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The protective impact of glutamine on anti-tuberculosis drug-induced nephrotoxicity in Wistar rats

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Abstract

This study assessed the protective effect of glutamine (GTN) against rifampicin/isoniazid/pyrazinamide/ethambutol (RIPE)-induced nephrotoxicity in rats. Thirty adult Wistar rats (200±20 g) of both sexes were grouped into 6 of 5 rats/group. The rats were treated daily for 30 days as follows: Group 1 (Vehicle control [normal saline 0.2mL]), group 2 (GTN 200 mg/kg), group 3 (RIPE 150, 75, 400 and 275 mg/kg in vehicle), group 4 (GTN 50 mg/kg +RIPE), group 5 (GTN 100 mg/kg +RIPE) and group 6 (GTN 200 mg/kg +RIPE). After treatment, blood samples were obtained and assessed for serum renal biomarkers. Kidneys were harvested, weighed and assessed for oxidative stress markers and histology. RIPE significantly ($p<0.01$) decreased body weight and significantly ($p<0.01$) increased kidney weight when compared to the control. Serum urea, creatinine, uric acid levels and kidney malondialdehyde levels were significantly ($p<0.001$) increased in RIPE-treated rats when compared to the control. Serum total protein, albumin, kidney glutathione, catalase, superoxide dismutase and glutathione peroxidase levels were significantly decreased ($p<0.001$) in RIPE-treated rats when compared to the control. RIPE caused tubular necrosis and collapsed glomeruli in the kidneys of rats. However, body and liver weights were significantly restored in GTN 100 mg/kg +RIPE and GTN 200 mg/kg +RIPE-treated rats at $p<0.05$ and $p<0.01$, respectively when compared to RIPE. Serum and kidney oxidative stress markers were restored in GTN 50 mg/kg +RIPE, GTN 100 mg/kg +RIPE and GTN 200 mg/kg +RIPE-treated rats at $p<0.05$, $p<0.01$ and $p<0.001$ respectively, when compared to RIPE. GTN restored kidney histology. GTN protects against RIPE-induced nephrotoxicity in a dose-related fashion.

Keywords: anti-tuberculosis drug, kidney, toxicity, glutamine, rat

1. Introduction

The kidneys, which receive 20 - 25% of cardiac output are the primary organs for maintaining homoeostasis of extracellular fluid volumes. The kidneys perform two major functions; excretion of the end-products of metabolism and the control of the concentration of body fluids. The kidneys are also involved in drugs and metabolites excretion through glomerular filtration and tubular secretion (1). The regulatory effect of the kidneys on drugs and their metabolites predisposes them to nephrotoxicity. Drug-induced nephrotoxicity is recognized as a contributor to acute and chronic kidney diseases. Nephrotoxicity reflects damage to different sections of the nephron, which may results to renal failure (2).

Rifampicin/isoniazid/pyrazinamide/ethambutol (RIPE) is a frequently used anti-tuberculosis regimen. It is used for treating tuberculosis for two months followed by isoniazid/rifampicin for eight months (3). One of the primary complications of using RIPE is the development of nephrotoxicity, which may be acute or chronic (4-6). Rifampin has the highest propensity for nephrotoxicity, which can be augmented by partner anti-tuberculosis drugs (7). In animal studies, RIPE has been associated with impaired renal biomarkers, kidney inflammatory reactions and oxidative stress marked by lipid peroxidation (3,8). RIPE may cause kidney morphological changes such as tubular necrosis (9,10).

Glutamine (GTN) is one of the most abundant amino acid in human body. It is involved in the regulation of protein synthesis, acid–base balance, immune function and adaptation to stress (11,12). It is as essential chemical substance for glutathione, which is an important antioxidant in the body (13). Glutathione is a potent ubiquitous antioxidant that is important for many drugs and endogenous substance metabolisms (14, 15). GTN has potent antiulcer, antioxidant, antibacterial, cardioprotective, anticancer, and anti-apoptotic potential (16). Moreover, its nephroprotective effect has been reported in animal studies (17). It decreased renal cell apoptosis and inflammatory markers in cisplatin-induced nephrotoxicity in rats (18). It stabilized antioxidant status and reduced renal lipid peroxidation in cisplatin- treated rats (19). GTN decreased tubular necrosis, stabilized renal biochemical markers and decreased oxidative stress in gentamycin –treated rats (20). The current study evaluated the protective effect of GTN on RIPE-induced nephrotoxicity in Wistar rats.

2. Materials and Methods

2.1. Drugs/chemicals and animals

Glutamine (GTN) (Qualikems Fine Chemical Private Limited

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Nandesari, Vadodara, Gujarat, India) and Rifampicin, isoniazid/ pyrazinamide/ethambutol (RIPE) (Lupin Limited Chikalthana, Aurangabad India) were used. Approval was obtained from the Research Ethics Committee (NDU/PHARM/PCO/AEC/068) of the Department of Pharmacology/Toxicology, Faculty of Pharmacy, Niger Delta University, Nigeria. Thirty Wistar rats of both sexes (200±20g) were used. The rats were supplied by the animal handling unit of the Department of Pharmacology/Toxicology, Faculty of Pharmacy, Niger Delta University, Nigeria. The rats were randomized into six groups of 5 rats /group and were housed under standard laboratory conditions (12 h light: 12 h dark cycle at 25±2°C). The rats were orally treated daily for 30 days as follows: Group 1 (Vehicle control [normal saline 0.2mL]), group 2 (GTN 200 mg/kg) (20), group 3 (RIPE 150, 75, 400 and 275 mg/kg in vehicle) (3), group 4 (GTN 50 mg/kg +RIPE), group 5 (GTN 100 mg/kg +RIPE) and group 6 (GTN 200 mg/kg +RIPE). The rats were weighed and exposed to anesthesia at the end of treatment. Blood samples were collected from the heart and analyzed for serum renal biomarkers. The rats were dissected and kidneys were harvested, weighed and assayed for oxidative stress markers and histology. The kidneys assayed for oxidative stress markers were rinsed in cold saline and homogenized in chilled phosphate buffer (0.1 M, pH 7.4) containing KCl (1.17%). The homogenates were centrifuged (15000 rpm for 30 min at 4 ^{O}C) and the supernatants were collected and assayed for oxidative stress markers.

2.2. Evaluation of renal biomarkers

Serum creatinine, urea, uric acid, total protein and albumin concentrations were measured using an auto analyzer.

2.3. Oxidative stress marker assay

Malondialdehyde (MDA) was estimated using an established method described by Buege and Aust (21). Super oxide dismutase (SOD) was assessed using the procedure described by Sun and Zigman (22). Glutathione (GSH) was assayed according to the method reported by Sedlak and Lindsay (23). Glutathione peroxidase (GPx) was measured using the procedure described by Rotruck *et al*. (24). The method reported by Aebi (25) was used for the estimation of catalase (CAT).

2.4. Histology of the kidney

Kidney samples were cleaned and stored in a solution of formalin saline (10%) for 24hr. Thereafter, the kidney samples were dehydrated in graded concentration of alcohol solutions, processed and embedded in paraffin wax. Kidney tissues were sectioned (3µm thickness) and stained with Haematoxylin and Eosin on slides. The slides were examined using a light microscope.

3. Results

3.1. Effects of glutamine on body and kidney weights of antituberculosis drug-treated rats

GTN had no effects (p>0.05) on the body and kidney weights when compared to the control (Table 1). On the other hand, RIPE significantly (p<0.01) decreased body weight and significantly (p<0.01) increased kidney weight when compared to the control (Table 1). However, treatment with GTN 100 mg/kg +RIPE, and GTN 200 mg/kg +RIPE significantly restored the body and kidney weights at p<0.05 and p<0.01, respectively when compared to RIPE (Table 1).

Table 1. Effects of glutamine on body and kidney weights of anti-tuberculosis drug-treated rats

Treatment (mg/kg)	FBW(g)	AKW(g)	RKW (%)
Control	210.8±16.7	0.75±0.07	0.36±0.07
GTN 200	220.6±14.9	0.73±0.03	0.33±0.05
RIPE	120.7± 16.6#	1.88±0.04#	1.56±0.08#
GTN 50 + RIPE	140.1±15.5	1.70±0.01	1.21±0.06
GTN 100+RIPE	175.0±15.2*	1.10±0.06*	0.63±0.04*
GTN 200+RIPE	200.2±17.5**	0.78±0.09**	0.39±0.02**

Data as mean ± SEM (Standard error of mean), n=5, GTN: Glutamine, RIPE: Rifampicin/isoniazid/pyrazinamide/ethambutol, FBW: Final body weight, AKW: Absolute kidney weight, RKW: Relative kidney weight, # p<0.01 differ significantly when compared to the control, *p<0.05 and **p<0.01 differ significantly when compared to RIPE

3.2. Effects of glutamine on renal biomarkers of antituberculosis drug-treated rats

GTN had no significant (p>0.05) effects on serum renal biomarkers when compared to the control. In contrast, RIPE significantly (p<0.001) increased serum creatinine, urea and uric acid levels when compared to the control (Fig. 1a-1c). On the other hand, treatment with GTN 50 mg/kg +RIPE, GTN 100 mg/kg +RIPE and GTN 200 mg/kg +RIPE significantly decreased serum creatinine, urea and uric acid levels at p<0.05, p<0.01 and p<0.001, respectively when compared to RIPE (Fig. 1a-1c). RIPE significantly (p<0.001) decreased serum total protein and albumin levels when compared to the control (Fig. 1d and 1e). However, treatment with GTN 50 mg/kg +RIPE, GTN 100 mg/kg +RIPE and GTN 200 mg/kg +RIPE significantly increased serum total protein and albumin levels at p<0.05 and p<0.01 and p<0.001, respectively when compared to RIPE (Fig. 1d and 1e).

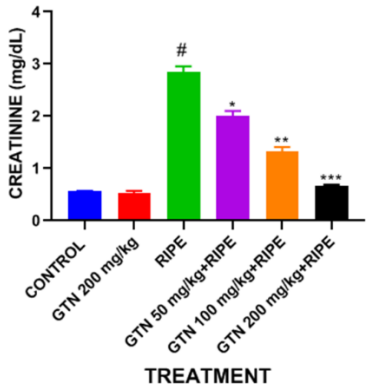

Fig. 1a. Effect of glutamine on serum creatinine of anti-tuberculosis drug-treated rats. Data as mean ± SEM (Standard error of mean),n=5, GTN:Glutamine,RIPE:Rifampicin/isoniazid/pyrazinamide/ethambutol, #p<0.001 differ significantly when compared to the control, *p<0.05, **p<0.01, and ***p<0.001 differ significantly when compared to RIPE

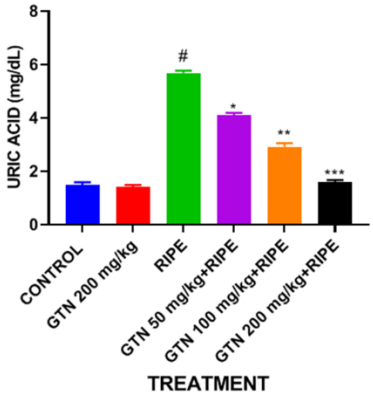

Fig. 1b. Effect of glutamine on serum uric acid of anti-tuberculosis drug-treated ratsData as mean ± SEM (Standard error of mean), n: 5, GTN:Glutamine,RIPE:Rifampicin/isoniazid/pyrazinamide/ethambutol, #p<0.001 differ significantly when compared to the control, *p<0.05, **p<0.01, and ***p<0.001 differ significantly when compared to RIPE

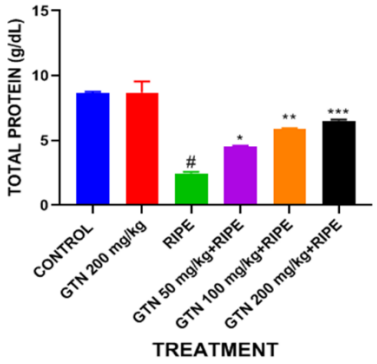

Fig. 1d. Effect of glutamine on serum total protein of anti-tuberculosis drug-treated ratsData as mean ± SEM (Standard error of mean), n: 5, GTN:Glutamine,RIPE:Rifampicin/isoniazid/pyrazinamide/ethambutol, #p<0.001 differ significantly when compared to the control, *p<0.05, **p<0.01, ***p<0.001 differ significantly when compared to RIPE

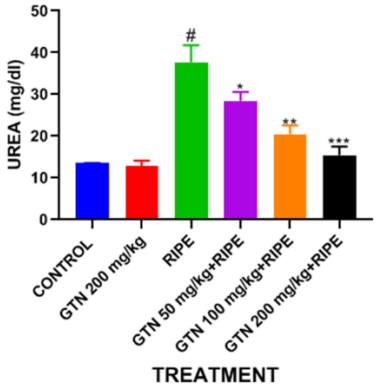

Fig.1c. Effect of glutamine on serum urea of anti-tuberculosis drug-treated rats.Data as mean ± SEM (Standard error of mean), n: 5, GTN: Glutamine, RIPE: Rifampicin/isoniazide/pyrazinamide/ethambutol, #p<0.001 differ significantly when compared to the control, *p<0.05, **p<0.01, and ***p<0.001 differ significantly when compared to RIPE

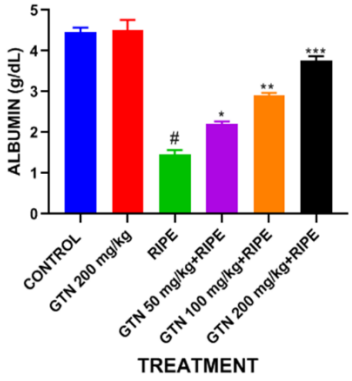

Fig. 1e. Effect of glutamine on serum albumin of anti-tuberculosis drug-treated rats. Data as mean ± SEM (Standard error of mean), n: 5, GTN:Glutamine,RIPE:Rifampicin/isoniazid/pyrazinamide/ethambutol, #p<0.001 differ significantly when compared to the control, *p<0.05, **p<0.01, and ***p<0.001 differ significantly when compared to RIPE

3.3. Effect of glutamine on kidney oxidative stress markers of anti-tuberculosis drug-treated rats

GTN didn't produce significant (p>0.05) effects on kidney SOD, CAT, GPx, GSH and MDA levels when compared to the control. RIPE significantly (p<0.001) decreased kidney SOD, CAT, GPx, and GSH levels when compared to the control (Table 2). However, treatment with GTN 50 mg/kg +RIPE, GTN 100 mg/kg +RIPE and GTN 200 mg/kg +RIPE significantly increased kidney SOD, CAT, GPx, and GSH levels at p<0.05 and p<0.01 and p<0.001, respectively when compared to RIPE (Table 2). RIPE produced significant (p<0.001) decrease in kidney MDA level when compared to the control (Table 2). Interestingly, GTN 50 mg/kg +RIPE, GTN 100 mg/kg +RIPE and GTN 200 mg/kg +RIPE significantly decreased kidney MDA levels at p<0.05, p<0.01, and p<0.001, respectively when compared to RIPE (Table 2).

Table 2. Effect of glutamine on kidney oxidative stress indices of anti-tuberculosis drug-treated rats

Treatment (mg/kg)	GSH µmole/mg protein	GPx U/mg protein	SOD U/mg protein	CAT U/mg protein	MDA nmole/mg protein
Control	12.80±1.07	25.40±2.14	30.50±3.10	25.46±3.11	0.23±0.01
GTN 200	13.31±1.11	25.51±3.32	31.00±3.21	25.67±3.33	0.22±0.07
RIPE	5.52±0.28#	10.62±0.38#	13.48±1.14#	8.48±0.10#	1.44±0.09#
GTN 50+RIPE	7.67±0.18*	14.80±1.10*	18.52±1.19*	12.56±1.39*	1.00±0.07*
GTN 100+RIPE	9.72±0.29**	19.91±1.07**	22.54±2.09**	17.72±1.19**	0.60±0.06**
GTN 200+RIPE	11.75±1.07***	24.60±2.13***	28.66±2.10***	23.52±2.29***	0.33±0.01***

Data as mean ± SEM (Standard error of mean), n=5, GTN: Glutamine, RIPE: Rifampicin/isoniazid/pyrazinamide/ethambutol, GSH: Glutathione, GPx: Glutathione peroxidase, SOD, Superoxide dismutase, MDA: Malondialdehyde. #p<0.001 differ significantly when compared to the control, *p<0.05, **p<0.01, and ***p<0.001 differ significantly when compared to RIPE.

3.4. Effect of glutamine on kidney histology of anti-tuberculosis drug-treated rats

The kidneys of the control (Fig. 6A) and GTN (200 mg/kg) treated rats (Fig. 6B) showed normal renal tubules and glomeruli. On the other hand, the kidney of RIPE-treated rats showed tubular necrosis, lipid accumulation, collapsed glomeruli and widened Bowman's space (Fig. 6C). The kidney of GTN 50 mg/kg+ RIPE-treated rats showed tubular necrosis, collapsed glomeruli, and widened Bowman's space (Fig. 6D). The kidneys of GTN 100 mg/kg+ RIPE-treated rats (Fig. 6E) and GTN 200 mg/kg+ RIPE –treated rats (Fig. 6F) rats showed normal tubules and normal glomeruli.

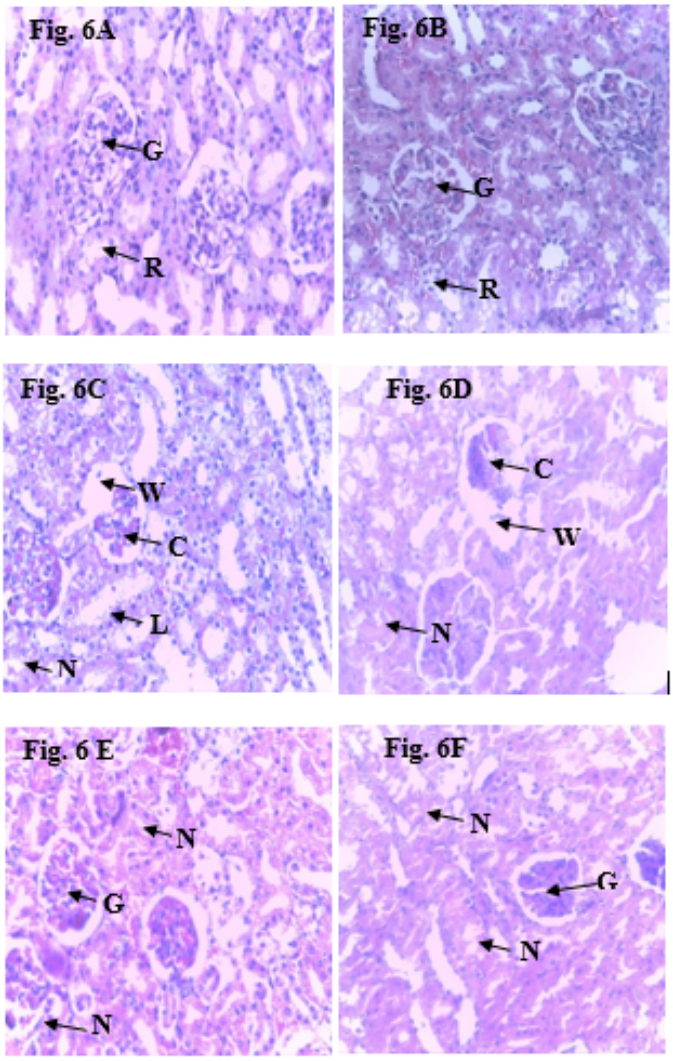

Fig 6A-F. are kidney micrographs of the control and experimental rats. 6A: Control, 6B: Treatment with GTN (200mg/kg), C: Treatment with RIPE, D: Treatment with GTN (50mg/kg) + RIPE, E: Treatment with GTN (100mg/kg) + RIPE. Treatment with GM (200mg/kg) + RIPE. R: Normal renal tubule, G: Normal glomerulus, N: Tubular necrosis, LP: Lipid accumulation, C: Collapsed glomerulus, W: Widened Bowman's space. x 400

4. Discussion

Nephrotoxicity is one of the common challenges associated with the use of RIPE (26). GTN may play an important role in protecting cells from chemical-induced damage (18). The current study assessed the protective effect of GTN on RIPE-induced nephrotoxicity in rats. The assessments of body and organ weights are essential in toxicity studies (27). In this study, RIPE decreased body weight and increased kidney weight in treated rats. This is consistent with decreased body weight and increased kidney weight in isoniazid-rifampicin-treated rats reported by Prince *et al.* (26). The observed decrease in body weight might have been caused by the appetite suppressive effect of RIPE. On the other hand, increase in the kidney weight might be a consequence of RIPE-induced renal inflammation. Studies showed that the induction of renal inflammation might be one of the mechanisms by which RIPE perturbs renal function (26). But GTN supplementation restored body and kidney weights in a dose–related fashion. GTN supplementation might have stimulated appetite and down-regulated the induction of renal inflammation by RIPE. Over the years, the assessments of biochemical markers have been used to diagnose and establish the types and magnitude of perturbations caused by underlying ailments and drugs (28). This study assessed creatinine, urea, uric acid, total protein and albumin levels to ascertain the toxic impact of RIPE on the renal status of rats (28). RIPE remarkably increased serum creatinine, urea, uric acid levels and decreased serum total protein and albumin levels in rats. These are signs of renal dysfunction caused by RIPE. Similarly, Martins and Sabina (29) reported altered levels of the aforementioned parameters in rats treated with anti-tuberculosis drugs. But GTN Supplementation restored renal biomarkers in RIPE-treated rats in a dose-related fashion.

Reactive oxygen species (ROS) are produced due to exposure to many exogenous chemicals or plenty of endogenous metabolic processes including redox enzymes and electron transport chain (30). Excess ROS are usually neutralized by endogenous antioxidants such as CAT, SOD, GPx and GSH. The inability of antioxidants to neutralize ROS can cause oxidative stress leading to cellular damage (30). In this study, RIPE caused notable decreases in kidney SOD, CAT, GSH and GPx levels. This is consistent with depleted kidney antioxidants caused by antituberculosis drugs in rats reported by Sharma *et al.* (31). The depleted kidney antioxidants in RIPE-treated rats may be due to excess ROS production leading to oxidative stress (31). Oxidative damage has been speculated to be involved in the etiology of many chronic diseases and drug-induce toxicities (32). However, in a dose-related fashion, kidney antioxidants were restored in GTN supplemented rats. Studies have shown that GTN can increase the antioxidant capacity of tissues, by enhancing CAT, SOD, GSH, and GPx activities (33, 34). GTN might have inhibited the production of ROS by RIPE thereby incapacitating its capacity to deplete kidney antioxidants. Lipid peroxidation (LPO) is a free radical oxidation of poly unsaturated fatty acids that has been associated with some diseases. It is a self-sustaining process that is capable of causing extensive tissue damage. Many toxic aspects of LPO are attributed to reactive aldehydes produced from oxidized lipids, which react with cellular nucleophiles such as nucleic

acids, proteins and GSH forming a variety of adducts. The formation of adducts has been associated with a number of disease conditions (35). MDA, one of the reactive aldehydes has been used as a yardstick for LPO (36). In this study, RIPE increased kidney MDA levels in treated rats. This is in agreement with increased MDA concentration in antituberculosis drug-treated rats reported by Hussein *et al*. (37). The elevated levels of kidney MDA may be due to the oxidation of poly unsaturated fatty acids caused by RIPE. However, GTN supplementation decreased kidney MDA levels in a dose-related fashion. This may be due to the ability of GTN to inhibit the lipid peroxidative activity of RIPE in the kidneys of treated rats.

Histology is necessarily a largely descriptive and interpretive science. It gives structural manifestations of diseases at the light-microscopic level (38). In this study, the histological assessment of the kidneys of RIPE-treated rats showed tubular necrosis, lipid accumulation, collapsed glomerulus and widened Bowman's space, which correlate with observed changes in evaluated biomarkers. Adikwu *et al.* (28) reported similar kidney histological changes in anti-tuberculosis drug-treated rats. This observation may be due to the induction of oxidative stress by RIPE through ROS production causing structural and functional damage to kidney biomolecules (DNA, lipids, and proteins) (28). However, supplementation with GTN restored kidney histology. GTN might have restored kidney histology by safeguarding kidney biomolecules from the menace of oxidative stress induced by RIPE.

GTN supplementation protects against RIPE-induced nephrotoxicity in a dose-related fashion. It may have clinical use for RIPE associated nephrotoxicity.

Ethical statement

Approval was obtained from the Research Ethics Committee (NDU/PHARM/PCO/AEC/068) of the Department of Pharmacology/Toxicology, Faculty of Pharmacy, Niger Delta University, Nigeria.

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Conflict of interest

The authors declare there was no conflict of interest

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Authors' contributions

Concept: E. A., M.M., T. B. N., Design: E. A., M.M., T. B. N., Data Collection or Processing: E. A., M.M., Analysis or Interpretation: E. A., M.M., T. B. N., Literature Search: E. A., M.M., T. B. N., Writing: E. A., M.M., T. B. N.

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