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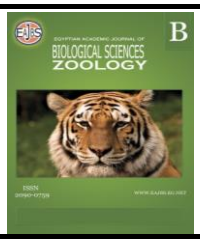

Protective Effects of *Tribulus terrestris* Against Gentamicin Mediated Nephrotoxicity, Oxidative Damage and Apoptosis in Male Rats

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ABSTRACT

Gentamicin (GNT) is an aminoglycoside antibiotic that is that's employed in the treatment of diverse forms of bacterial infections. However, gentamicin evoked renal toxicity. Therefore, the present experiment investigated the protecting impact of *Tribulus terrestris* and vitamin C against GNT-induced renal toxicity in albino rats. Six groups with eight rats each were used for this purpose; they included the normal control group that received physiological saline, the second group received *Tribulus terrestris* (200 mg kg orally), the third group received Vit. C (200 mg kg, orally). The fourth group injected gentamicin sulfate (100mg/kg I/P). The fifth group received ***Tribulus terrestris*** (200 mg kg orally) then injected with gentamicin sulfate, the sixth group received Vit. C (200 mg kg orally) then injected with gentamicin sulfate. The results showed that GNT significantly decreases serum levels of total protein and albumin. On the other hand, there were significant increases in levels of BUN, creatinine, and uric acid. Significant decreases were recorded in the levels of catalase (CAT) and superoxide dismutase (SOD). In addition, there was a substantial increase in the kidney contents of malondialdehyde (MDA). Serum levels of beta 2 microglobulin (β2M), nitric oxide (NO), and kidney injury molecule-1 (KIM-1) were substantially magnified but glutathione S transferase (GST) was significantly declined. Also, GNT caused histopathological changes and increased the expression of caspase-3 in the kidney tissues. However, administration of *Tribulus terrestris* as well as vitamin C, ameliorated the GNT-induced nephrotoxicity, perhaps via their antioxidant properties.

INTRODUCTION

Today, diseases associated with kidney and urinary tracts are more common in each developed and developing countries (Harambat *et al.*, 2012). The incidence increased

regularly with the increasing variety of medications induced nephrotoxicity (Jha V, 1995).

Gentamicin (GNT) is an aminoglycoside antibiotic used for the treatment of severe bacterial infections chiefly those of gram-negative organisms, however, restricted in use as it considered as a Drug-induced acute renal failure (ARF) (Ali *et al.,* 2011).

GNT induced reduction in creatinine clearance and this is indicative of reduced kidney perfusion and a remarkable tissue injury. These facts were bolstered by histopathological results, which demonstrate the loss of cellular components of renal tubules. Additionally, gentamicin increases the plasma urea level (Ogundipe *et al.,* 2017).

As what mentioned in (Bonventre and Weinberg, 2003; Ogundipe *et al.,* 2017) that gentamicin lowered plasma protein which was accompanied by a significant increased urine total protein, these could result from injury of glomerular basement membrane responsible for the filtration mechanism and absorptive failure of proximal convoluted tubules with oozing of plasma protein into the urine.

GNT elicited significant kidney injury in rats measured by the tubular injury score, proteinuria, and tubular injury markers as Neutrophil gelatinase-associated lipocalin (NGAL) and KIM-1 (Kidney Injury Molecule-1). As well as the pathological tubular injury (Chen *et al.,* 2017).

GNT nephrotoxicity is caused by variant pathways, such as decrease of renal blood flow, oxidative stress with a decline in the effectiveness of renal antioxidant enzymes (Christo *et al.,* 2011), inflammation, lipid peroxidation, the nuclear factor kappa B pathway and apoptosis (El-Kashef *et al.,* 2015; Rangan *et al.,* 2009).

That is why the focus of today's clinical trials is on the consumption of compounds with powerful antioxidant activity to overcome GNT nephrotoxicity and block drug-induced oxidative damage (Yanagida *et al.,* 2004).

Tribulus terrestris (Zygophyllaceae) is a valuable herb far-famed for its uses in traditional medicine in varied parts of the world as it is highly rich in components having potential biological importance, for example, saponins, flavonoids, alkaloids, and other nutrients (Kostova and Dinchev, 2005). Many therapeutic actions have reports for the plant extracts since dry fruits extract of *Tribulus terrestris* showed free radical scavenging activity (Vangalapati *et al.,* 2014). In addition, it has anti-inflammatory, immunomodulatory, diuretic, anti-urolithic, anti-diabetic, absorption enhancing, hypolipidemic, cardiotonic, hepatoprotective, analgesic, antispasmodic, antibacterial, anthelmintic, larvicidal and anti-carcinogenic activities (Chhatre *et al.,* 2014; El-Shaibany *et al.,* 2015).

Tribulus terrestris extract possesses anti-oxidative, apoptosis inhibitory, and vasodilator effects (Shalaby and Hammouda, 2014). The fruit takes off gravel from the urine and stones in the bladder. Additionally, it is recorded to possess cooling, diuretic, tonic, and aphrodisiac properties (Kavitha *et al.,* 2011).

Another experiment reported that the extract of *Tribulus terrestris* could have a protective impact on cisplatin-induced apoptosis of the kidney. This could be associated with the existence of antioxidant contents acting through numerous central and peripheral mechanisms (Ghanbari *et al.,* 2016).

Vitamin C is a hydrophilic six-carbon compound structurally associated with glucose (Elias and Oputiri, 2013) that exists in elevated concentration in citrus, soft fruits, and leafy green vegetables while, kidney and liver are good animal sources of the vitamin (Stangeland *et al.,* 2009). Vitamin C does its antioxidant effect by preventing lipid peroxidation and oxidative cell damage (Xavier *et al.,* 2007). Vitamin C displays anti-inflammatory impacts, inhibits endothelial dysfunction, and decreases the danger of cardiovascular illnesses (Ozkanlar and Akcay, 2012). Additionally, numerous studies revealed that vitamin C medications or therapies improved kidney function, reduced renal inflammation, and improved impaired renal function in hypertensive rats (Tian *et al.,* 2007).

Hence, the present study carried out with the hypothesis that *Tribulus terrestris* and vitamin C hold powerful antioxidant capacity that would alleviate oxidative damage resulted from GNT induced nephrotoxicity in rats.

MATERIALS AND METHODS

Ethical Statement:

Animal care and housing, as well as the experimental protocol, were approved by Animal Care and Ethics Review Committee at the Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt (The approval NO. 201690) in accordance with guidelines for the care and use of laboratory animals of National Institute of Health.

Isoflurane was used for anesthesia during sample collection and all precautions were taken to minimize discomfort. At the end of the work, rats were euthanized by decapitation to avoid pharmacological interference with experiments.

Animal Care and Treatments:

48 male Wistar rats, weighing 200–250 g (8–10 weeks old) were obtained from the experimental animal center of Helwan University, Egypt. They were housed in standard plastic cages and maintained on a 12-h light/dark cycle at 22°C–24° C they provided with rodent diet and water *ad libitum*

Experimental Design:

Acclimatization continued for 2 weeks then, rats were randomly allocated into six groups (each of 8 rats). The first group is a normal control group orally received physiological saline (using stomach tube). The second group (Tri) orally received ***Tribulus terrestris*** (Fruit Powder Liquid Extract from Hawaii Pharm LL, USA) at a dose of 200 mg kg^{-1} BW for 10 days (Abdel-Kader et al., 2016). The third group (Vit C) orally received Vit C **(**Puritan's Pride INC. Ronkonkoma, NY 11741, USA) at a dose of 200 mg kg^{-1} BW (Derakhshanfar et al., 2013) for 10 days. The fourth group (GNT) at the 5th day of the experiment were injected gentamicin sulfate (Sigma, St Louis, MO) for induction of acute renal disease at a dose of 100mg/kg I/P for 5 successive days in distilled water (Singh et al., 2012). The fifth group (GNT-Tri) orally received ***Tribulus terrestris*** at a dose of 200 mg kg^{-1} BW for 10 days and after 5 days gentamicin sulfate was injected at a dose of 100mg/kg I/P for 5 successive days in distilled water and the sixth group (GNT-Vit C) orally received Vit. C at a dose of 200 mg kg^{-1} BW for 10 days after 5 days gentamicin sulfate was injected at a dose of 100mg/kg I/P for 5 successive days in distilled water.

Acute Oral Toxicity:

Twelve male Wistar rats were used to determine the median lethal dose (LD_{50}) of ***Tribulus terrestris*** extract. Graduated doses started with 200 mg /kg b. wt and increased by the multiplying factor (1.3) up to 2120 mg /Kg b. wt. was administered orally using a stomach tube. Animals were observed for clinical signs of toxicity and mortality for 24 hours. The highest dose was repeated two times. The LD_{50} was determined using the modified up-and-down method (Bruce, 1985).

Body Weight:

Experimental rats were weighed at the beginning and the end of the experimental period.

Serum Collection and Tissue Preparation:

At the tenth day of the experiment, blood samples were collected from the medial canthus of the eye (retro-orbital bleeding) under isoflurane anesthesia in non-heparinized tubes and left for 30 min at room temperature to clot, then samples let to stand at refrigeration for one hour then centrifuged at 3000 rpm for 20 min to obtain clear sera, which were preserved at −20 °C until used for biochemical and immunological assays.

After collection of the blood, the rats were killed by cervical decapitation under isoflurane anesthesia and the kidneys were immediately removed from each rat and washed in physiological saline. One kidney from each rat was homogenized in 5 ml of phosphate buffer (pH 7.4) on the ice, using an electric homogenizer. Homogenates were then centrifuged at 3000 rpm for 15 min at 4 °C and the resulting supernatants were kept at −20 °C until later use. The remaining kidneys were immediately fixed in 10% neutral buffered formalin for histopathological and immunohistochemistry examination.

Assessment of Serum Biochemical Parameters:

The stored sera were used for evaluation of renal injury biomarkers Serum total proteins (TP), serum albumin (ALB) were measured according to Henry (Henry, 1964) all previous tests using reagent kits supplied by StanBio-Laboratories incorporation, USA; Renal functions; creatinine and uric acid were estimated according to Caraway (Caraway, 1963). BUN is measured according to (Tietz, 1995) they were measured using kits of Bio Merieux (France). The previously mentioned parameters were measured using (5010, V5+ Photometer, BM Co. Germany).

Measurement of Antioxidant Parameters In Kidney Tissue:

Levels of Catalase (CAT), Super-oxide Dismutase (SOD), and Malondialdehyde (MDA) were assayed using a spectrophotometer (5010, V5+ Photometer, BM Co. Germany) using commercial test kits by (Bio-Chain, Inc., USA) and measured according to manufacturer's instructions.

KIM-1 measured according to(Zhou et al., 2008) using Cell Biolabs, Inc. (USA).

The total serum GST activity was determined by a photometric method. GST activity was measured using a spectrophotometer at 340 nm with the standard substrate (1-chloro-2,4-dinitrobenzene, CDNB) and co-substrate (reduced glutathione, GSH), as qualified by (Habig *et al.,* 1974) using kits provided by ALPCO (USA).

NO is measured using colorimetric assay kits provided by Cayman Chemical Company (USA) (Nims *et al.,* 1995).

β_2M was measured by ELISA using a solid-phase sandwich ELISA kits from Crystal Chem according to the manufacturer's protocol.

Measurement of Serum Interleukins:

Serum interleukin-6 (IL-6) was measured in serum by ELISA using a solid-phase sandwich ELISA test kit obtained from immune-biological laboratories Co. Ltd., Japan. Interleukin 10 (IL-10) ELISA kit (My BioSource Co, San Diego, California, USA). The procedures were performed according to the manufacturer's protocol.

Histopathological Examination:

The kidney was examined then dissected and fixed in 10% neutral buffered formalin. Following fixation, the specimens were washed carefully in running tap water, dehydrated in an ascending series of alcohol, cleared in xylene and then embedded in paraffin wax. Sections of 5μ thickness each were cut and stained with hematoxylin and eosin. Sections were then investigated under the light microscope according to Bancroft and Gamble (Bancroft and Gamble, 2007). Scoring was done according to Cekmen *et al.* (2013). The severity of lesions was started from 0 to 3 according to the pathological changes of tubular. Slides were examined and assigned for the severity of changes using scores on a scale of none (0), mild (1), moderate (2), and severe (3) damages, in which (0) denotes no change. The paraffin sections were then stained using primary monoclonal antibodies against caspase-3 (rat caspase-3 antibody cat no. MBS261814 California, San Diego (USA)). The binding of antibodies was visualized by avidin-biotin complex (ABC kit, Vector Laboratories) and the immunostaining reaction was labeled with diaminobenzidine (DAB) as a chromogen and counterstained with Mayer’s Hematoxylin (Abdel-Daim *et al.,* 2017) for quantitative analysis, the intensity of immunoreactive parts was used as a criterion of cellular

activity after subtracting background noise. The measurement was done using an image analyzer (Image J program). From each slide of both experimental groups, 7 fields were randomly selected. The total field and immunohistochemical (IHC) stained areas were calculated and the percentage of IHC stained area calculated as follows: IHC stained area% = IHC stained area/total area X 100. (Elgawish *et al.*, 2015)

Statistical Analysis:

The obtained data subjected to one-way ANOVA to evaluate the effect of *Teribulus terrestris* extract, Gentamicin, and vitamin C differences between means was tested at the 5% probability level using Duncan Multiple Range Test. The entire statistical analysis was done using SPSS program version 16 (SPSS, Richmond, VA, USA). The scoring of histopathological changes is analyzed using the IBM-SPSS version 22 using cross-tabulation through Qui square. P value is considered significant when is <0.05 and the confidence interval is 95%.

RESULTS

Acute Oral Toxicity:

Oral administration of ***Tribulus terrestris*** extracts up to 2.12 gm/kg b. wt. did not exhibit any toxic signs or mortalities within 24 hrs. Also, there were no signs of delayed toxicity when rats were observed for two weeks.

Serum Biochemical Results:

The effects of gentamicin nephrotoxicity, as well as the protective effects of ***Tribulus terrestris*** and vitamin C on serum biochemical analysis, are presented in table 1.

Our results revealed significant decreases (P≤0.05) in serum total protein and albumin in gentamicin intoxicated rats when compared to the control group. On the other hand, significant increases (P≤0.05) in serum BUN, creatinine, and uric acid levels were recorded in the same group when compared to the control group.

Table 1: The effects of gentamicin nephrotoxicity as well as the protective effects of ***Tribulus terrestris*** and vitamin C on serum biochemical analysis

Groups / Parameters	Tp (gm/dl)	ALB (gm/dl)	BUN (mg/dl)	Creat (mg/dl)	Uric Acid (mg/dl)
Normal control	5.07±0.07^{a}	2.80±0.06^{a}	22.00±1.15^{d}	0.76±0.03^{d}	2.17±0.09^{d}
Tri	5.10±0.21^{a}	2.77±0.15^{a}	24.67±0.88^{d}	0.81±0.01^{d}	2.20±0.11^{d}
Vit C	4.90±0.06^{a}	2.60±0.06^{a}	24.50±0.29^{d}	0.81±0.01^{d}	2.27±0.15^{d}
GNT	2.6±0.06^{d}	1.34±0.03^{d}	45.67±1.20^{a}	2.73±0.03^{a}	4.55±0.13^{a}
GNT-Tri	3.57±0.17^{b}	1.97±0.03^{b}	31.67±0.88^{c}	1.37±0.09^{c}	3.13±0.07^{c}
GNT-Vit C	3.17±0.09^{c}	1.63±0.07^{c}	37.33±1.76^{b}	1.97±0.03^{b}	3.93±0.03^{b}

Means in the same row with different superscripts ($^{a-d}$) are significantly different (p≤ 0.05); values are presented as means ±SE.

Pre-treatment with ***Tribulus terrestris*** and vitamin C at doses of 200 and 200 mg/kg respectively, 5 days before gentamicin intoxication, ameliorate the negative impacts in most of the measured serum biochemical parameters. The results showed that both ***Tribulus terrestris*** and vitamin C effectively declined gentamicin-induced renal toxicity.

Tribulus terrestris pre-administration at a dose of 200 mg/kg significantly (P≤0.05) increased the serum total protein and albumin more than vitamin C pre-administration also, there were significant decreases (P≤0.05) in serum renal products; BUN, creatinine and uric acid compared to the vitamin C pre-administration group.

There were no significant changes in serum biomarkers in rats received either ***Tribulus terrestris*** or vitamin C when compared to the normal control group, indicating the safety of ***Tribulus terrestris*** and vitamin C at the selected doses used in this experiment.

Renal Lipid Peroxidation and Antioxidant State:

The effects of gentamicin nephrotoxicity, as well as protective effects of ***Tribulus terrestris*** and vitamin C on renal tissue homogenate lipid peroxidation and antioxidant parameters, are shown in table 2.

Table 2: The effects of gentamicin nephrotoxicity as well as protective effects of ***Tribulus terrestris*** and vitamin C on renal tissue homogenate lipid peroxidation and antioxidant parameters.

Groups / Parameter	CAT (U/g)	SOD (U/g)	MDA (nmol/g)
Normal control	1.44±0.03^{a}	3.60±0.06^{a}	0.28±0.00^{c}
Tri	1.46±0.02^{a}	3.55±0.01^{a}	0.28±0.01^{c}
Vit C	1.49±0.02^{a}	3.63±0.08^{a}	0.27±0.00^{c}
GNT	0.81±0.02^{d}	2.22±0.01^{d}	0.43±0.00^{a}
GNT-Tri	1.03±0.02^{b}	3.04±0.04^{b}	0.36±0.01^{b}
GNT-Vit C	0.91±0.02^{c}	2.80±0.00^{c}	0.37±0.01^{b}

Means in the same row with different superscripts ($^{a-c}$) are significantly different ($p \leq 0.05$); values are presented as means ±SE.

A significant elevation ($P \leq 0.05$) in renal MDA content was recorded in the gentamicin intoxicated group compared with the control group. On the other hand, renal SOD and CAT were significantly ($P \leq 0.05$) reduced.

Concerning *Tribulus terrestris* pre-administrated group renal MDA was decreased as well as vitamin C pre-administered group while, SOD and CAT were elevated compared to the gentamicin intoxicated group but, we observed they showed more increase in ***Tribulus terrestris*** pre-administrated group than vitamin C pre-administered group.

There were no significant changes in renal oxidative stress parameters in rats received either ***Tribulus terrestris*** or vitamin C when compared to the control group.

Biomarkers of Acute Kidney Injury:

The effects of gentamicin nephrotoxicity, as well as protective effects of ***Tribulus terrestris*** and vitamin C on biomarkers of acute kidney injury, are shown in table 3. There were significant increments ($P \leq 0.05$) in the levels of β2M, NO, and KIM-1 in gentamicin treated group in comparison with the control group. on the other hand, there was a significant reduction in the level of GST enzyme in this group as compared with the control one.

Both *Tribulus terrestris* pre-administrated group and vitamin C pre-administered group showed a significant reduction in the levels of β2M, NO, and KIM-1 in comparison with gentamicin intoxicated group, moreover, we observed that *Tribulus terrestris* pre-administrated group showed significant decrease than vitamin C pre-administered group.

GST enzyme showed a significant increase in both *Tribulus terrestris* pre-administrated and vitamin C pre-administered groups when compared with gentamicin intoxicated group also, there was a significant increment in *Tribulus terrestris* pre-administrated group than vitamin C pre-administered group.

There were no significant changes in biomarkers of acute kidney injury in rats received *Tribulus terrestris* and vitamin C when compared to the control group.

Table 3: The effects of gentamicin nephrotoxicity as well as protective effects of ***Tribulus terrestris*** and vitamin C on biomarkers of acute kidney injury.

Groups / Parameters	β2 Micro. (µg/ml)	GST (nmol/ml)	NO (nmol/µl)	KIM-1 (pg/ml)
Normal control	2.64±0.05^{d}	48.34±0.44^{a}	54.13±0.34^{d}	0.277±0.003^{d}
Tri	2.39±0.20^{d}	47.77±0.44^{a}	54.97±0.57^{d}	0.279±0.009^{d}
Vit C	2.54±0.01^{d}	49.11±0.67^{a}	53.97±0.32^{d}	0.271±0.002^{d}
GNT	5.16±0.07^{a}	32.42±0.57^{d}	90.87±0.79^{a}	0.549±0.007^{a}
GNT-Tri	3.17±0.06^{c}	41.77±0.83^{b}	72.36±0.66^{c}	0.348±0.004^{c}
GNT-Vit C	3.76±0.04^{b}	37.19±0.44^{c}	76.34±0.38^{b}	0.388±0.003^{b}

Means in the same row with different superscripts ($^{a-d}$) are significantly different ($p \leq 0.05$); values are presented as means ±SE.

Immunological Profile for Acute Renal Injury:

The effects of gentamicin nephrotoxicity, as well as protective effects of *Tribulus terrestris* and vitamin C on the immunological profile for acute renal injury, are shown in table 4.The results of this study showed significant increases ($P \leq 0.05$) in serum levels of IL-6 and IL-10 in gentamicin intoxicated rats when compared to the control group. *Tribulus terrestris* pre-administration and vitamin C pre-administration significantly ($P \leq 0.05$) decreased the elevation in the levels of IL-6 and IL-10, also, we observed that *Tribulus terrestris* pre-administration caused more significant decrease than vitamin C pre administered group.

There were no significant changes in the immunological profile for acute kidney injury in rats received *Tribulus terrestris* or vitamin C when compared to the control group.

Table 4: The effects of gentamicin nephrotoxicity as well as protective effects of ***Tribulus terrestris*** and vitamin C on immunological profile for acute renal injury.

Groups / Parameters	(pg/ml)	(pg/ml)
Normal control	8.33±0.17^{d}	31.38±0.65^{d}
Tri	8.56±0.12^{d}	31.00±0.58^{d}
Vit C	8.31±0.01^{d}	30.06±0.04^{d}
GNT	17.57±0.14^{a}	83.63±0.74^{a}
GNT-Tri	11.10±0.19^{c}	51.33±1.17^{c}
GNT-Vit C	13.28±0.15^{b}	60.05±0.80^{b}

Means in the same row with different superscripts ($^{a-d}$) are significantly different ($p \leq 0.05$); values are presented as means ±SE.

The Weight Of The Kidney:

The effects of gentamicin nephrotoxicity, as well as protective effects of *Tribulus terrestris* and vitamin C on the weight of the kidney and Caspase-3 enzyme for immunohistochemistry, are shown in table 5.

Gentamicin nephrotoxicity intoxicated rats showed a significant increment in the weight of the kidney in comparison with the control rats. On the contrary, there were significant decreases in the kidney weight in both pre-administered groups, moreover, we found that ***Tribulus terrestris*** pre-administered group showed a significant decrease than that of vitamin C pre-administered group.No significant changes in weights of the kidney were reported

in rats received *Tribulus terrestris* or vitamin C when compared to the control group.

Table 5: The effects of gentamicin nephrotoxicity as well as protective effects of *Tribulus terrestris* and vitamin C on weight of the kidney and Caspase-3 enzyme in renal tissues for immunohistochemistry.

Groups / Parameters	Weight of kidney (gm)
Normal control	0.40±0.06^{c}
Tri	0.43±0.08^{c}
Vit C	0.45±0.03^{c}
GNT	0.97±0.03^{a}
GNT-Tri	0.61±.01^{b}
GNT-Vit C	0.72±0.04^{b}

Means in the same row with different superscripts ($^{a\text{-}c}$) are significantly different ($p \leq 0.05$); values are presented as means ±SE.

Histopathological Studies:

The effects of gentamicin nephrotoxicity, as well as protective effects of *Tribulus terrestris* and vitamin C on scores of histopathological changes among different experimental groups, are shown in Fig 1A.

Examined H&E sections of kidneys of the normal control group, *Tribulus terrestris***,** and vitamin C groups revealed that kidney tissues showing renal cortices formed of glomeruli and tubules, glomeruli showed capillary tuft within thin Bowman's space and mesangial cells, tubules lined by columnar cells with abundant eosinophilic cytoplasm & interstitium showed thin-walled blood vessels and stroma (Fig. 1A (i), (ii) and (iii)).

On the other hand, the examination of kidneys of the gentamicin nephrotoxicity group showed that the sections in kidney cortex indicating a moderate hydropic degeneration and vacuolation of tubular epithelial cells and areas of hemorrhage/ congestion, glomeruli with a focal moderate increase in cellularity with degeneration and dilation of Bowman's space and hyaline casts in tubules. Also, they show stroma with focal moderate lymphocytic infiltrate with focal shrinkage of the glomerulus and tubular degeneration and calcification (Fig. 1A (iv), (v) and (vii))

Tribulus terrestris treated gentamicin nephrotoxicity group showed marked improvement with residual mild pathological changes in the form of mild focal hydropic degeneration of tubular epithelial cells, glomeruli showed a slight increase in cellularity and stroma showed mild congestion and minimal lymphocytic infiltrate (Fig. 1A (viii)).

Vitamin C treated gentamicin nephrotoxicity group revealed moderate improvement with residual pathological changes: moderate hydropic degeneration of tubular epithelial cells, glomeruli showed moderate degenerative changes, stroma showed mild congestion and mild interstitial lymphocytic infiltrate (Fig. 1A (ix)).

Fig. 1B shows the representation of the pathological changes of the kidneys due to gentamicin and the protective effect of Vitamine C and the ***Tribulus terrestris.***

Immunohistochemistry:

The effects of gentamicin nephrotoxicity, as well as protective effects of ***Tribulus terrestris*** and vitamin C on the weight of the kidney and Caspase-3 enzyme for immunohistochemistry, are shown in table 5.

Results showed that gentamicin intoxicated rats recorded a significant increase in the area and % of the stained area when compared to control.

Pre-treatment with *Tribulus terrestris* and vitamin C significantly decrease these results.

Moreover, *Tribulus terrestris* pre-treated group revealed a significant decrease than the vitamin C pre-treated group.There were no significant changes in immunohistochemistry in rats received *Tribulus terrestris* or vitamin C when compared to the control group Fig. 2A.

The expression of caspase3 in the kidney as shown in figure 2A. The normal control group showed weak to mild focal positivity of tubular epithelial cells for caspase 3 highlighted by the cytoplasmic brown staining. The group demonstrated negative immunoreaction (Fig. 2A (i)). Treatment with tribulus showed weak focal positivity of tubular epithelial cells for caspase 3 highlighted by the cytoplasmic brown staining (Fig. 2A (ii)). Vit C treated group showed mild focal positivity of tubular epithelial cells for caspase 3 highlighted by the cytoplasmic brown staining (Fig. 2A (iii)). Gentamicin nephrotoxicity group showed moderate to marked diffuse positivity of tubular epithelial cells for caspase 3 highlighted by the cytoplasmic brown staining (Fig. 2A (iv)). Tribulus treated gentamicin nephrptoxicated group showed decreased weak focal positivity of tubular epithelial cells for caspase 3 highlighted by the cytoplasmic brown DAB staining (Fig. 2A (v)). Vitamin C treated gentamicin nephrotoxicated group showed decreased weak focal positivity of tubular epithelial cells for caspase 3 highlighted by the cytoplasmic brown DAB staining (Fig. 2A (vi)).

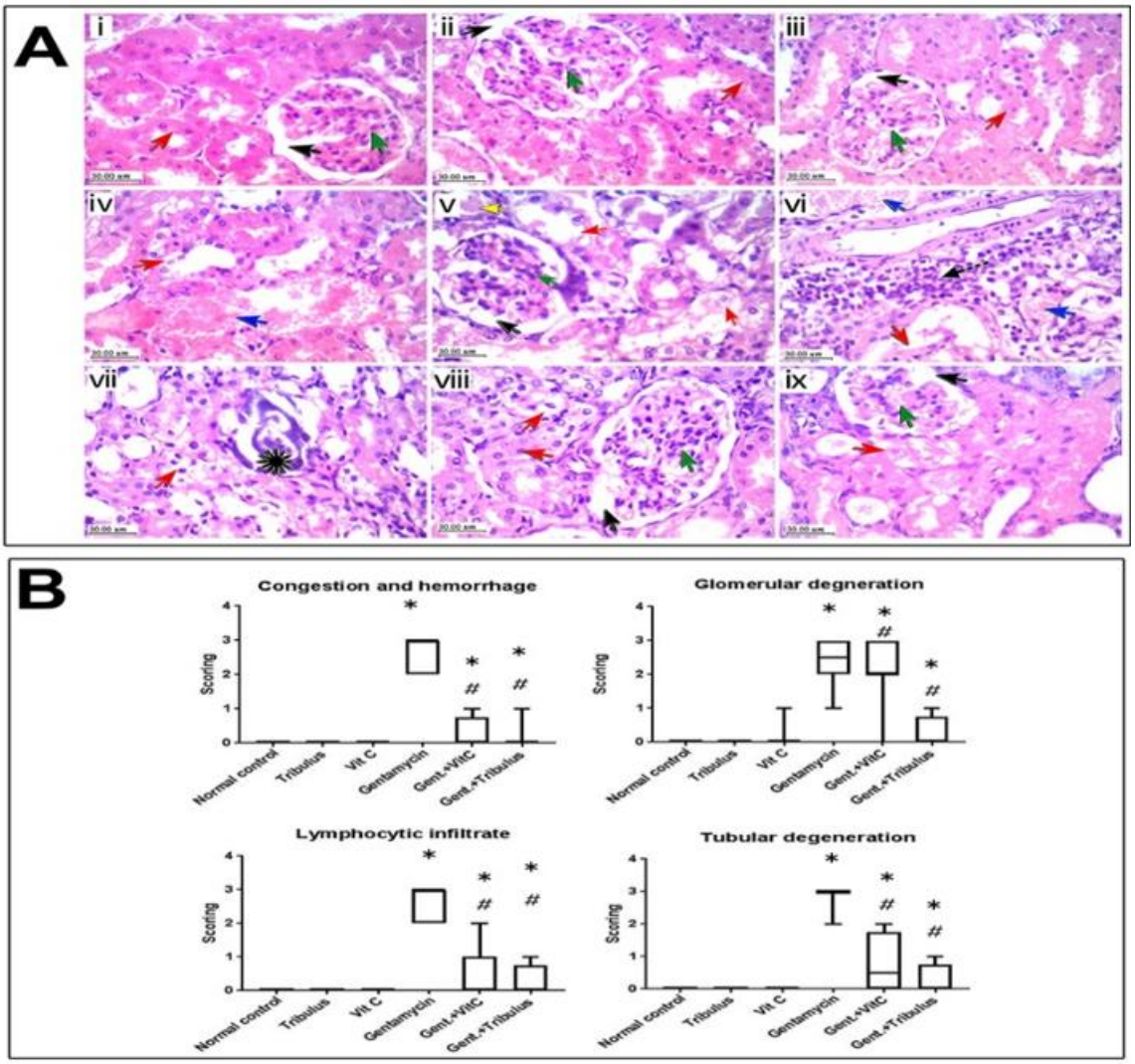

Fig. 1A: *Tribulus terrestris* effect on kidney histopathology in normal and GNT nephrotoxicity group. Photomicrographs of examined groups stained with H&E. (i, ii and iii) normal control, ***Tribulus terrestris,* and** Vit C groups they all showed normal renal tissue. (iv, v, vi and vii) represent the GNT nephrotoxicity group. Hydropic degeneration and vacuolation of tubular epithelial cells (red arrow) and areas of hemorrhage/ congestion (blue arrows), degeneration and dilation of Bowman's space (black arrow) and hyaline casts in tubules (yellow arrow). Focal moderate lymphocytic infiltrate (dashed arrow) with focal shrinkage of the glomerulus (green arrow), tubular epithelial cells (red arrow) showed degeneration and calcification (Astrisk). (viii), represents the (GNT + Tri) group. It showed marked improvement with residual mild pathological changes. (ix) represents the (GNT + Vit C) group in which Kidney showed moderate improvement with residual pathological changes. Scale bar represents 30 μm.

Fig. 1B: Boxplot graph for median histologic score in renal sections shows the pathological changes in the different study groups: congestion & hemorrhage, glomerular degeneration, lymphocytic infiltrate and tubular degeneration. The most affected group was the gentamycin group. For each rat kidney section, 10 fields were randomly selected and examined under x 400 magnification, and the median was calculated. Figure is done by Graph prism 7.03. * is significant compared to the normal control group ($p<0.05$) and # is significant to Gentamycin group ($p<0.05$). Tribulus and Vitamin C groups have the same of normal control group values (all zero score), so controls are represented by the normal control group.

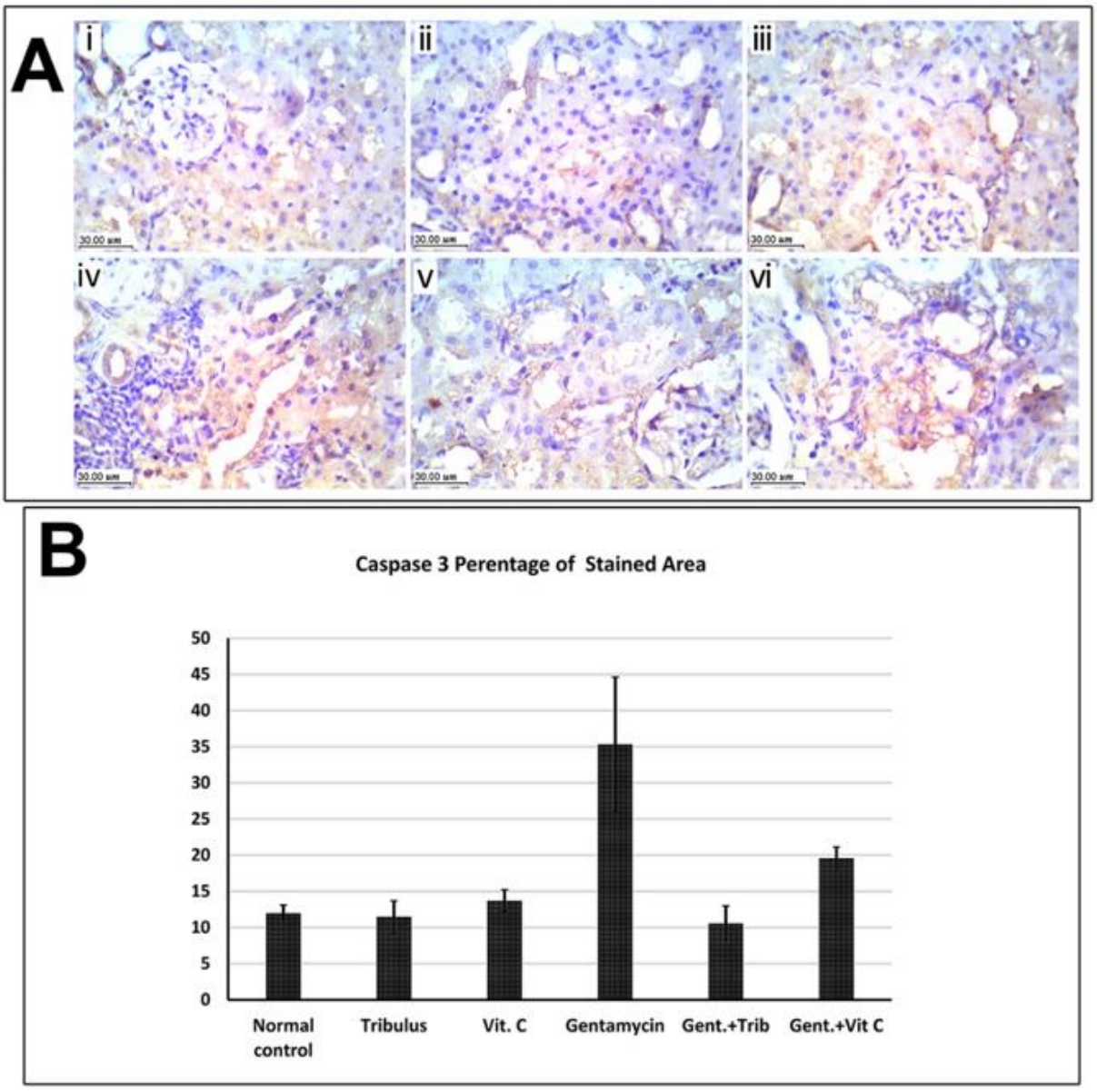

Fig. 2A: Representative photomicrographs of kidney tissue stained for Caspase 3, from different experimental groups. (i) normal control, (ii) tribulus treated group, Vit C treated group, (iii GNT nephrotoxicity group. (iv) (GNT + Tri) group and (v) (GNT + Vit C) group group. Original magnification 400x, scale bar 30 µm.
Fig.2B: Shows the Caspase 3 percentage of stained area. The GNT group showed marked high area of staining while these areas are decreased when Tribulus and Vit. C are added

DISCUSSION

This study erected that the administration of *Tribulus terrestris* and vitamin C could reduce the severity of nephrotoxicity induced by gentamicin attributable to their potent antioxidant and anti-inflammatory effect moreover, our result revealed that *Tribulus terrestris* was a stronger nephroprotective agent than vitamin C.

In the current study, we revealed that rats treated with gentamicin showed a reduction in renal function, with marked proximal tubule damage and increased levels of serum

creatinine, BUN, and uric acid with increment in total protein excretion in urine due to inability of kidneys to filter creatinine and non-protein waste products (Perrone *et al.,* 1992).

Many mechanisms such as angiotensin II generation, prostaglandin, and kallikrein-kinin systems or the potent vasodilator NO have been involved in GNT induced nephrotoxicity (Moreira *et al.,* 2014).

Gentamicin treatment resulted in nephrotoxicity that was evident with significant increment in serum creatinine, BUN, and uric acid levels with an increment of total protein excretion in urine due to the inability of kidneys to filter creatinine and non-protein waste products (Perrone *et al.,* 1992) in addition to, the decrease of glomerular filtration rate.

Accordingly, *Tribulus terrestris* extract improves plasma parameters, such; as creatinine and blood urea nitrogen as it can improve renal blood flow due to its inhibitory effect on the activity of the angiotensin-converting enzyme, stimulating the release of nitric oxide from vascular endothelium as well as hyperpolarization of the vascular smooth muscles leading to vasodilatation (Phillips *et al.,* 2006).

Pretreatment with vitamin C and *Tribulus terrestris* resulted in less harm in the biochemical parameters which may be attributed to its protective effect on the cell by the prevention of free radical production.

Renal toxicity induced by gentamicin as result of generating Reactive Oxygen Species (ROS) and starting lipid peroxidation in the kidneys (Balakumar *et al.,* 2008) as well as a decrease in the levels of renal reduced glutathione (GSH) and GST (Manikandan *et al.,* 2011) giving rise to the interaction between ROS and cellular contents, like lipids, proteins, carbohydrates, and nucleic acids causing damage of renal tissue (Gutteridge, 1995) and this confirmed by significant increase of MDA level with **a** significant decrease in SOD and CAT enzymes levels in gentamicin treated group.

Lipid peroxidation characterized as the procedure of oxidative debasement of polyunsaturated fatty acids and leads prompts weakened membrane function and structural integrity. In the present investigation, lipid peroxidation essentially lifted GNT exposure, *Tribulus terrestris* diminished the level of free radicals responsible for lipid peroxidation, and thus decreased the level of malondialdehyde. This reveals that *Tribulus terrestris* has the potential of scavenging free radicals and lessening GNT-instigated free-radical damage, additionally affirmed with the histological outcomes.

High Liquid Layer Chromatography investigation of *Tribulus terrestris* demonstrated the existence of phenolic acids as chlorogenic acid, caffeic acid, and 4-hydroxybenzoic acid as significant constituents. chlorogenic acid debilitates chronic ventricular remodeling after myocardial infarction and furthermore secures against ischemia reperfusion injury in the liver of rat, by means of its antioxidant properties. Caffeic acid enhances brain damage in focal cerebral ischemia. 4-Hydroxybenzoic acid is a potent scavenger of hydroxyl radical and secures against cerebral ischemia and reperfusion. The presence of these phenolic acids in *Tribulus terrestris* is required to add to the defensive property as they were beneficial in avoiding cell death (Reshma *et al.,* 2015).

Phytochemical investigation of *Tribulus terrestris* uncovered the presence of phenolic components together with saponin and flavonoids, separation and recognizable proof of the flavonoids gave numerous compounds such as quercetin and rutin which gave powerful antiradical capacity. Quercetin is an anti-oxidative flavonoids disseminated in the plant kingdom. Which has been appeared to tweak several signals transduction pathways which are related to the inflammation process (Ammar *et al.,* 2018).

It is a well-known fact that there is a positive correlation between the phenolic content and antioxidant activity of the material (Croft, 1998). There is an increasing interest in the efficacy of antioxidants to prevent the deleterious effect of oxidants in the human body. inside the body, superoxide and hydroxyl radicals are generated as a by-product of cellular

respiration. *Tribulus terrestris* was found to have high phenolic content and better radical scavenging activities (Reshma *et al.,* 2015).

Pre-administration of *Tribulus terrestris* extract improves oxidative stress via numerous mechanisms that incorporate a decreased level of free radicals like superoxide and preservation of total antioxidant capacity through main tainting near-normal activity level of endogenous enzymatic/non-enzymatic antioxidant. The later impacts might be credited to a higher level of total phenolic content in the *Tribulus terrestris* extract as found by phytochemical examination (Tag *et al.,* 2015). The results showed that GNT reduced the SOD and GST activities in renal tissues. The rebalancing of elevated antioxidant enzyme activity *Tribulus terrestris* treatment further is due to the protective nature of this plant against free radical-induced GNT administration (Kamboj *et al.,* 2011).

In addition, vitamin C treated groups manifested substantial elevation in antioxidant enzymes activities with a reduction in MDA levels as vitamin C may inhibit the chain reactions of GNT-generated free radicals as both animal (Odigie *et al.,* 2007) and human (Dogun and Ajala, 2005) studies have shown that vitamin C is a powerful antioxidant which mediates its antioxidant impact by scavenging (ROS) and restored the renal content of GSH in addition, activities of SOD, GST, and GPx across their normal values(Kensara, 2013). This effect was evident by the decline in renal MDA level, plasma urea, and plasma creatinine

Two roles have been played by NO, physiological and pathophysiological roles (Carlstrom *et al.,* 2009) and have a significant role in the function of renal tubular and renal hemodynamics regulation (Patel *et al.,* 1999). Group treated with gentamicin revealed significant increment in the level of NO; acute renal failure occurred due to gentamicin is as a result of the free radical nature of NO (Nakas-Ićindić et al., 2005) Also, NO produced cytotoxic peroxynitrite, as a result of interaction with superoxide radical which resulting in tubular cells injury and renal failure (Christo *et al.,* 2011).

Pre-administration of *Tribulus terrestris* extract showed a significant reduction in NO production Our results are concurrent with that of {Sik Kim, 2017 #114} they found that methanolic extract of *Tribulus terrestris* prevented the production of NO. also, they recorded eleven phenolic amides including a new compound, cis-terrestriamide, were isolated and characterized by the fruits of *Tribulus terrestris* and exhibited the inhibition NO production.

Pre-administration of vitamin C, with its antioxidant properties and through regulation of the production of NO, is protecting against the effects of GNT in these animals. We suppose that the use of vitamin C to protect or to decrease the renal injury resulted from GNT (Moreira *et al.,* 2014).

Recently, there are several markers used for early diagnosis of acute kidney injury (AKI) have been proposed such as Beta 2- macroglobulin (β2M) and kidney injury molecule-1 (KIM-1).

β2M is filtered by the glomerulus and almost reabsorbed and catabolized by the proximal tubular cells (Miyata *et al.,* 1998)**,** this process impaired in AKI. Elevation in the level of excretion of β2M I the urine has been recorded to be an early marker of tubular injury (Chapelsky *et al.,* 1992) before the elevation in serum creatinine level by 4−5 days (Tolkoff-Rubin *et al.,* 1988). KIM-1 is presented on the tubular epithelial surface in the kidney. KIM-1 is under detection levels in normal kidneys, while in ischemic kidney KIM-1 was increased as a marker for tubular injury (Vaidya *et al.,* 2006) so, KIM-1 could be considered as a biomarker for nephrotoxicity (Prozialeck et al., 2007) also, the Food and Drug Administration has recently considered KIM-1 as a marker for renal injury (Vaidya et al., 2010) and this support our result of β2M and KIM-1 levels in GNT treated rats.

Also, our results designate that the renal damage induced by gentamicin is due to the inflammatory process and this is confirmed by substantial elevation of renal pro-inflammatory markers such as IL-6 and IL-10 in gentamicin treated rats. Gentamicin

produced a marked elevation in the ratio of monocyte and macrophage leakage into the kidney and stimulated the expression of pro-inflammatory indicators such as TNF, IL-10, IL-6, and iNOS (nitric oxide induced) (Geleilete et al., 2002). These inflammatory molecules contribute in the development of renal impairment by elevating leukocytic attraction and adhesion to the site of inflammation in renal cells also, increment in the level of expression of adhesion molecules on the surface of the cell such as monocyte chemoattractant protein (MCP-1) and vascular cell adhesion molecule (VCAM-1), which act as chemotactic agents for macrophages, which increased in kidney of gentamicin treated rats (Park *et al.,* 2009).

Group treated with *Tribulus terrestris* decreased the levels of IL-6 and IL-10 significantly, N-trans-ρ-caffeoyl tyramine (CT) isolated from *Tribulus terrestris* was found to decrease the production of IL-6 and IL-10 so, it prevents the inflammatory process through acting as COX-2 (cyclooxygenase-2) selective inhibitor, so it may be a safe naturally-isolated drug that perhaps be used in the treatment of inflammatory diseases {Ko, (2015) #115}

Group treated with vitamin C revealed a significant decline in the levels of IL-10 and IL-6 as vitamin C mediates the oxidative-interaction that occurred among inflammatory cells and endothelial cells also, weakens the up-regulation of crucial adhesion molecule by deadening these radicals (Patel *et al.,* 1991).

In the present study, a significant decline in the intensity of histopathological and morphometric alterations induced by GNT was recorded in rat kidney treated with vitamin C and *Tribulus terrestris* when compared with GNT group.

Pretreatment with *Tribulus terrestris* significantly ameliorated renal dysfunction as well as decreasing the damage of renal tubules, oxidative stress, and apoptosis in GNT-treated rats. So, *Tribulus terrestris* decreases apoptosis of GNT by reducing the count of apoptotic cells which may be due to its antioxidant activity as it contains flavonoids (Harborne and Williams, 2000) which can control a set of enzymes participated in cell division, proliferation, detoxification, inflammation and immune response (Jung-Suk *et al.,* 2004)**.**

Recent studies indicated that *Tribulus terrestris* extract reduced oxidative stress and cell apoptosis in heart muscles as *Tribulus terrestris* extract resulted in the reduction of pro-apoptotic proteins such as Bax and caspase-3 through activating protein kinase C and increasing the level of Bcl-2 anti-apoptotic protein. Hence, *Tribulus terrestris* extract may exert soothing effects on cellular damages and oxidative stress in kidney tissues (as in heart muscles) through a similar mechanism or other mechanisms that need to be investigated in future studies (Zhang *et al.,* 2010). Our results of caspase-3 in different study groups are consistent with these studies.

Conclusion

In the current study, it is obvious that gentamicin induced nephrotoxicity, oxidative damage, and apoptosis in rats. In summary, these findings showed that both of vitamin C and *Tribulus terrestris* have potent antioxidant and antiapoptotic effects that ameliorated GNT-induced nephrotoxicity as well as *Tribulus terrestris* exhibit more antioxidant and antiapoptotic effects than vitamin C so, this study may provide an evidence to support clinical therapeutic value of *Tribulus terrestris* in treatment of renal dysfunction rather than its aphrodisiac activity.

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