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Effect of Vitellaria paradoxa (Shea butter) rich diet on gentamicin induced nephrotoxicity in white Wistar rats

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Abstract

Introduction and Objective. Nephrotoxicity means failure of the excretory function of the kidney due to toxic effects of medication or chemicals. As available drugs are associated with some negative side-effects, it is expedient to look for natural remedies with fewer or no side-effects. The aim of this study is to investigate the effect of Shea butter rich diet on gentamicin induced nephrotoxicity in rats.

Materials and method. Phytochemical analysis was carried out for various phytoconstituents on the Shea butter used for the study. Thirty (30) male white Wistar rats were divided into six groups with five rats in each group. Nephrotoxicity was induced by intraperitoneal administration of 80mg/kg body weight gentamicin for 7 days. Group 1 received only standard rat chow and water. Group 2 received 80mg/kg body weight gentamicin for 7 days, followed by standard rat chow and water only for 14 days. Group 3 received 80mg/kg body weight gentamicin for 7 day, followed by 20mg/kg body weight furosemide for 14 days. Groups 4, 5 and 6 received 80mg/kg body weight gentamicin for 7 days, followed by varied concentrations of 10, 20 and 30% Shea butter rich diet and water for 14 days. After the last dose, blood samples were collected and analyzed for serum urea, creatinine and electrolytes.

Results. Phytochemical screening revealed the presence of saponin, phenol, tannin, alkaloid, terpenoid, flavonoid, and reducing sugar. Induction of nephrotoxicity was marked by elevated levels of serum urea, creatinine and electrolytes (sodium, potassium, chloride and bicarbonate ions). Administration of the Shea butter rich diet, however, significantly decreased their levels in a dose dependent manner.

Conclusions. Results of the study showed that Shea butter rich diet was effective in ameliorating gentamicin-induced nephrotoxicity in rats.

Key words

creatinine, electrolytes, gentamicin, nephrotoxicity, serum, Shea butter, urea

INTRODUCTION

The term renal failure means the failure of the excretory function of the kidney, resulting in retention of nitrogenous waste products of metabolism in the blood. Additionally, there is failure in the regulation of fluid and electrolyte balance alongside endocrine dysfunction. The renal function is mostly grouped into acute and chronic renal failure. Acute kidney injury is a rapidly progressive loss of renal function generally characterized by oliguria (decreased urine production), as well as fluid and electrolyte imbalance [1].

Kidney disease is increasingly recognized as a public health problem globally. The declaration of World Kidney Day to be commemorated yearly beginning in March 2006 sends a clear and concise message to the public, government health officials, physicians, allied health professionals, patients and families, that Chronic Kidney Disease (CKD) is common, harmful, and treatable [2].

Over 850 million people globally have some form of kidney disease, which is roughly twice the number of people living with diabetes (422 million), as well as 20 times more than the global prevalence of cancer (42 million) or people living with HIV/AIDS (36.7 million) [3]. The global prevalence of chronic kidney disease (CKD) is 10.4% and 11.8% among men and women, respectively. Acute Kidney Disease (AKI), experienced by approximately 13.3 million people annually, may result or lead to CKD or kidney failure in the future. The number of people who require dialysis or kidney transplant ranges from 5.3 – 10.5 million annually, although many do not receive these treatments due to lack of resources or finances [3].

Gentamicin is an antibiotic drug used to treat a host of bacterial infections [4]. The drug works by stopping bacteria from making protein and subsequently, killing it. It irreversibly binds the 30S subunit of the bacterial ribosome, thus interrupting protein synthesis. While specific steps in the protein synthesis affected may vary between specific aminoglycoside agents, as it is with their affinity and degree of binding, aminoglycoside's presence in the cytosol generally perturbs peptide elongation at the 30S ribosomal subunit, leading to inaccurate mRNA translation and so biosynthesis of proteins that are truncated or that bear altered amino acid

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compositions at points? [4]. Specifically, binding impairs translational proofreading, resulting in misreading of the RNA message, premature termination, or both, and therefore leading to a truncated protein product. However, the use of gentamicin is associated with kidney diseases and other more severe reactions, such as low blood count, ear disorders, neuromuscular and nerve damages that limit its frequent use [5]. Gentamicin-induced nephrotoxicity involves renal oxidative stress, which is accompanied by reduction in the renal antioxidant defence mechanisms. In addition, glomerular damage, induction of acute tubular necrosis, and renal inflammation are the major events implicated in gentamicin nephrotoxicity [6]. Gentamicin induces lysosomal phospholipidosis that disrupts normal renal function [7]. It is evident that the renal accumulation of gentamicin is implicated in the induction of nephrotoxicity [8].

Fats and oils are increasingly becoming important in nutrition and commerce because they are sources of dietary energy, antioxidants, biofuels and raw materials for the manufacture of many industrial products. They are useful in cosmetics, food, pharmaceuticals and chemical industries [9]. With increasing awareness on the importance of vegetable oils in the food, cosmetic and pharmaceutical industries, there is need to search for indigenous plant species that can provide such oils and characterize them.

Shea butter is an off-white or creamy-coloured fat extracted from the nut of African Shea tree (*Vitellaria paradoxa,* formerly *Butryspermum paradoxum, B.parkii* and *B. paradoxa*) [10]. Shea tree grows naturally in the wild of the dry Savannah belt of West Africa, from Senegal in the West to Sudan in the East, and onto the foothills of the Ethiopian mountains [11]. The West African tree is classified as the subspecie *'paradoxa'* and the East African one as *'nilotica'* [12]. A Shea butter-rich diet was used in this study to investigate its effect on gentamicin-induced nephrotoxicity in rats.

MATERIALS AND METHOD

Drugs, reagents and chemicals. Gentamicin, furosemide (Muri Pharmacy and Laboratories, Nigeria), working standards of potassium, sodium, bicarbonate, chloride ions, creatinine and urea (Randox reagents) were used for the study. All chemicals were of analytical grade

Plant material and experimental animals. The fruit plant of *Vitellaria paradoxa* (Shea butter) was obtained from farms around Mbamba, Yola Adamawa State, Nigeria. The fruit plant was authenticated in the plant science department of the Modibbo Adama University, Yola.

Thirty male white Wistar rats (weighing between 100 ± 20g/body weight) were used for the study, procured from the National Veterinary Research Institute, Vom, Jos Plateau State, Nigeria. The rats were kept in cages barred with steel nets and allowed to acclimatize. They were constantly supplied with feed (Vital feed, Jos) and clean water *ad libitum* for 7 days.

Preparation of oil extract and formulation of Shea butter rich diet. Oil from the fruit plant of *Vitellaria paradoxa* was extracted locally using the method described by Aculyet al. [13]. The fruits were first depulped and the nuts processed into kernels by drying and pounding. The dried kernels were reduced into smaller particles by using a pestle and mortar. The reduced kernels were continuously kneaded in a big container and hot water was added to help separate out the butter oil from the paste. As the butter oil floats to the top of the container, the oil which was in a curd state was removed. The butter oil curd was then melted in an open pot over a slow fire. A period of slow boiling removed all the remaining water in the oil by evaporation. The Shea butter oil which appeared creamy at this point was ladled from the top of the pot into a clean container and kept in a cool place to harden.

Shea butter oil of different concentrations (10, 20 and 30%) was incorporated into the animal feed and mixed thoroughly. The incorporation of the oil into the feed was based on the daily feed intake of the animals (percentage of feed was substituted with that of the Shea butter oil). The formulated Shea butter diet of different concentrations (10, 20 and 30%) were used as feed for animals induced with nephrotoxicity.

Phytochemical Screening of Shea butter

Test for Alkalo. Mayer's reagent test for alkaloid was used, 1ml of sample (Shea butter) containing a few drops of 1% HCl in a test tube was stirred on a steam bath and the solution obtained filtered; the filtrate was then treated with one drop of Mayer's reagent. Turbidity of the filtrate on addition of Mayer's reagent confirmed the presence of alkaloid [14].

Test for saponins. Two (2) ml of sample was measured into a test tube and shaken vigorously. The test was positive when the characteristic honeycomb froth persisted for at least 30 minutes [15].

Test for reducing sugars. Exactly 0.5ml each of Fehling's solution A and B was measured into a test tube. 0.5 ml of the sample was added to the solution and heated in a water bath. A brick-red precipitate denoted the presence of reducing sugars [16].

Test for flavonoids. The alkaline reagent test was used. Three (3) drops of dilute NaOH was added to 1 ml of the sample (Shea butter) which produced an intense yellow colour. On addition of a few drops of dilute HCl, the sample became colourless, indicating the presence of flavonoids [16].

Test for tannins. Exactly 0.5 ml of sample was heated in a steam bath for 5 minutes. Two (2) drops of 5% $FeCl_3$ was added. A greenish precipitate indicated the presence of tannins [17].

Test for terpenoids. Five (5) ml of sample was added to 2 ml of chloroform and 3 ml of conc. H_2SO_4 (boiled in water bath). The formation of grey coloured solution indicated the presence of terpenoid [18].

Nephrotoxicity study. Nephrotoxicity was induced in rats by administering high doses of gentamicin (80mg/kg/body weight/day) for 7days intraperitoneally.

Group-1: Sham – given standard diet and water only.
Group-2: Negative – given 80mg/kg/body weight/day gentamicin for 7 days + Normal diet and water.
Group-3: Positive – given 80mg/kg/body weight/day gentamicin for 7 days + 20mg/kg/body weight/day furosemide for 7 days.

Group-4: 10% Shea butter diet group – given 80mg/kg body weight/day gentamicin for 7 days + 10% Shea butter rich diet and water for 7 days.
Group-5: 20% Shea butter diet group – given 80mg/kg/ body weight/day gentamicin for 7 days + 20% Shea butter rich diet and water for 7 days.
Group-6: 30% Shea butter diet group – given 80mg/kg body weight/day gentamicin for 7 days + 20% Shea butter rich diet and water for 7 days.

After the last dose, the rats were fasted overnight. They were sacrificed using urethane as anaesthesia and blood samples were collected by cardiac puncture into sample bottles. Serum was obtained from the blood with the aid of a bench top centrifuge and was used for the estimation of biochemical parameters. Serum creatinine concentration was estimated using Jaffe's kinetic method while serum urea was estimated by the diacetyl monoxime DAM method [19]. The level of serum electrolytes was determined by the ion selective electrode principle of COBAS 6000 (c501) [20].

Statistical analysis. One-way Analysis of Variance (ANOVA) was used. The grouped data was expressed as mean ± standard error of mean (SME); the statistical significance of the difference was evaluated using Statistical Package for Social Sciences (SPSS) Version 24.

RESULT

Phytochemical analysis. Table 1 shows the result of the phytochemical analysis performed on the Shea butter used for the study. The phytochemical analysis revealed the presence of alkaloid, saponin, reducing sugar, flavonoid, tannin, alkaloid, terpenoid and phenol.

Effect of Shea butter rich diet on serum urea and creatinine. Table 2 shows the effect of gentamicin on kidney parameters (urea and creatinine). There was a significant increase in the levels of serum urea (15.90 ± 0.17) and creatinine (129.23 ± 0.46) in the negative control group, compared to the sham group (5.23 ± 0.12 urea; 90.03 ± 0.15 creatinine) after 7 days administration of gentamicin. Administration of Shea butter rich diet was marked, however, by significant decrease ($p< 0.05$) in the levels of serum urea and creatinine across all Shea butter diet treated groups, (11.37 ± 0.20 urea and 115.13 ± 0.72 creatinine in the 10% Shea butter diet treated group), (9.01 ± 0.69 and 103.53 ± 0.33 in the 20% Shea butter rich diet treated group) and (5.23 ± 0.19 and 95.17 ± 0.18 in the 30% Shea butter diet treated group) in a dose dependent manner. The 30% Shea butter rich diet treated group almost normalized the levels of serum urea and creatinine, as seen in the Table, in comparison to the Furosemide (standard drug) group (5.32 ± 0.19 urea and 94.17 ± 0.17 creatine). The result revealed that the Shea butter rich diet had dose dependent activity.

Effect of Shea butter rich diet on serum electrolytes. Table 3 shows the effect of gentamicin on serum electrolytes (Na^+, K^+, Cl^- and HCO_3). There was electrolyte imbalance as the levels of Na^+ (153.67 ± 1.45), K^+ (15.07 ± 0.18), Cl^- (115.07 ± 0.15) and HCO_3 (40.05 ± 0.13) were all significantly increased in group II (negative control) animals, compared to those in group I (Sham) – Na^+ (133.00 ± 1.15), K^+ (4.20 ± 0.15), Cl^- (100.33 ± 0.88) and HCO_3^- (20.20 ± 0.15) after administration of gentamicin. However, the levels of serum Na^+, K^+, Cl^- and HCO_3^- were significantly decreased ($p< 0.05$) in a dose dependent manner following treatment with Shea butter rich diet, (146.77±0.91 Na^+, 11.03±0.15 K^+, 113.00±1.15 Cl^- and 34.83±0.12 HCO_3^- for 10% Shea butter group), (141.67±0.91 Na^+, 9.03±0.09 K^+, 109.33±0.68 Cl^- and 29.10±0.12 HCO_3^- for 20% Shea butter group) and (137.83±0.17 Na^+, 6.80±0.15 K^+, 104.77±0.19 Cl^- and 24.40±0.26 HCO_3^- for 30% for Shea butter group). The result revealed that the Shea butter rich diet had dose dependent activity.

Table 1. Phytochemical constituents of Shea butter (*Vitellaria paradoxa*) oil

Phytochemicals	Result
Alkaloid	+
Saponin	+
Reducing sugar	+
Flavonoid	+
Tannin	+
Terpenoid	+
Phenol	+

+ = present

Table 2. Effect of Shea butter rich diet on serum urea (mmol/L) and creatinine (µmol/L)

GROUP	UREA	CREATININE
I (NORMAL CONTROL)	5.23 ± 0.12^{BC}	90.03 ± 0.15^{BC}
II (NEGATIVE CONTROL)	15.90 ± 0.17^{A}	129.23 ± 0.46^{A}
III (FUROSEMIDE)	5.32 ± 0.19^{BC}	94.17 ± 0.17^{BC}
IV (10% SHEA BUTTER DIET)	11.37 ± 0.20^{AB}	115.13 ± 0.72^{AB}
V (20% SHEA BUTTER DIET)	9.01 ± 0.69^{B}	103.53 ± 0.33^{AB}
VI (30% Shea butter diet)	5.85 ± 0.19^{bc}	95.17 ± 0.18^{bc}

Values are Mean ± SEM (n = 5); [a] Significantly ($p> 0.05$) increased compared to normal; [b] Significantly ($p< 0.05$) decreased compared to negative control; [c] Significantly ($p< 0.05$) decreased compared to groups IV and V.

Table 3. Effect of Shea butter rich diet on serum electrolytes in mmol/l.

Group	Sodium (Na^+)	Potassium (K^+)	Chloride (Cl^-)	Bicarbonate (HCO_3^-)
I (Normal control)	133.00 ± 1.15^{b}	4.20 ± 0.15^{b}	100.33 ± 0.88^{b}	20.20 ± 0.15^{b}
II (Negative control)	153.67 ± 1.45^{a}	15.07 ± 0.18^{a}	115.07 ± 0.15^{a}	40.05 ± 0.13^{a}
III (Furosemide, Standard drug)	132.00 ± 1.15^{bc}	4.17 ± 0.12^{b}	100.67 ± 0.67^{b}	22.77 ± 0.18^{bc}
IV (10% Shea butter diet)	146.77 ± 0.91^{ab}	11.03 ± 0.15^{ab}	113.00 ± 1.15^{a}	34.83 ± 0.12^{a}
V (20% Shea butter diet)	141.67 ± 0.45^{ab}	9.03 ± 0.09^{b}	109.33 ± 0.68^{ab}	29.10 ± 0.12^{b}
VI (30% Shea butter diet)	137.83 ± 0.17^{b}	6.80 ± 0.15^{b}	104.77 ± 0.19^{b}	24.40 ± 0.26^{b}

Values are mean ± SEM (n = 5); [a] significantly increased compared to normal control; [b] significantly ($p< 0.05$) decreased compared to negative control; [c] significantly ($p<$

0.05) decreased compared to Shea butter diet treatment groups (10, 20 and 30%)

DISCUSSION

Phytochemicals are produced by plants as defence mechanisms against pathogens and are the basic source for the establishment of several pharmaceutical industries [21]. Saponins are known to possess anti-carcinogenic and other pharmacological properties, such as cardioprotective, antiviral and anti-inflammatory [22]. Saponins also play a vital role in the treatment of malaria [23]. Marina et al., [24]. found tannin to be effective in the treatment of acute diarrhea in children. The treatment of sore throat, haemorrhage and wound healing has also been linked to tannins [25]. Alkaloids have been reported to have higher anticancer activities [26], and are also known to have anti-microbial effects [27]. Asif et al., indicated in a study that alkaloids are also an anti-hypertensive agent [28], terpenoids and triterpenoids have demonstrated antibacterial activities [29], and flavonoids and phenols are known to act as antioxidants and play major roles in ameliorating damages caused by oxidative stress/free radicals [30]. The presence of reducing sugars confirmed the presence of carbohydrate. Sugars are mostly monosaccharide and disaccharides, reducing sugars in particular play an important role in energy generation.

The exact mechanism by which gentamicin induces nephrotoxicity is unknown. Tubular cytotoxicity is the result of many interconnected actions, triggered by drug accumulation in epithelial tubular cells [31]. Results of the study corroborate the findings of Quiros et al., in which a significant nephrotoxic effect of gentamicin, administered at a dose of 80 mg/kg body weight, was observed in other animal species [31]. The amelioration of gentamicin induced nephrotoxicity by Shea butter rich diet, as seen in this, study was dose dependent. The low content of urea and creatinine observed could be attributed to the high saponin content of Shea butter. Saponins, apart from lowering serum cholesterol, also reduces proteinuria (high protein in urine) by forming sparingly digestible saponin-protein complexes in the intestine [32]. Given that urea is a nitrogenous byproduct of protein and amino acid catabolism, the decrease in the levels of serum urea was attributed to the high content of saponin in the Shea butter diet.

The nephrotoxicity of aminoglycoside antibiotics, and especially that of the most used compound, gentamicin, is well documented [33]. Oxygen-free radicals are important mediators of gentamicin-induced acute renal failure [33]. Shea butter has high antioxidant activity and contains flavonoid and phenol which, apart from protection against cancer, have been found to prevent damage to cells caused by free radicals through their free radical scavenging activities [34]. As gentamicin nephrotoxicity involves the generation of free radicals [35], the nephrotoxic ameliorative effect of Shea butter could be attributed to the antioxidant compounds it contains.

CONCLUSION

The result obtained in this study show that a Shea butter rich diet ameliorated the nephrotoxic effect of gentamicin. The amelioration was dose dependent (Tab. 2 and 3). The maximal effect was achieved at incorporating 30% concentration of the Shea butter oil into the animal feed which almost normalized the biochemical parameters that were assayed in the experimental animals. Dietary modification with Shea butter can therefore prevent, or at least, ameliorate drug induced nephrotoxicity (specifically gentamicin).

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