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ORIGINAL ARTICLE

Modulation of gentamicin-induced renal dysfunction and injury by the phenolic extract of soybean (*Glycine max*)

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ABSTRACT

Gentamicin (GM) is one of the most important of the aminoglycoside antibiotics used widely for the treatment of serious and life-threatening infections and whose clinical use is limited by its nephrotoxicity. As the pathogenesis of GM-induced renal dysfunction and injury involves reactive oxygen species, the polyphenolic constituents of soybean with antioxidant property may protect against GM-induced renal toxicity. We therefore tested this hypothesis using phenolic extract of soybean (PESB) on GM-induced nephrotoxicity rat model. Administration of GM (80 mg/kg, s.c.) for 12 days to rats induced marked renal failure, characterized by a significantly increased plasma creatinine, urea and Na^+ ions levels, with K^+ depletion. This was also associated with decreases in the activity of the renal antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST)] measured and depletion of both blood and renal reduced glutathione (GSH) levels. The activities of membrane-bound glucose-6-phosphatase (G6Pase) and 5^1-nucleotidase (5^1-NTD) enzymes as well as γ-glutamyltransferase (γ-GT) and aspartate aminotransferase (AST) (enzymes that are located in the proximal tubule) were decreased. Renal histology examination further confirmed the damage to the kidney as it reveals severe necrosis of the proximal renal tubules with deposition of colloid casts. These alterations were ameliorated in rats pretreated with PESB. The decrease in the activities of SOD, CAT, GST as well as GSH depletion observed in GM-treated rats was prevented in the rats pretreated with PESB. The activities of γ-GT, AST and G6Pase were also increased in the kidney. These protective effects were dose dependent except for G6Pase activity and GSH levels that were preserved only at 500 mg/kg dose of PESB, and 5′-NTD activity that was dose dependently decreased. Furthermore, the extent of tubular damage induced by GM was reduced in rats that also received PESB. The lower dose (500 mg/kg) of the extract, however, appeared to provide better histological protection. These results suggest that the PESB has protective effects on GM-mediated nephropathy and this may be related to the action of the antioxidant polyphenolic content of the soybean.

INTRODUCTION

Aminoglycosides are a class of clinically important antibiotics used in the treatment of infections caused by Gram-positive and Gram-negative organism and have long been one of the commonest causes of drug-induced nephrotoxicity which accounts for nearly 10–15% of drug toxicity [1]. Infact, they are one of the principal causes of hospital-acquired acute renal failure. Although a clear recognition of the patient and treatment-related

risk factors, combined with once-a-day schedule and effective monitoring procedures, have improved the situation over what prevailed in the early 1980s, the safety of the aminoglycosides still compares less to that of the other broad-spectrum antibiotics.

Gentamicin (GM) is an aminoglycoside antibiotic that is still commonly used in the treatment of life-threatening infections due to Gram-negative organisms [2]. However, about 30% of patients treated with this drug for more than 7 days show some signs of nephrotoxicity [3], and this side effect and other complications that arise from the use of this drug limit its clinical usefulness. GM-induced nephrotoxicity is usually characterized by tubular necrosis, primarly localized to the proximal tubule [4]. Marked decreases in glomerular filtration rate (GFR) and alterations in intraglomerular dynamics have also been observed [5].

Several clinical and experimental strategies have been employed to reduce or protect against GM-induced renal dysfunction and injury. Some of these strategies include: (i) decreasing or preventing GM accumulation by the kidneys, and (ii) preventing or decreasing the lysosomal phospholipidosis induced by the cell-associated GM and other means of protection. Most of the attempts to reduce GM nephrotoxicity without compromising the antibacterial action have been met with little success. Changes in the structure of the aminoglycosides cause loss of antibacterial effect [2]. There is therefore a need for search for effective, safe and practical agents that can reduce GM nephrotoxicity without compromising its efficacy as an antibacterial agent. Among the other main approaches used so far to protect against aminoglycoside nephrotoxicity, the most consistent effects have been observed with the use of antioxidants and especially deferroxamine [6]. Based on the findings that GM forms complexes with mitochondrial Fe^{2+} to catalyze the formation of free oxygen radicals, iron chelators were tested and were proved to be effective in the prevention of aminoglycoside-induced ototoxicity [7]. Extension of this finding to nephrotoxicity appears to be possible [8], but biophysical and biochemical considerations [9], suggest that the protective effect of deferroxamine may be critically dependent on the dosage of GM. Other compounds were also used on account of their antioxidant effects, but the mechanisms have not always been unambiguously established. Means of protection based on a correlation of the functional abnormalities or on an increase in cell regeneration capabilities have also been attempted, but no clinical application has so far been made [6].

Flavonoids continue to draw attention as possible, very useful therapeutic agents for combating pathologic states associated with free radical production [10]. The role of dietary flavonoids in the prevention of several chronic diseases has been the subject of intense research interest and the soy phenolics have been the focus of particular attention [11]. Furthermore, there is increasing evidence that dietary phytoestrogens present primarily in soybeans as isoflavones have a beneficial role in chronic renal disease [12]. Nutritional intervention studies have shown that consumption of soy-based protein reduces proteinuria and attenuates renal functional or structural damage in animals and humans with various forms of chronic renal disease [12].

To the best of our knowledge, the influence of soybeans and its products on GM toxicity have not been investigated. We have therefore, in the present study, examined the possible protective effect of soybean phenolic extract in a rat model of GM-mediated nephropathy, an attempt aimed at improving the therapeutic indices of the aminoglycosides by identifying agents with antioxidant property that could protect the kidney from their undesirable side effects.

MATERIALS AND METHODS

Chemicals

Gentamicin sulphate (Formulations Plc, Surrey, UK), glucose-6-phosphate (G6P), adenosine monophosphate (AMP), adrenaline and 1-chloro-2,4-dinitrobenzene (CDNB) were obtained from Sigma Chemical Co. (St Louis, MO, USA), 5′,5′-dithiobis-2-nitrobenzoic acid, reduced glutathione (GSH), and metaphosphoric acid were purchased from MRS Scientific Ltd (Wickford, UK) γ-glutamyltransferase (γ-GT) and aspartate aminotransferase (AST) kits were obtained from Randox Laboratories Ltd (Crumlin, UK). Other chemicals include hydrogen peroxide (H_2O_2), sodium acetate, magnesium chloride, trichloroacetic acid, ammonium molybdate, ferrous sulfate, potassium dichromate ($K_2Cr_2O_7$), glacial acetic acid, ethylenediaminetetraacetic acid, sodium chloride (NaCl) and were of analytical grade.

Soybean extraction

Soybean seeds, *Glycine max* (family Papilionacaea), were obtained from cultivated plants in Ibadan, Nigeria and were authenticated at the Department of Botany, University of Ibadan Herbarium (UIH), Nigeria. A voucher specimen is deposited in the same department.

The seeds were blended into powder and packed into the soxhlet extractor and defatted with petroleum ether (b.p. 40–60 °C). The defatted soybean was then extracted in absolute methanol and the methanolic extract evaporated in a water bath to obtain the solid extract. The extract was fractionated by column chromatography over silica gel with gradient elusion using ethylacetate : formic acid (85 : 15, v/v). Eluates (10 mL) were collected into eight major fractions and concentrated at room temperature. Separated fractions were subjected to thin layer chromatography [silica gel60, 250 μm F254; Merck, Darmstardt, Germany; mobile phase, ethylacetate : formic acid (85 : 15, v/v)]. Chromatograms were visualized under UV light at 254 nm to detect the presence of polyphenols. Finally, the presence of phenolic compounds with antioxidant property was identified by spraying with DPPH (2,2-diphenyl-picrylhydrazyl) solution (1 mg/mL) which gave distinct yellow color [13]. DPPH assay has been used as an indicator of free radical scavenging activity [14]. DPPH radical is reduced from a stable free radical which is purple in color to diphenylpicryl hydrazine which is yellow. The purified fractions (phenolic extract of soybean; PESB) were pooled together from which a stock solution was prepared and administered to rats.

Determination of total phenolic content of extract

The total phenolic content of the extract of soybean was determined according to the method described by Yen et al. [15]. The result was expressed as (+)-catechin equivalent.

Animals and treatment schedule

Albino rats of the Wistar strain weighing between 110 and 275 g were obtained from the preclinical animal house of the Faculty of Basic Medical Sciences of the University of Ibadan, and fed commercially available standard pelleted feed and water ad libitum. Rats were randomly divided into five groups of five animals each. Group I (control) received normal saline (0.9% NaCl) subcutaneously (s.c). Group II received GM (80 mg/kg/day, s.c). Groups III and IV received 500 and 1000 mg/kg/day PESB orally, respectively, and after 1 h, GM (80 mg/kg, s.c.) was administered. Group V was treated with 1000 mg/kg/day of PESB only. All treatments were given for 12 days. On the 12th day, after treatment, blood samples were collected for determination of plasma concentrations of urea, creatinine, Na^+ and K^+ ions.

Collection of blood samples and preparation of post-mitochondrial fraction (PMF) from kidney samples

Rats were killed by cervical dislocation and dissected. Blood samples were collected by cardiopuncture into heparinized tubes and kidneys immediately removed, rinsed in ice-cold 1.15% KCl, blotted and weighed. The kidneys were then minced with scissors in 3 volumes of ice-cold 100 mM potassium phosphate buffer, pH 7.4 and homogenized in a potter-Elvehjem homogenizer. The homogenates were later centrifuged at 12 500 ***g*** for 15 min at 4 °C and the supernatants, termed the PMF, were aliquoted and used for the enzyme assays.

Biochemical evaluation

Blood urea nitrogen and creatinine were measured using commercial kits available. Sodium and potassium concentrations were determined by a flame photometer. Catalase (CAT) activity was determined according to the method of Clairborne [16]. The assay was based on the ability of CAT to induce the disappearance of H_2O_2, which was followed spectrophotometrically. The level of superoxide dismutase (SOD) activity was determined based on the ability of SOD to inhibit the spontaneous oxidation of adrenaline to adrenochrome as described by Magwere et al. [17]. Glutathione-S-transferase (GST) activity was estimated by the method of Habig et al. [18] using CDNB as substrate. Estimation of GSH level was carried out according to the method described by Beutler et al. [19]. Glucose-6-phosphatase (G6Pase) activity was determined following the method of Swanson [20] and 5^1-nucleotidase (5′-NTD) activity was evaluated by the method of George et al. [21] using AMP as substrate. AST activity was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine [22], while γ-GT activity was estimated following the principle described by Szasz [23].

Histological assessment

Kidneys from rats of all the groups were fixed in 10% formaldehyde, dehydrated in graded alcohol and embedded in paraffin. Fine sections were obtained, mounted on glass slides and counter-stained with hematoxylin and eosin for light microscopic analyses. The slides were coded and were examined by a histopathologist who was blinded to the treatment groups. Renal histological damage (tubular necrosis) was assessed on a score previously described [24] as follows: zero (0) = no cell necrosis; 1 = mild usually single-cell necrosis in sparse

tubules; 2 = moderate, more than one cell involved in sparse tubules; 3 = marked tubules exhibiting total necrosis in almost every power field; 4 = massive total necrosis.

Statistics

All variables were tested for normal distribution using the Kolmogorov–Smirnov test ($P > 0.05$) and for homogeneity of variance among groups using the Levene's test ($P > 0.05$). The groups were compared using one-way ANOVA. If significant differences were found ($P < 0.05$), the treatment groups were compared with the control group using Dunnett's test. All the statistics were carried out in SAS (The SAS System for windows, v8; SAS Institute Inc., Cary, NC, USA).

RESULTS

The effect of GM treatment on renal function test is illustrated in *Figures 1* and *2*. GM produced a significant increase in plasma creatinine ($P < 0.001$) and urea ($P < 0.01$) concentrations and a moderate increase in plasma Na^+ ion ($P < 0.01$) with mild depletion in K^+ ion concentration when compared with the control rats. Pretreatment with PESB (500 mg/kg) significantly improved renal function in the GM-treated rats. The larger dose (1000 mg/kg) of the extract, however, did not seem to offer better protection than the lower dose. The result on total protein is shown in *Figure 2*.

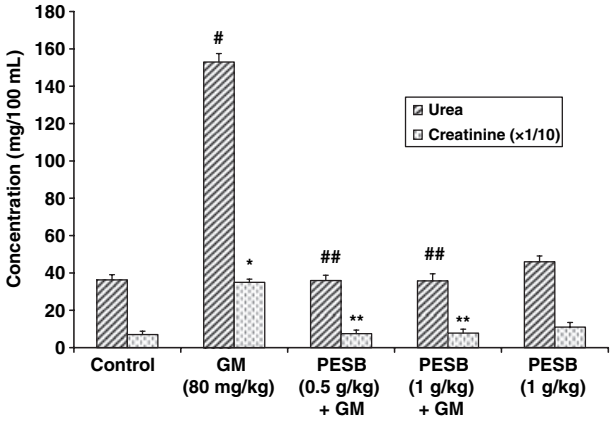

Figure 1 Effect of phenolic extract of soybean (PESB) on plasma creatinine and urea of normal and gentamicin (GM)-treated rats. $^*P < 0.001$ when compared with control; $^{**}P < 0.001$ when compared with gentamicin-only group; $^{\#}P < 0.01$ when compared with control; $^{\#\#}P < 0.01$ when compared with gentamicin-only group.

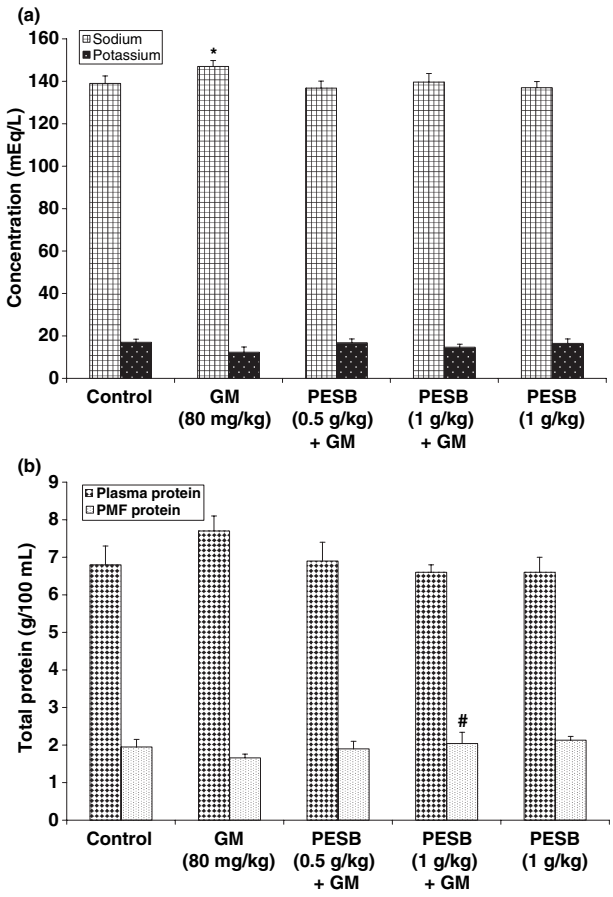

Figure 2 Effect of phenolic extract of soybean (PESB) on (a) plasma sodium and potassium ions concentrations and (b) plasma and kidney post-mitochondrial fraction (PMF) total protein of normal and gentamicin (GM)-treated rats. $^*P < 0.05$ when compared with control; $^{\#}P < 0.05$ when compared with gentamicin-only group.

The result for the total phenolic content of PESB was 50.5 ± 1.2 mg/g extract. *Table I* shows the effect of GM administration on blood and renal GSH levels as well as on GST activity in the kidney. There was a significant depletion in GSH level both in the blood ($P < 0.001$) and kidney ($P < 0.01$) in GM-only-treated rats when compared with control. Pretreatment with 500 mg/kg PESB, however, prevented the GM-induced depletion of both blood and kidney GSH levels ($P < 0.001$ and <0.05, respectively). PESB at a dose of 1000 mg/kg alone produced an insignificant decrease in blood GSH, but significant in the kidney ($P < 0.01$) when compared with control. There was further depletion in GSH level in the GM + PESB (1000 mg/kg) group both in the blood and in the kidney. The activity of GST was significantly decreased ($P < 0.01$) in rat kidney by GM. PESB

Table I Effect of phenolic extract of soybean (PESB) on normal and gentamicin (GM)-induced changes in reduced glutathione (GSH) level and glutathione-S-transferase (GST) activity.

Treatment group	Blood GSH (μg/mg protein)	PMF GSH (μg/g kidney wt)	GST activity (nmol/g kidney wt)
Control (saline)	0.89 ± 0.08	7.9 ± 1.4	2.80 ± 0.18
GM (80 mg/kg)	0.28 ± 0.07*	2.3 ± 0.4*	1.23 ± 0.22*
PESB (500 mg/kg) + GM (80 mg/kg)	0.97 ± 0.07**	3.7 ± 0.9**	1.78 ± 0.15**
PESB (1000 mg/kg) + GM (80 mg/kg)	0.13 ± 0.02**	1.4 ± 0.9**	4.33 ± 0.37**
PESB (1000 mg/kg)	0.67 ± 0.17	2.5 ± 0.4*	3.43 ± 0.45

Values are expressed as mean ± SEM for five rats in each group. PMF = post-mitochondrial fraction.
*$P < 0.001$ when compared with control.
**$P < 0.001$ when compared with gentamicin-only group.

Table II Effect of phenolic extract of soybean (PESB) on normal and gentamicin (GM)-induced changes in superoxide dismutase (SOD) and catalase (CAT) activity in rat.

Treatment group	Blood SOD[a]	PMF SOD[a]	PMF CAT[b]
Control (saline)	13.4 ± 3.3	29.2 ± 7.8	8.7 ± 1.8
GM (80 mg/kg)	6.7 ± 4.1*	20.0 ± 4.8*	4.0 ± 0.5*
PESB (500 mg/kg) + GM (80 mg/kg)	10.4 ± 4.8[#]	27.8 ± 2.6[#]	10.7 ± 1.8[#]
PESB (1000 mg/kg) + GM (80 mg/kg)	13.3 ± 4.2[#]	33.3 ± 6.7**	11.7 ± 3.2**
PESB (1000 mg/kg)	13.4 ± 3.3	23.3 ± 4.1	10.0 ± 1.4

Values are expressed as mean ± SEM for five rats in each group. PMF = post-mitochondrial fraction.
[a]Activity expressed as units of enzyme required to inhibit auto-oxidation of adrenaline to adrenochrome. 1 unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the auto-oxidation of adrenaline to adrenochrome.
[b]Activity expressed as μmol H_2O_2 consumed/min/mg protein.
*Significantly different from control ($P < 0.05$); **Significantly different from GM-treated rats ($P < 0.01$); [#]Significantly different from GM-treated rats ($P < 0.05$).

Table III Effect of phenolic extract of soybean (PESB) on gentamicin (GM)-induced changes in glucose-6-phosphatase (G6Pase), 5^1-nucleotidase (5^1-NTD), γ-glutamyltransferase (γ-GT) and aspartate aminotransferase (AST) activity in rat kidney.

Treatment group	G6Pase (mmol/g protein/min)	5′-NTD (mmol/g protein/min)	γ-GT (units/mL)	AST (units/mL)
Control (saline)	113.23 ± 5.4	150.94 ± 20.6	5.39 ± 0.9	1.45 ± 0.07
GM (80 mg/kg)	101.68 ± 2.2*	146.63 ± 6.3	2.16 ± 0.4*	1.26 ± 0.04*
PESB (500 mg/kg) + GM (80 mg/kg)	112.64 ± 3.1	141.74 ± 8.5	2.89 ± 0.3	1.41 ± 0.05
PESB (1000 mg/kg) + GM (80 mg/kg)	91.98 ± 5.7[#]	132.18 ± 7.1	3.99 ± 0.8[#]	1.43 ± 0.05[#]
PESB (1000 mg/kg)	85.88 ± 2.6*	131.42 ± 6.1	5.36 ± 0.2	1.43 ± 0.04

Values are expressed as mean ± SEM for five rats in each group.
*Significantly different from control ($P < 0.01$); [#]Significantly different from GM-treated rats ($P < 0.01$).

administered before treatment with GM dose dependently increased GST activity in these rats and the effect was significant (see *Table I*).

The changes observed in whole blood SOD as well as renal SOD and CAT activities are recorded in *Table II*. The extract dose dependently prevented GM-induced decrease in SOD (both in the blood and in the kidney) and renal CAT activities of rats. PESB at both doses significantly attenuated the GM-decreased blood SOD and PMF CAT activities.

The activities of G6Pase, 5′-NTD, γ-GT and AST in the GM-treated rats and the influence of PESB is shown in *Table III*. GM treatment caused significant decreases in the activities of G6Pase, γ-GT and AST whereas mild insignificant decrease was observed in 5′-NTD activity when compared with the control. PESB at a dose of

500 mg/kg increased G6Pase activity non-significantly in the GM-treated rats but caused further decrease in the enzyme activity at a dose of 1000 mg/kg ($P < 0.05$). The extract, however, produced an insignificant but dose-dependent decreases in the activity of 5′-NTD in these rats. Pretreatment with PESB protected against GM-induced decreases in renal γ-GT and AST activities and these effects were dose dependent. The protective effect of the extract against the GM-induced decrease in γ-GT and AST was significant at 1000 mg/kg dose when compared with the GM-only group ($P < 0.01$).

Histological sections from rats treated with GM show kidneys with massive tubular necrosis and tubules showing evidence of thyroidization. Kidney sections from rats pretreated with the 500 and 1000 mg/kg doses of PESB before GM administration show mild to moderate and moderate tubular necrosis, respectively. The 500 mg/kg dose of the extract appears to show better histological protection against the GM-induced renal damage. The glomeruli are spared and the interstitium free from infiltration by chronic inflammatory cells in all the groups (*Figure 3*).

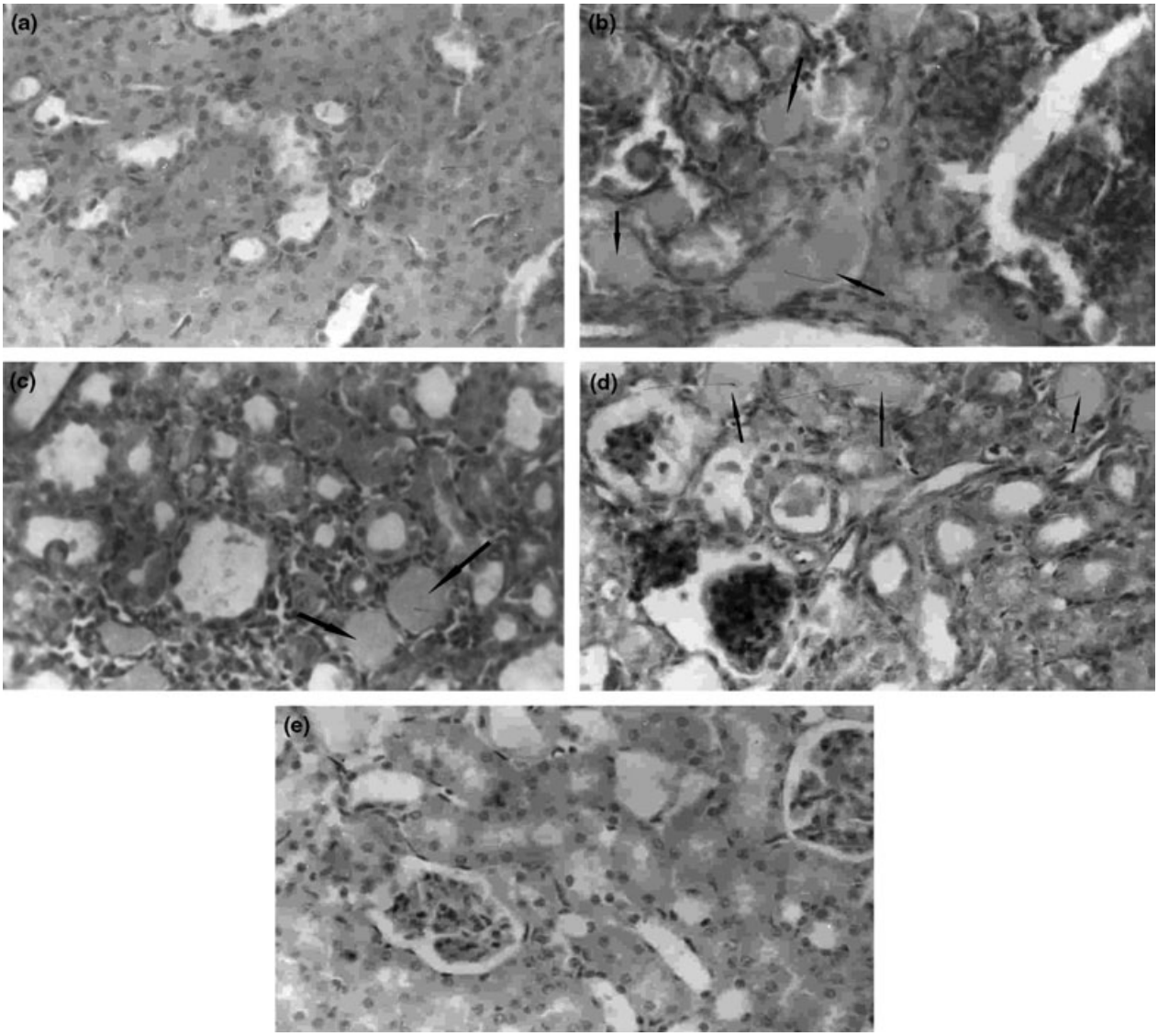

Figure 3 Photomicrograph of rat kidney section (×400). (a) Control (normal saline) rat kidney section. (b) Gentamicin-treated (80 mg/kg) group showing severe tubular necrosis. (c) Gentamicin (80 mg/kg) plus phenolic extract of soybean (PESB) (500 mg/kg)-treated group with tubules showing mild to moderate necrosis. (d) Gentamicin (80 mg/kg) plus PESB (1000 mg/kg)-treated group with moderate tubular necrosis. (e) PESB (1000 mg/kg)-treated group. Arrows indicate some of the tubules showing necrosis.

DISCUSSION

The present study evaluated the effect of defatted PESB on GM-induced nephrotoxicity. The results from this study show that rats treated with GM developed marked acute renal failure as evident by the significant elevation in plasma creatinine and urea (indicators of impaired glomerular function). This was also associated with significant hypernatraemia and mild K^+ ion depletion. Renal histology also revealed massive tubular necrosis with deposition of colloid casts within the tubular lumina. Pretreatment with 500 mg/kg/day of defatted PESB significantly improved renal function and histology. A higher dose of the extract (1000 mg/kg/day), however, did not offer greater protection.

The relationship between GM-induced alteration of antioxidant defense system and nephrotoxicity was further established in this present study. GM nephropathy was associated with decreased activity of SOD and CAT in the kidney, as already observed in several studies. SOD and CAT enzymes are major primary antioxidant defense components that primarily catalyze the dismutation of superoxide radical (O_2^-) to H_2O_2 and decomposition of H_2O_2 to H_2O, respectively [25,26]. The decreased SOD and CAT activity induced by GM probably results in accumulation of O_2^- and H_2O_2 which react with metal ions to promote additional radical generation, with the release of the particularly reactive hydroxyl radicals [27]. Hydroxyl radicals react at nearly diffusion-limited rates with any component of the cell, including lipids, DNA and proteins. The net result of this non-specific free radical attack is a loss of cell integrity, enzyme function, and genomic stability [28]. The involvement of these reactive oxygen species (ROS) in the impairment of GFR has been reported [29]. Moreover, the elevation in glomerular antioxidant enzymes protects renal function against the injury induced by ROS [30]. The protective effect of PESB therefore could be attributed to its antioxidant properties. The increase in the activity of SOD and CAT produced by the extract in the GM-treated rats may prevent exaggerated production of ROS and oxidative damage. In addition, the amount of renal nitric oxide, which plays an important role in the regulation of GFR [31], may be decreased as a consequence of its reaction with the excessive amount of O_2^- because of the low activity of SOD [32]. It is therefore reasonable to suggest that PESB may also be preserving renal nitric oxide levels by increasing SOD activity, thus preventing O_2^- accumulation and reducing the production of the toxic reaction product, peroxynitrite anion ($ONOO^-$) arising from the reaction between $NO^\bullet$ and O_2^-.

The results of this study further reveal that GM nephrotoxicity is associated with decrease in renal GST activity and excessive GSH utilization resulting in depletion of cellular GSH level. As GSH is a hydroxyl radical scavenger, our result correlates with other findings that hydroxyl radical may play a role in GM nephrotoxicity [33]. Pretreatment with PESB (500 mg/kg) reduced the GM-induced renal depletion of GSH and decrease in GST activity.

However, treatment with the extract alone (1000 mg/kg) produced a moderate increase in GST activity, and a reduction of both blood and renal GSH. The apparent decrease in both renal and blood GSH levels with concomitant increase in GST activity following treatment with PESB at 1000 mg/kg dose in the GM-treated rats may be associated with increased utilization of GSH both as a hydroxyl radical scavenger and as a substrate for GST-catalyzed reactions. This process usually facilitates detoxification and excretion but may also be involved in the biosynthesis of certain compounds such as leukotriene C_4 [34], and prostaglandins [35] which can be transformed to the dehydration prostaglandin products [36] which are potentially toxic [37]. Furthermore, many GSH conjugates undergo further enzymatic modification by hydrolysis of the glutathione-S-conjugate at the γ-glutamyl bond. This reaction is catalyzed by the enzyme γ-GT. In addition to hydrolysis, γ-GT can catalyze transpeptidation or auto-transpeptidation. γ-GT is an enzyme localized in the cell membrane of many cell types including kidney tubules. The kidney, in fact, has the highest activity in several mammals studied, including humans [38]. The decreased activity of this enzyme due to GM treatment as observed in this study may also play a role in GM nephrotoxicity by impairing further enzymatic modification of cytotoxic GSH conjugates. The activity of AST, which is also located in the proximal tubule [39], was decreased. In this study, the GM-depressed activities of γ-GT and AST and were preserved in rats treated with PESB. 5′-NTD and G6Pase (suitable marker enzymes for plasma membranes) were similarly decreased in the GM-treated rats. The inactivation of these microsomal marker enzymes reveals microsomal damage from GM-induced oxidative stress, causing disruption in membrane function and its eventual collapse. All these may contribute to the proximal tubular damage during GM treatment as also observed in several studies. The PESB in this study increased G6Pase activity, though not significant, at a

dose of 500 mg/kg/day but caused a significant decrease in the activity of the enzyme at 1000 mg/kg/day in the GM-treated rats. The activity of 5′-NTD, however, was decreased dose dependently, though not significant in these rats. Treatment with the extract alone (1000 mg/kg/day dose) decreased the activities of these enzymes, the effect being stronger on G6Pase. Although, PESB at high dose preserved the activities of antioxidant enzymes and renal function, but its ability at this dose to decrease the activities of 5′NTD and G6Pase when administered alone and also to decrease GSH level when administered together with GM suggest that PESB in large doses may probably produce pro-oxidant effect. The reason for this observation is not completely understood presently. Long-term studies involving high doses of the extract are needed to evaluate the chemoprotective potential of soybeans.

In conclusion, it seems possible therefore that the defatted PESB at a relatively low and effective dose has a specific stabilizing effect on the cell membrane and/or other vital cellular macromolecules and can thus protect against GM-induced microsomal damage or tubular necrosis. The protective effects of the extracts appear to be related to the antioxidant polyphenolic content present in soybean.

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