as well Table 3. Furthermore, Figure 3 shows a clear increase in Bax expression which indicates a necrosis mechanism triggered by gentamicin treatment while that reaction did not appear in the control group, guarana seed treated group and, in the gentamicin,+ guarana seed extract cotreated group as shown in Figure 3. These findings together are nicely consistent with tens of previously published papers over the last three decades that elucidated gentamicin toxicity.

Conclusion

In conclusion, the present study confirms that gentamicin can damage renal tissues when given at 100 mg/kg/day and the guarana seed extract may capable of preventing that event when cotreated with the gentamicin at 300 mg/kg/day or even a slightly more as long as this study has elucidated that extract is safe. We think that further work needs to be done to determine which active components are responsible for that antioxidation effect.

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