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Renoprotective Effects Of Orally Administered Aqueous Extract Of *Tridax Procumbens* On Gentamicin Induced Nephrotoxicity In Albino Wistar Rats

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ABSTRACT

The renoprotective effects of orally administered aqueous extract of *Tridax procumbens* was investigated in Wistar rats of mixed sexes, aged between 2 to 4 months. The animals were divided into five groups of four animals each according to body weights. Group I received water only (baseline control), groups II to V received subcutaneous gentamicin (80mg/kg body weight) for eight days and on day 4 8 groups III, IV, and V received vitamin C (500mg/kg body wt.), 200mg/kg and 400mg/kg body wt of *T. procumbens* extract respectively, 30 minutes prior to the subcutaneous injection of gentamicin (80mg/kg body wt.). On Day 9 of the study, blood samples were collected from all the animals and serum urea and creatinine levels estimated; the animals were then sacrificed and the kidneys harvested and processed for histological examination. Results of the biochemical analysis revealed that aqueous extract of *T. procumbens* at a dose of 400mg/kg body wt. caused a significant reduction in serum urea and creatinine levels ($p < 0.05$) when compared with the negative control group. Histological examination also revealed that the extract was able to protect the kidneys of the rats against kidney injury induced by gentamicin. The aqueous extract of *Tridax procumbens* shows promise as an adjuvant therapy along side with aminoglycosides as it is able to protect the kidney against gentamicin induced nephrotoxicity.

KEY WORDS: *T. procumbens,* Aqueous extract, Renoprotection, Gentamicin, Rats

Amino glycosides including gentamicin are very important agents for the treatment of gram negative bacterial infections. Nephro-toxicity is the major side effect of amino glycosides, accounting for 10-15% of all cases of acute renal failure (Homes and Weinberg 1986). The specificity of gentamicin renal toxicity is apparently related to its preferential accumulation in the renal convoluted tubules and its effect on biological membranes.

Plant-based drugs have been used against various diseases for a long period of time. The primitive man used herbs as therapeutic agents and medicament, which they were able to procure easily. Nature has provided abundant plant wealth for all living creatures, which possess medicinal virtues. The essential values of some plants have long been published; however, a large number of them remain unexplored as yet. Therefore, there is a necessity to explore their uses and to conduct pharmacognostic and pharmacological studies to ascertain their therapeutic properties (Bhagwat *et al* 2008).

Tridax procumbens Linn (compositae) is common grass found in tropical eastern, southern, and western part of Nigeria, growing primarily during raining season. Its common name is coat buttons because of the appearance of its flowers (Saxena and Albert 2005). The extracts of *Tridax procumbens* have been reported to have various pharmacological effects, antimicrobial activity against both gram-positive and gram-negative bacteria, and stimulate wound healing (Taddel and Rosas 2000, Udopa 1991, Diwan *et al* 1982, Diwan *et al* 1983). Flavones, glycoside, polysaccharide, monosaccharides and asteraceae have been isolated from the leaves of the plant (Ali *et al* 2001, Yadawa and Saurabh 1998, Ali and Jahangir 2002, Raju and Davidson 1994).

Traditionally, the local Yoruba population of Western States of Nigeria uses the leaves of the plant as treatment to reduce blood pressure (Salahdeen et *al* 2004.) In an extensive search of the literature, there is dearth of scientific information on renoprotective effect of *T. procumbens* to the best of our knowledge. In view of this, the present study seeks to evaluate the protective effect of the leaf extract of *T. procumbens* against gentamicin-induced kidney injury in rats.

MATERIALS AND METHODS

Experimental Animals

Twenty albino rats of the Wistar strain, of both sexes, weighing 110 to 270g were obtained and housed at the Animal House, College of Medicine,

University of Nigeria Enugu Campus. They were fed on standard commercial rat feed [Guinea Feed Nigeria®, Plc] and water *ad libitum*. They were allowed for a period of one week for acclimatization, under room temperature (25 ± 3°C) and adequate lighting conditions. All animals were handled in this study according to international guideline for handling experimental animals (APS, 2002).

Plant Collection and Taxonomy

Fresh leaves of *Tridax procumbens L.* were collected from the University of Nigeria, Enugu campus environs during the month of March. The leaves were identified by the Department of Botany, University of Nigeria Nsukka.

Plant Extraction

2000g of the fresh leaves were washed and macerated to a paste using a gasoline powered grinding machine. About 500mL of water were then added to the paste, homogenized by stirring and allowed for about 30 minutes for extraction to take place. Filtration was carried out using a muslin cloth sieve. The resulting extract was stored at 4 ± 2°C prior to subsequent administration to the animals and throughout the course of the experiment.The concentration of the aqueous extract was determined evaporating 4ml of the extract in an evaporating dish of known weight in an oven (Gallen Kamp®, UK) to dryness at 70°C. The dish containing the residue was allowed to cool and the weight of the residue was obtained by subtracting the weight of the empty dish from the weight of the dish and residue. The extractive value of the aqueous extract (AE) was 112mg/ml.

Experimental Design

The experiment lasted for about two weeks. The animals were divided into five (5) groups of four (4) animals each, labeled I -V. Animals in groups III to V received 500mg/kg Ascorbic acid (Vitamin C), 200mg/kg AE and 400mg/kg AE respectively once daily or 3 days via an oral cannula. This was done to stabilize the plasma drug concentration. On the 4th day, drug administration continued, and thirty minutes later, 80mg/kg /day subcutaneous injection of gentamicin was given to induce renal injury as described by Harlalka et al (2007) with slight modification. Group II received only 80mg/kg gentamicin subcutaneously once daily for 8 days and served as negative control. Group I received no treatment and served as the baseline control. The test substance, Ascorbic acid and Gentamicin were administered to these animals for another four (4) days. On the 9th day; blood was collected from all the animals by retro-orbital puncture into plain tubes. Sera were separated from cells as soon as possible and stored frozen prior to analysis. After blood collection, the animals were euthanized under diethyl ether anesthesia and their kidneys excised, rinsed in physiological saline and fixed in 10% formal saline prior to histological processing.

Biochemical Assays

Serum urea test was carried out the serum samples using the diacetyl monoxime method (Baker *et al,* 1985); serum creatinine levels were measured used the Jaffe's method as modified by Ochei and Kolhatkar (2000).

Tissue Processing

The tissues were processed using an Automatic tissue processor (Leica TP1020 model). They were embedded in paraffin wax tissue blocks and sectioned to 5µm thick using a rotary microtome (Heitz 150 Rotary Microtome, Cambridge model). Sections were stained using the Haematoxylin and Eosin (H and E) technique as modified by Baker *et al,* (1985) for light microscopy. The sections were examined using Swift® binocular microscope with in-built lighting system. The sections were then photographed using a Samsung S850® digital photomicroscope.

Statistical Analysis

Data generated were analyzed using the SPSS software. All data were expressed as the mean value ± SEM. The level of significance was determined by the student't' test while the main effects on treatment groups were determined by the One way Analysis of Variance (ANOVA), followed by the Turkey Post Hoc multiple comparison tests. $P<0.05$ was considered significant.

RESULTS

In Table 1, results show that subcutaneous injection of 80mg/kg gentamicin sulphate to animals for 5 days resulted in renal injury. The negative control group (II) had the highest increase in these renal function parameters with mean values of 9.17 ± 1.08 mmol/l and 107.50 ± 26.50 µmol/l fo

Table 1:Blood urea and creatinine concentration of various treatment groups compared with base line control.

Groups	Blood Urea (mg/dl) Mean ± SEM	Serum creatinine (mg/dl) Mean ± SEM
I (Baseline control)	4.65 ± 0.15	71.00 ± 0.02
II (negative control)	9.17 ± 1.08**	107.50 ± 26.50*
III (500mg/kg Vit.C)	6.20 ± 0.49	66.25 ± 13.25
IV (200mg/kg)	5.80 ± 0.61	66.50 ± 4.50
V (400mg/kg)	5.73 ± 1.13	70.75 ± 17.75

*P<0.05 ** P<0.01

Table 2: Blood urea and creatinine concentration of treatment groups compared with negative control

Groups	Blood Urea (mg/dl) Mean ± SEM	Serum creatinine (mg/dl) Mean ± SEM
I (Baseline control)	4.65 ± 0.15**	71.00 ± 0.02*
II (negative control)	9.17 ± 1.08	107.50 ± 26.50
III (500mg/kg Vit.C)	6.20 ± 0.49	66.25 ± 13.25*
IV (200mg/kg)	5.80 ± 0.61	66.50 ± 4.50*
V (400mg/kg)	5.73 ± 1.13*	70.75 ± 17.75*

*P<0.05 ** P<0.01

Table 3:Blood urea and creatinine concentration of various treatment groups compared with the positive control [500mg/kg b.wt. Ascorbic acid (Vit. C)]

Groups	Blood Urea (mg/dl) Mean ± SEM	Serum creatinine (mg/dl) Mean ± SEM
I (Baseline control)	4.65 ± 0.15	71.00 ± 0.02
II (negative control)	9.17 ± 1.08	107.50 ± 26.50*
III (500mg/kg Vit.C)	6.20 ± 0.49	66.25 ± 13.25
IV (200mg/kg)	5.80 ± 0.61	66.50 ± 4.50
V (400mg/kg)	5.73 ± 1.13	70.75 ± 17.75

*P<0.05

Table 4:Scoring of the Histological changes observed on the Kidney tissues from the various groups

Groups	Engorged glomerulus with decreased Bowman's capsular space	Inflammatory cellular infiltration	Tubular erosion and dilation	Necrosis and glomerular erosion	Presence of eosinophilic tubular casts
I (Baseline control)	-	-	-	-	-
II (negative control)	++	+++	+++	++	-
III (500mg/kg Vit.C)	+	++	+	-	+
IV(200mg/kg)	+	++	++	-	-
V (400mg/kg)	-	++	+	-	-

Key: Absence (-); Mild (+); Moderate (++); Severe (+++)

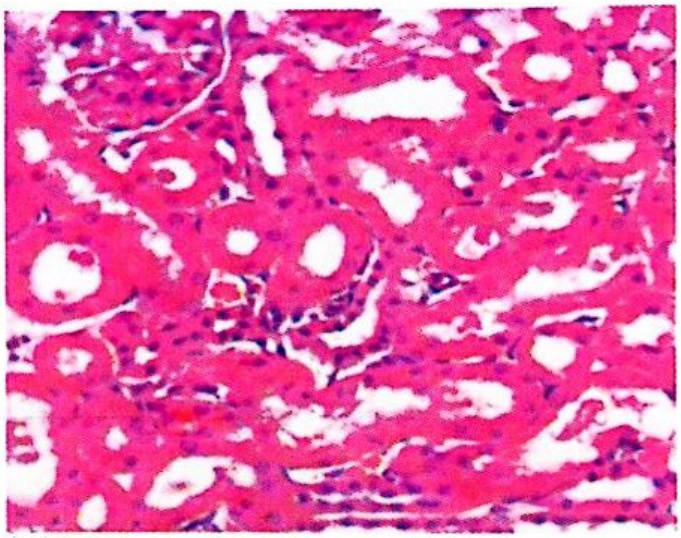

Figure 1: Control Kidney section from baseline control showing normal histoarchitecture

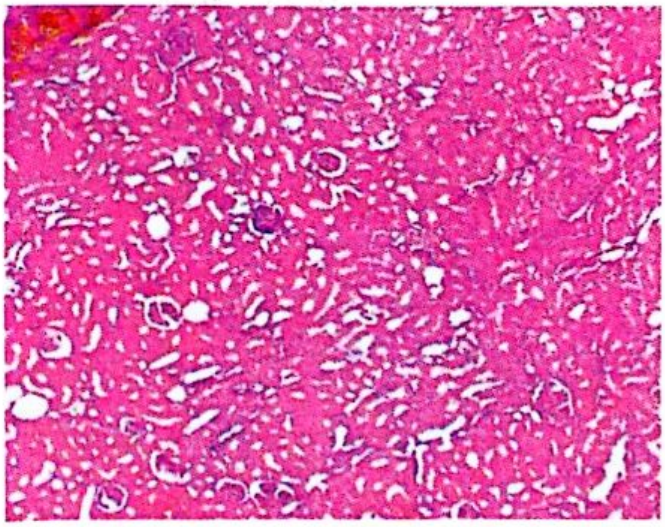

Figure 2: Kidney section from Gentamicin only treated group (Negative control) showing dilated and eroded tubules with engorgement of some of the glomeruli.

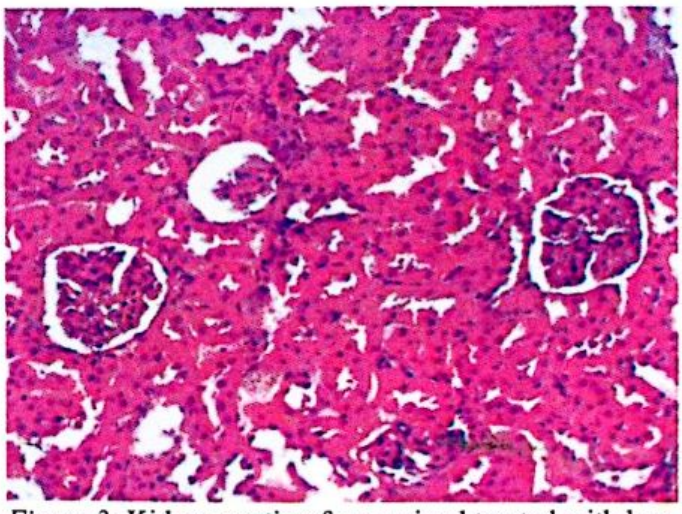

Figure 3: Kidney section from animal treated with low dose *T. procumbens* showing eroded tubules and infiltration of inflammatory cells.

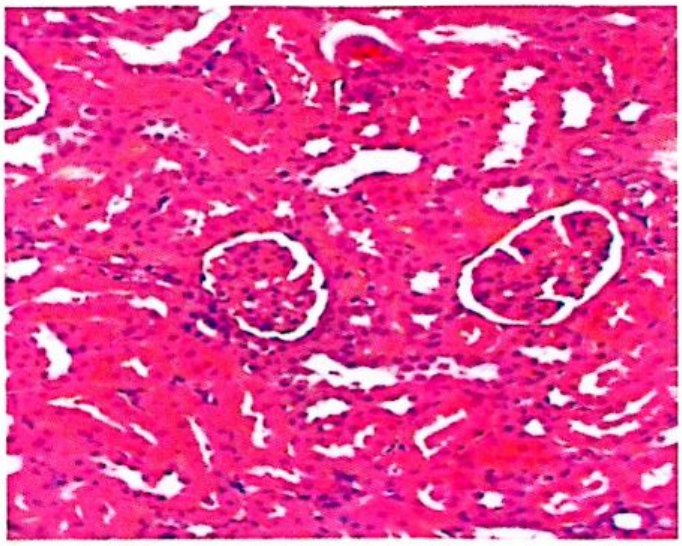

Figure 4: Kidney section from animal treated with high dose *T. procumbens* showing mild inflammatory cellular infiltration with mild tubular erosion.

urea and creatinine respectively. These were statistically significant when compared with baseline control ($P<0.01$; $P<0.05$). Serum urea values for animals in groups III, IV and V were lower than that of the negative control but only group V was statistically significant ($P<0.01$). Statistically significant decreased levels of Serum Creatinine levels were also observed in groups III, IV and V when compared with the negative control group ($P<0.05$) (Table 2). A comparison of the kidney function parameters of all the treatment groups with Group III [500mg/kg b.wt. of Ascorbic acid (Vit. C)] showed comparable renoprotective potency with groups IV and V (Table 3).

Histopathological findings in the treatment groups include infiltration of mononuclear leucocytic cells between the renal tubules, necrosis, engorged glomeruli with reduced Bowman's capsular space; Tubules appear eroded, widened and oedematous. The features for negative control group were severe (Figure 2) while those for the extract treated groups were moderate and minimal (Figures 3 and 4) respectively. The control kidney section revealed normal histoarchitecture (Figure 1).

DISCUSSION

The present study investigated the possible renoprotective effect of orally administered aqueous extract of *Tridax procumbens* on gentamicin induced nephrotoxicity in rats. The study also attempted to compare this effect, (if present), to that afforded by Ascorbic acid (Vitamin C), a known anti-oxidant drug.

Gentamicin at a dose of 80mg/kg body wt produced significant nephrotoxicity as evidenced by increased serum urea and creatinine levels, degenerative and inflammatory changes seen in the renal corpuscles, tubules and interstitium. This tissue injury may have been caused by one or a combination of such factors as reactive oxygen species (ROS), increased lipid peroxidation of tubular cell mitochondria, defective alterations in lysosomal enzymes and lysosomal membrane permeability of tubular epithelial cells, and/or inhibition of protein synthesis in the renal tubules. (Morin *et al* 1980, Shah 1984, Shah *et al* 1987; Fantone and Ward 1982, Kumar *et al* 2000)

Results of the experiment show that pre-treatment of the rats with aqueous extract of *T. procumbens* and subsequent co-administration of extract and gentamicin produced significant renoprotective effect. The groups treated with the extract at dose of 400mg/kg body weight, showed significantly reduced mean serum urea and creatinine levels ($p<0.05$) when compared with that of the group treated with gentamicin alone. Histological observation of kidney sections also reveals that this group had less marked glomerular and tubular lesions when compared with the extensive kidney injury observed in the gentamicin treated group.

T. procumbens extracts have been shown to contain, among many other phytochemical components, flavonoids (Ikewuche *et al* 2009). Flavonoids are known to possess anti-oxidant properties (Buhler and Miranda 2000). Also in some earlier publications, the leaf extracts of *T. procumbens* have been shown to possess anti-oxidant properties through *in vivo* animal experiments (Kumar *et al* 2001, Bhagwat *et al*, 2008, Ravikumar *et al* 2005) and *in vitro* experiments (Taddel and Rosas 2000, Udopa 1991, Helmantha 2005). Kumar *et al* (2000) reported that anti-oxidants are able to protect the kidneys against gentamicin induced kidney nephrotoxicity and against the cell damaging effects of reactive oxygen species. The renoprotective effect shown by the aqueous extract of *T. procumbens* in the present study may possibly have been via a free radical scavenging mechanism.

The AE treated groups seem to show comparable renoprotection when compared with Vit. C treatment group. The high dose AE treatment group (400mg/kg b.wt.) protected the renal tissue from injury better than Vit. C. Vitamin C has also been shown to possess antioxidant properties both *invivo* and *invitro*, (Padayatty *et al 2002*, Weyers *et al 2007*, Geogerti *et al* 2003). It can be inferred from this study that *T. procumbens* confers a better renoprotection than Vit. C.

In conclusion, the present study provides evidence that co-administration of the aqueous extract of *T. procumbens* along with gentamicin significantly protected the rats against nephrotoxicity induced by gentamicin. Cautious use of the plant material should however be exercised, as animal experimentation which even though predictive, may not always correspond to humans. It is therefore recommended that further studies should be carried out to isolate the biologically active component(s) responsible for the observed renoprotective effect and to elucidate its mechanism of action, pharmacokinetics, pharmacodynamics and possible side effects.

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