

# Anti-nephrotoxic activity of some medicinal plants from tribal rich pockets of Odisha

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## ABSTRACT

**Background:** Gentamicin, a strong cationic drug accumulated at biological membranes causes net increase in oxidative stress and lipid peroxidation leading to necrotic changes in renal tubules and consequently precipitates acute nephrotoxicity. Several phytoconstituents and plants extracts demonstrated significant anti-oxidant and cyto-protective activities. *Vitex negundo* Linn. (VN), *Oroxylum indicum* Vent. (OI) and *Barringtonia acutangula* Linn. (BA) are widely found throughout the Asian sub-continent including India, used extensively in different forms of Indian traditional medicine like Ayurveda and Unani. **Objective:** Nephroprotective activity of extracts of VN roots, OI whole plant and BA leaves were investigated against experimentally induced acute nephrotoxicity [Gentamicin (i.p; 80mg/kg for 7 days)] in Wistar rats as test animals. **Materials and Methods:** The rats were treated with Cystone (5 mL/kg; p.o) taken as positive control and methanol-dichloromethane (1:1) extracts of VN, OI and BA (200 mg/kg; p.o) as test drugs for 7 days. Following the said treatments, biochemical parameters of urine (volume, creatinine and lactate dehydrogenase (LDH)) and serum (urea, creatinine, albumin and total protein) were estimated. Renal anti-oxidant markers viz., superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and lipid peroxidation (LPO) in renal tissue were assayed. Tissue sections of kidneys from different groups were made and histopathological features were observed. **Result:** The extracts of VN, OI and BA significantly attenuated the nephrotoxicity by elevation of body weight, CAT, GPx and SOD or lowering urine LDH and creatinine, serum urea; serum creatinine and LPO respectively. Histopathological score of VN, OI and BA treated groups were 1 +, 2 + and 2 + respectively against 4 + of the toxic group. **Conclusion:** The findings suggested the significant nephroprotection of VN roots followed by OI whole plant and BA leaves.

**Key words:** Gentamicin, nephroprotection, *Oroxylum indicum* Vent. whole plants and *Barringtonia acutangula* Linn. leaves, *Vitex negundo* Linn. roots

## INTRODUCTION

Acute renal failure (ARF) is a major complication of kidney, encountered globally. Aminoglycoside induced nephrotoxicity is one of the leading cause of ARF, accounting about 10-15% of total cases of ARF across the world.<sup>[1]</sup> Gentamicin (aminoglycoside antibiotic) was introduced in 1963 and despite of its fatal side effects like, nephrotoxicity and ototoxicity, it has been used successfully for last 4 decades typically against gram-negative infections because of its good bactericidal efficacy and low cost. The exact mechanism of gentamicin induced nephrotoxicity

is yet to be elucidated completely. However, the etiology behind gentamicin induced nephrotoxicity is rested on the fact that aminoglycosides (gentamicin) are strong cationic drugs accumulated at biological membranes (especially at S<sub>1</sub>-S<sub>2</sub> segments of proximal tubule) causes net increase in oxidative stress and lipid peroxidation leading to necrotic changes in renal tubules and consequently precipitates acute nephrotoxicity.<sup>[2]</sup>

*Vitex negundo* Linn. (VN) (Family: Verbenaceae; locally known as *Begunia*), *Oroxylum indicum* Vent. (OI) (Family: Bignoniaceae; locally known as *Tatelo*) and *Barringtonia acutangula* Linn. (BA) (family: Barringtoniaceae; locally known as *Hinjal*) widely found throughout the Asian sub-continent including India.<sup>[3-5]</sup> VN, OI and BA are found to be used extensively in different forms of Indian traditional medicine<sup>[6,7]</sup> like Ayurveda and Unani. Seeds and

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leaves of VN are used externally in case of rheumatism and inflammation. Different parts of VN are used as tonic, diuretic, expectorant, vermifuge, febrifuge and antihelminthic.<sup>[8]</sup> Similarly, different parts of OI are used as astringent, tonic, anti-inflammatory, anti-rheumatic, anti-leucoderma, anti-helminthic, expectorant, piles, bronchitis, asthma, stomachic,<sup>[9,10]</sup> gastropathy and ulcers, diarrhoea, dysentery,<sup>[11]</sup> jaundice,<sup>[12]</sup> etc. Report suggests that various plant parts (root, seed, bark and leaf) of BA are used as carminative, expectorant, bitter tonic, emetic, fish poison and also used in the treatment of seminal weakness, diarrhoea, gonorrhoea and urinary tract infection.<sup>[13]</sup> The seeds and leaves are useful in vitiated conditions of *pitta* and *kapha*, colic, intestinal worms, wounds, ulcers, hallucination, as an anthelmintic, leprosy, splenomegaly, cough, bronchitis, dysmenorrhoea, intermittent fevers, ophthalmitis, syphilis, cephalalgia, and lumbago. In recent past several studies have been undertaken on VN, OI and BA with respect to their phytochemical properties and pharmacological activities, suggested antibacterial,<sup>[14]</sup> antifungal, cyto-toxic, analgesic, anti-inflammatory, anti-oxidant free radical scavenging, anti-histaminic, anti-androgenic activities<sup>[14,15]</sup> of VN, anti-cancer, free radical scavenging, immune-stimulant effects<sup>[16,17]</sup> of OI and anti-nociceptive, anti-diarrheal, neuro-pharmacological, hepatoprotective, anti-oxidant activities of BA respectively.<sup>[18]</sup>

Based on the ethnopharmacological studies and reported works the nephroprotective activity of VN, OI and BA was undertaken. Therefore, in the present context anti-nephrotoxic activity of methanol-dichloromethane (1:1) extracts of VN roots, OI whole plant and BA leaves were evaluated against gentamicin induce acute nephrotoxicity in rats with a brief exposure to their renal anti-oxidant activities (*in-vivo*).

## MATERIALS AND METHODS

### Plant materials

Fresh plant materials of VN roots, OI whole plant and BA leaves were collected in the month of September from Bhartapur Forest Range (Bhubaneswar, Odisha, India), Satakoshia Forest (Dhenkanal, Odisha, India) and coastal area of Jajpur district (Odisha, India) respectively. Plant materials were identified at Regional Research Laboratory (Institute of Mineral and Material Technology, Bhubaneswar, Orissa, India) and a voucher specimens of VN (Voucher No.: 9995/RRL (B)), OI (Voucher No.: 12500/RRL (B)) and BA (Voucher No.: 10001/RRL (B)) were deposited therewith. After authentication fresh plant materials of VN, OI and BA were collected, washed under running tap water, shade dried and pulverized in a mechanical grinder to get coarse plant materials.

### Animals

Healthy albino male rats of Wistar strain weighing between 150-200 g and Swiss albino mice weighing between 20-25 g were used in the nephroprotective activity and oral acute toxicity study respectively. The animals were kept under standard laboratory condition ( $12 \pm 1$  h, day-night schedule; temperature maintained at  $25 \pm 2^\circ\text{C}$ ; housed in large spacious hygienic cages with access to food and water *ad libitum*).

The Institutional Animal Ethics Committee approved the experimental protocol and the condition in the animal house was approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) with a registration number of 1210/PO/ac/08/CPCSEA.

### Chemicals and assay kits

Chemicals used in the study were of analytical grade (Merck Specialties Pvt. Ltd., India). Cystone (Polyherbal-reference drug; Himalya Drug Company, India), Genticyn injection (contains 80 mg of gentamicin sulphate per mL; Piramla Health Care (P) Ltd., India), biochemical estimation (urea and creatinine) kits (E-coline, Merck, India) and protein estimation kit (S.D Fine Chemicals, India) were purchased from local chemist and supplier. Catalase (CAT), Lactate dehydrogenase (LDH) and Glutathione peroxidase (GPx) assay kits were procured from Bio-Assay Systems, USA. Superoxide dismutase (SOD) and Lipid peroxidation (LPO) estimation kits were obtained from Enzo Life Sciences, Germany.

### Preparation of plant extracts and test doses

Pulverized plant materials of VN, OI and BA were extracted<sup>[13]</sup> separately at  $60^\circ\text{C}$  taking methanol-dichloromethane (1:1) as solvent over 24 h. Methanol-dichloromethane (1:1) extracts of VN, OI and BA were collected by removing excess solvent with the help of rotary evaporator (Heidolph Labrota-4000 Efficient) and preserved in air tight containers (yield: VN - 18.39%; OI - 15.87%; BA - 12.6%). Calculated amount of extracts of VN, OI and BA were suspended in 0.5% (v/v) of CMC to prepare the test doses.

### Oral acute toxicity study

Oral acute toxicity study<sup>[19]</sup> of the extract was carried out on albino mice at a dose range of 100-4000 mg/kg of body weight and  $\text{LD}_{50}$  was calculated.

### Anti-nephrotoxic activity

Total 36 rats were divided randomly into six groups of six animals in each. Group 1 (Normal): Each rat received 0.5% sodium CMC (5 mL/kg; p.o.; once daily) for 7 days. Group 2 (Toxic): Each rat received gentamicin (80 mg/kg; i.p.; once daily) for 7 days. Group 3 (reference drug treated), Group 4 (VN-200), Group 5 (OI-200) and Group 6 (BA-200) received Cystone<sup>[20,21]</sup> (5 mL/kg; p.o.;

once daily), VN extract (200 mg/kg; p.o.; once daily), OI extract (200 mg/kg; p.o.; once daily) and BA extract (200 mg/kg; p.o.; once daily) respectively along with gentamicin (80 mg/kg; i.p.; once daily) for 7 days.

### Renal function test(s)

Renal parameters like urine LDH, urine creatinine, serum creatinine, serum urea, serum albumin and serum total protein with physical parameters like, urine volume, renal somatic index and change in body weight was considered in the study. Estimation of different biochemical parameters of serum (urea, creatinine, albumin and total protein) and urine (creatinine) were carried out with the help of a biochemical semi-auto analyzer (EBRA-Chem-5 plus-V2, West-Germany) according to standard procedures<sup>[22]</sup> by using commercially available kits.

Urine from each test animals were collected over 24 h by keeping them in individual metabolic cages on 7<sup>th</sup> day followed by estimation of above mentioned urine parameters. Blood samples were collected from test animals under ether anaesthesia on 8<sup>th</sup> day by direct cardiac puncture before sacrifice and the above mentioned serum parameters were tested.

### Histopathological study

Kidneys from each rat were taken out surgically before sacrifice. Fatty adherents from the kidneys were removed (without washing) and the kidneys were weighed using a digital balance to calculate the Renal Somatic Index (RSI).<sup>[21]</sup> RSI was calculated as:  $RSI = (\text{kidney weight (g)} \div (\text{final body weight (g)})) \times 100$ .

For histological studies, a small transverse piece (3 mm<sup>3</sup> approximately) of tissue from each kidney were separated out surgically with precision so as to ensure minimal damage to the other part. Remaining parts of the kidneys were immediately processed for renal antioxidant study. Kidney tissue samples were fixed by using a formosal solution (10 v/v formaldehyde in normal saline), embedded with paraffin wax followed by preparation of tissue sections using a microtome and stained with Haemotoxylline-Eosin.<sup>[23]</sup> Tissue sections were examined (100X) under a polarized-light-microscope (LEICA-DM-750, Germany) and photomicrographs were taken using a digital camera. Histological features include tubular congestion, tubular cast, epithelial desquamation, glomerular congestion, blood vessel congestion and inflammatory cell infiltration were observed from the tissue sections and their corresponding histopathological scores<sup>[21,24]</sup> were assigned as follows: 0- tissue sections showing normal tubular and glomerular architectures with normal blood vessels and no tubular cast, 1- epithelial cell degeneration with tubular casts, 2- tubular epithelial

desquamation (<50%) with inflammatory cell infiltration, 3- tubular desquamation (>50%) with necrotic changes (few), 4- tubular necrosis (predominant/recognizable/abundant).

### Renal anti-oxidant activity

Renal anti-oxidant activity was evaluated by estimating the levels of renal anti-oxidant markers namely catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) along with the amount of lipid peroxidation (LPO) in renal tissue.

Prior to the homogenization the kidneys (after taking the tissue sample for histological study) were washed with phosphate buffered saline (pH: 7.4) to remove blood. Renal tissue homogenates were prepared by churning the kidneys in ice cold assay buffer (provided with assay kits) solution separately (10-20% of tissue homogenates were prepared for different assays as per the instructions) using a tissue homogenizer (Glass-Teflon Potter Elvehjem Tissue Homogenizer, Omni Int., India) and centrifuged at 14000 ×g (10-30 minutes at 4°C; time of centrifugation varies from one enzyme to another) in a ultra-freeze-centrifuge. The supernatants (containing cytosolic and mitochondrial fractions) were assayed directly for the renal anti oxidant markers (CAT, GPx and SOD) and LPO (10 µL of butylated hydroxytoluene provided as 500 nM acetonitrile was added to the samples and stored at -70<sup>o</sup> C for MDA estimation) according to the procedures indicated in kits using a Micro-plate reader (Sperctra-Max-340, Molecular Devices, USA).

Briefly, CAT activity was measured (OD<sub>570</sub>) as: CAT present in the given sample is directly proportional to the amount of H<sub>2</sub>O<sub>2</sub> decomposed and expressed in terms of nmol of H<sub>2</sub>O<sub>2</sub> per mg of protein per minute.<sup>[25]</sup> Activity of GPx was determined (OD<sub>340</sub>) on the basis of the change in OD following the consumption of NADPH and expressed in terms of nmol of oxidized NADPH per minute.<sup>[26]</sup> LPO in renal tissue was estimated (A<sub>586</sub>) in terms of malondialdehyde (MDA) production equivalent, in accordance with the BML-AK171 assay method (protocol provided with the assay kit) and expressed in terms of nmol per gram of protein. The principle of BML-AK171 assay<sup>[27]</sup> is as follows: two molecules of MDA react with one molecule of n-methyl-2-phenyl-indole at 45<sup>o</sup> C to form an intensely colored carbocyanine substance with  $\lambda_{\text{max}}$  of 586 nm and the change in absorbance of test sample was observed to calculate the MDA content. SOD activity was measured (A<sub>550</sub>) by its ability to inhibit the reaction dependent on the generation of superoxide ion by a xanthine-xanthine oxidase system. The reaction involved<sup>[28]</sup> were the conversion of WST-1 into WST-1-Formazan dye complex. Thus, the reduction in appearance of WST-1-Formazan is a measure of SOD present in the given sample.

## Statistics

Data obtained in the above studies were statistically analyzed and expressed as mean  $\pm$  SEM. Statistical significance was assessed by One-way ANOVA followed by Tukey-Kramer multiple comparison tests. Significance level was set at  $P < 0.05$ . Toxic group was compared with the normal group. Cystone treated group, VN-200, OI-200 and BA-200 groups were compared with toxic group.

## RESULTS

### Renal function test(s)

The results obtained from renal function tests are outlined in Table 1. Gentamicin administered rats (toxic group) exhibited significant weight loss ( $-5.75 \pm 1.54$ ;  $P < 0.01$ ) and decrease in urine volume output ( $3.75 \pm 0.46$ ;  $P < 0.001$ ) along with significant ( $P < 0.001$ ) elevation in urine LDH ( $78.61 \pm 5.25$ ), urine creatinine ( $2.51 \pm 0.24$ ), serum urea ( $88.75 \pm 6.16$ ) and serum creatinine ( $2.09 \pm 0.30$ ) when compared with normal rats (weight gain:  $4.46 \pm 0.99$ ; urine volume:  $10.91 \pm 0.83$ ; urine LDH:  $21.67 \pm 2.71$ ; urine creatinine:  $1.03 \pm 0.18$ ; serum urea:  $27.45 \pm 3.24$  and serum creatinine:  $0.62 \pm 0.14$ ).

Significant increase in body weight ( $2.41 \pm 1.76$ ;  $P < 0.05$ ) and urine volume ( $7.97 \pm 1.25$ ;  $P < 0.05$ ) was observed in Cystone (5mL/kg) treated group along with reduction in urine LDH ( $33.45 \pm 4.24$ ;  $P < 0.001$ ), urine creatinine ( $1.14 \pm 0.17$ ;  $P < 0.001$ ), serum urea ( $36.17 \pm 5.58$ ;  $P < 0.001$ ), serum creatinine ( $0.83 \pm 0.17$ ;  $P < 0.01$ ) and serum total protein ( $5.96 \pm 0.69$ ;  $P < 0.05$ ) levels as compared with the toxic group. On the other hand change in serum albumin level and RSI remained non-significant.

VN-200 group showed significant ( $P < 0.05$ ) elevation in body weight ( $2.67 \pm 1.43$ ) and urine volume ( $7.17 \pm 0.63$ ) as compared to toxic group. Attenuation in urine

LDH ( $26.35 \pm 2.21$ ;  $P < 0.001$ ), urine creatinine ( $1.40 \pm 0.25$ ;  $P < 0.01$ ), serum urea ( $42.98 \pm 4.02$ ;  $P < 0.001$ ) and serum creatinine ( $1.04 \pm 0.20$ ;  $P < 0.05$ ) levels were detected with non-significant changes in serum albumin, serum total protein levels and RSI value.

OI-200 group exhibited significant decrease in urine LDH ( $42.73 \pm 4.34$ ;  $P < 0.001$ ), urine creatinine ( $1.63 \pm 0.16$ ;  $P < 0.05$ ), serum urea ( $47.76 \pm 6.53$ ;  $P < 0.001$ ), and serum total protein value ( $5.75 \pm 0.48$ ;  $P < 0.05$ ) with net weight gain ( $3.12 \pm 2.29$ ;  $P < 0.01$ ) when compared with toxic group. However, non-significant changes were recorded in serum creatinine, serum albumin, urine volume output and RSI.

BA-200 group showed significant decrease in urine LDH ( $39.0 \pm 3.83$ ;  $P < 0.001$ ), urine creatinine ( $1.58 \pm 0.24$ ;  $P < 0.05$ ) and serum urea ( $57.83 \pm 3.86$ ;  $P < 0.01$ ) along with net weight gain ( $1.75 \pm 1.87$ ;  $P < 0.05$ ) when compared with toxic group. In contrast, non-significant changes were recorded in serum creatinine, serum albumin, serum total protein, urine volume output and RSI.

### Renal anti-oxidant activity

Oxidative stress experienced by different rat groups (evaluated in terms of renal anti-oxidant markers activities and amount of renal LPO) are cited in Table 2. Data obtained from toxic group revealed massive oxidative stress as shown by significant ( $P < 0.001$ ) reduction in renal anti-oxidant markers (CAT:  $85.7 \pm 6.25$ ; GPx:  $105.94 \pm 11.42$ ; SOD:  $6.85 \pm 1.04$ ) and significant ( $P < 0.001$ ) elevation in renal LPO ( $194.28 \pm 19.09$ ; expressed in terms of MDA) as compared to the normal group (MDA:  $69.26 \pm 14.15$ ; CAT:  $164.72 \pm 7.72$ ; GPx:  $220.97 \pm 9.67$ ; SOD:  $17.64 \pm 1.89$ ).

Significant increase in CAT ( $141.9 \pm 9.04$ ;  $P < 0.001$ ), GPx ( $197.21 \pm 12.86$ ;  $P < 0.001$ ) and SOD ( $13.78 \pm 1.31$ ;

**Table 1: Renal function test(s) profile of Vitex negundo, Oroxyllum indicum and Barringtonia acutangula treated rats**

Groups	Change in body weight (g)	Renal somatic index	Urine volume (mL)	Urine creatinine (g 24 h <sup>-1</sup> )	Urine LDH (IU)	Serum urea (mg dL <sup>-1</sup> )	Serum creatinine (mg dL <sup>-1</sup> )	Serum albumin (g dL <sup>-1</sup> )	Serum total protein (g dL <sup>-1</sup> )
Normal	4.46 $\pm$ 0.99	0.86 $\pm$ 0.03	10.91 $\pm$ 0.83	1.03 $\pm$ 0.18	21.67 $\pm$ 2.71	27.45 $\pm$ 3.24	0.62 $\pm$ 0.14	2.91 $\pm$ 0.12	5.23 $\pm$ 0.28
Toxic	-5.75 $\pm$ 1.54**	1.02 $\pm$ 0.05 <sup>ns</sup>	3.20 $\pm$ 0.46***	2.51 $\pm$ 0.24***	78.61 $\pm$ 5.25***	88.75 $\pm$ 6.16***	2.09 $\pm$ 0.30***	2.38 $\pm$ 0.35 <sup>ns</sup>	8.16 $\pm$ 0.46**
Cystone	2.41 $\pm$ 1.76 <sup>a</sup>	0.96 $\pm$ 0.08 <sup>ns</sup>	8.10 $\pm$ 1.25 <sup>b</sup>	1.14 $\pm$ 0.17 <sup>c</sup>	33.45 $\pm$ 4.24 <sup>c</sup>	36.17 $\pm$ 5.58 <sup>c</sup>	0.83 $\pm$ 0.17 <sup>b</sup>	2.57 $\pm$ 0.48 <sup>ns</sup>	5.96 $\pm$ 0.69 <sup>a</sup>
VN-200	2.67 $\pm$ 1.43 <sup>a</sup>	0.89 $\pm$ 0.08 <sup>ns</sup>	7.25 $\pm$ 0.63 <sup>a</sup>	1.40 $\pm$ 0.25 <sup>b</sup>	26.35 $\pm$ 2.21 <sup>c</sup>	42.98 $\pm$ 4.02 <sup>c</sup>	1.04 $\pm$ 0.20 <sup>a</sup>	2.72 $\pm$ 0.44 <sup>ns</sup>	6.34 $\pm$ 0.47 <sup>ns</sup>
OI-200	3.12 $\pm$ 2.29 <sup>a</sup>	0.81 $\pm$ 0.05 <sup>ns</sup>	6.70 $\pm$ 0.72 <sup>a</sup>	1.63 $\pm$ 0.09 <sup>a</sup>	42.73 $\pm$ 4.34 <sup>c</sup>	47.76 $\pm$ 6.53 <sup>c</sup>	1.23 $\pm$ 0.22 <sup>a</sup>	3.01 $\pm$ 0.29 <sup>ns</sup>	5.75 $\pm$ 0.48 <sup>a</sup>
BA-200	1.75 $\pm$ 1.87 <sup>a</sup>	0.90 $\pm$ 0.06 <sup>ns</sup>	5.48 $\pm$ 0.65 <sup>ns</sup>	1.58 $\pm$ 0.24 <sup>a</sup>	39.00 $\pm$ 3.83 <sup>c</sup>	57.83 $\pm$ 3.86 <sup>b</sup>	1.59 $\pm$ 0.10 <sup>ns</sup>	2.70 $\pm$ 0.25 <sup>ns</sup>	6.51 $\pm$ 0.39 <sup>ns</sup>
P value	0.0031	0.2252	<0.0001	0.0003	<0.0001	<0.0001	0.0001	0.8180	0.0038
F value	4.608	1.482	10.539	6.750	26.823	18.138	7.281	0.4385	4.438

\*= $P < 0.05$ ; \*\*= $P < 0.01$ ; \*\*\*= $P < 0.001$ ; for  $n=6$ ; (Toxic Vs Normal). a= $P < 0.05$ ; b= $P < 0.01$ ; c= $P < 0.001$ ; for  $n=6$ . (Toxic Vs all other groups); ns=non-significant. Toxic group was compared with normal group, Cystone, VN-200, OI-200 and BA-200 groups were compared with Toxic group. Normal: Normal rats received 0.5% of CMC (5mL/kg); Toxic=Gentamicin (80mg/kg) administered group; Cystone=Reference drug (Cystone; 5mL/kg) treated group; VN-200, OI-200 and BA-200 indicated methanol-dichloromethane (1:1) extracts of *Vitex negundo* (Linn.) root, *Oroxyllum indicum* (Vent.) whole plant and *Barringtonia acutangula* (Linn.) leaves were considered (200mg/kg) as treated groups respectively; LDH=lactate dehydrogenase



**Table 2: Renal anti-oxidant markers levels and renal lipid peroxidation in Vitex negundo, Oroxylum indicum and Barringtonia acutangula treated rats**

Groups	MDA (nmol/g protein)	CAT (H <sub>2</sub> O <sub>2</sub> nmol/ mg protein min <sup>-1</sup> )	GPx (oxidized NADPH nmol/ mg protein min <sup>-1</sup> )	SOD (U/mg protein)
Normal	69.26±14.15	164.72±7.72	220.97±9.67	17.64±1.89
Toxic	194.28±19.09***	85.70±6.25***	105.94±11.42***	6.85±1.04***
Cystone	121.8±12.74 <sup>a</sup>	141.9±9.04 <sup>c</sup>	197.21±12.86 <sup>c</sup>	13.78±0.81 <sup>a</sup>
VN-200	102.69±17.20 <sup>c</sup>	127.14±6.76 <sup>b</sup>	158.62±4.34 <sup>b</sup>	16.05±1.57 <sup>b</sup>
OI-200	130.40±8.47 <sup>a</sup>	119.28±5.09 <sup>a</sup>	167.95±10.23 <sup>c</sup>	15.13±1.95 <sup>b</sup>
BA-200	139.40±7.74 <sup>ns</sup>	130.31±4.03 <sup>c</sup>	174.61±4.75 <sup>c</sup>	8.50±1.05 <sup>ns</sup>
P value	<0.0001	<0.0001	<0.0001	<0.0001
F value	9.026	15.262	17.018	8.901

\*= $P<0.05$ ; \*\*= $P<0.01$ ; \*\*\*= $P<0.001$ ; for  $n=6$ ; (Toxic Vs Normal). a= $P<0.05$ ; b= $P<0.01$ ; c= $P<0.001$ ; for  $n=6$ . (Toxic Vs all other groups). ns=Non-significant. Toxic group was compared with normal group, Cystone, VN-200, OI-200 and BA-200 groups were compared with Toxic group. Normal=Normal rats received 0.5% of CMC (5mL/kg); Toxic=Gentamicin (80mg/kg) administered group; Cystone=Reference drug (Cystone, 5mL/kg) treated group; VN-200, OI-200 and BA-200 indicated methanol-dichloromethane (1:1) extracts of *Vitex negundo* (Linn.) root, *Oroxylum indicum* (Vent.) whole plant and *Barringtonia acutangula* (Linn.) leaves were considered (200mg/kg) as treated groups respectively; MDA=Malonaldehyde; SOD=Superoxide dismutase; CAT=Catalase; GPx=Glutathione peroxidase

$P < 0.05$ ) activities with decrease in LPO ( $121.8 \pm 12.74$ ;  $P < 0.05$ ) were observed in Cystone (5 mL/kg) treated rats.

VN-200 group exhibited significant ( $P < 0.01$ ) elevation in renal anti-oxidant markers activities (CAT:  $127.14 \pm 6.76$ ; GPx:  $158.62 \pm 4.34$  and SOD:  $15.13 \pm 1.57$ ) and reduction in renal LPO ( $102.69 \pm 17.20$ ) level as compared with toxic group.

LPO ( $130.40 \pm 8.47$ ) level in OI-200 group was decreased significantly ( $P < 0.05$ ) along with increase in the activities of CAT ( $119.28 \pm 5.09$ ;  $P < 0.05$ ) and GPx ( $167.95 \pm 10.23$ ;  $P < 0.01$ ). However, change in SOD ( $8.50 \pm 1.05$ ) remained statistically non-significant.

BA-200 group showed significant ( $P < 0.001$ ) elevation in CAT:  $130.31 \pm 4.03$  and GPx:  $174.61 \pm 4.75$  levels as compared with toxic group. However, SOD ( $8.50 \pm 1.05$ ) and reduction in renal LPO ( $139.40 \pm 7.74$ ) levels were found non-significant.

### Histopathological study

The histological features observed from the transverse sections (T.S) of kidneys of different rat groups are presented in Table 3 along with corresponding histopathological scores. Photomicrographs of the tissue sections are illustrated in Figure 1 (a-f). Histological features and histopathological scoring of (4+) observed from the toxic group suggests that the gentamicin administered rats had encountered massive histological damages as evidenced by predominant tubular and glomerular congestion with tubular casts, epithelial desquamation, blood vessel congestion and infiltration of few inflammatory cells. In contrast to the T.S of kidney of toxic rat, the VN-200 and Cystone groups exhibited minimal cellular damages. Analogues to the cystone treated group, VN-200 group (histopathological scoring: 1+) shows normal tubular arrangements and glomerular architectures

with normal blood vessels and absence of tubular cast. Histopathological scoring for OI-200 group and BA-200 is 2+ and the kidney T.S shows normal tubular arrangements with glomerular and blood vessel congestion implies a moderate level of renal tissue damage.

### Percentage (%) of increase/decrease in different parameters

Percentage of increase (↑) and decrease (↓) of different parameters from the base value that is from the toxic group were determined as:  $[(\text{value}_{\text{toxic}} - \text{value}_{\text{test}}) \div (\text{value}_{\text{toxic}}) \times 100]$  and were presented hereunder.

Body weight (↑) (VN: 146.4%; OI: 154.3% and BA: 130.4%); urine LDH (↓) (VN: 66.5%; OI: 45.6% and BA: 50.3%); urine creatinine (↓) (VN: 44.2%; OI: 35% and BA: 37%); serum urea (↓) (VN: 51.6%; OI: 46.2% and BA: 34.8%); serum creatinine (↓) (VN: ↓ 50.2%; OI: ↓ 32% and BA: non-significant); LPO (↓) (VN: 47.1%; OI: 32.8% and BA: non-significant); CAT (↑) (VN: 48.3%; OI: 39.2% and BA: 52%); GPx (↑) (VN: 49.7%; OI: 58.5% and BA: 64.8%) and SOD (↑) (VN: 120.9%; OI: 24.1% and BA: non-significant).

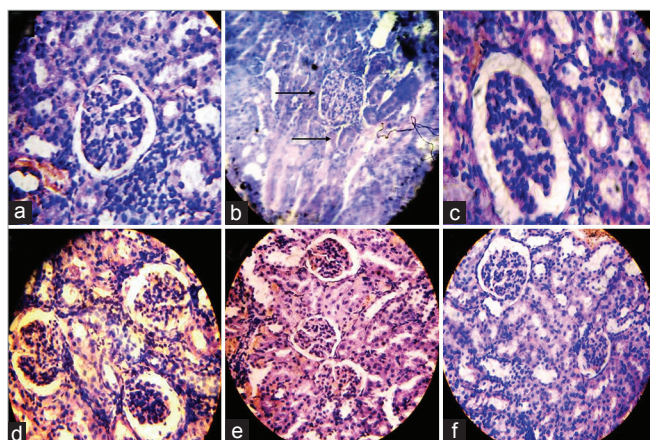
## DISCUSSION

Gentamicin being the most nephro-toxic antibiotic amongst all aminoglycosides, its over dosing eventually lead to acute nephrotoxicity and consequently precipitating ARF in human as well as animal subjects. The intracellular metabolism of drugs leads to the formation of reactive metabolites, which are toxic for cell, as are free radicals. The superoxide ion normally formed, during oxidation forms hydroxyl radicals which lead to lipid peroxidation. In turn, it causes oxidative deterioration of polyunsaturated lipids of membranes and causes the dramatic modification of structure and function. The toxic agent reduces the concentration of antioxidants, superoxide dismutase,

**Table 3: Histological features and histopathological scoring of transverse section of rat kidneys**

Groups →	Normal	Toxic	Cystone	VN-200	OI-200	BA-200
↓ Histological features and histopathological scoring						
Tubular congestion	-	++++	+	+	+	++
Tubular cast	-	++	-	-	+	+
Epithelial desquamation	-	++	+	+	+	+
Glomerular congestion	-	++++	-	-	++	++
Blood vessel congestion	-	++	+	+	+	++
Inflammatory cells	-	++	-	+	+	+
Histopathological scoring	0	4+	1+	1+	2+	2+

++=Indicated histological feature was present; --=Absence of histological features. Normal=Normal rats received 0.5% of CMC (5mL/kg); Toxic=Gentamicin (80mg kg<sup>-1</sup>) administered group; Cystone=Reference drug (Cystone; 5mL/kg) treated group; VN-200, OI-200 and BA-200 indicated methanol-dichloromethane (1:1) extracts of *Vitex negundo* (Linn.) root, *Oroxylum indicum* (Vent.) whole plant and *Barringtonia acutangula* (Linn.) leaves were considered (200mg/kg) as treated groups respectively



**Figure 1:** Photomicrographs of the tissue sections of (a) Normal: normal rats received 0.5% of CMC (5mL/kg); (b) Toxic: gentamicin (80mg/kg) administered group; (c) Cystone: reference drug (Cystone; 5mL/kg) treated group; (d) VN-200: methanol-dichloromethane (1:1) extract of *Vitex negundo* root (200mg/kg) treated group; (e) OI-200: methanol-dichloromethane (1:1) extract of *Oroxylum indicum* whole plant (200mg/kg) treated group; (f) BA-200: methanol-dichloromethane (1:1) extract of *Barringtonia acutangula* leaves (200mg/kg) treated group

glutathione, catalase, vitamin-E, ascorbic acid which is the protective tissues that reacts and removes reactive oxygen species. In our study, we observed acute renal damage in toxic group rats following gentamicin administration/overdosing manifested by: abnormal shifts in renal function tests (urine creatinine: ↑ 143.7; urine LDH: ↑ 262.7; serum urea: ↑ 223.3 and serum creatinine: ↑ 237%), renal antioxidant enzymes activities (CAT: ↓ 48; GPx: ↓ 52 and SOD: ↓ 180.5%) and high LPO (↑ 180.5%) in renal tissue with altered histopathological signs as compared to the normal rats. Final body weight and urine volume output of toxic group was also decreased by 228.9% and 65.6% respectively. In gentamicin intoxicated rats, elevated levels of serum urea and creatinine signifies depressed glomerular filtration; reduced CAT, GPx and SOD activities

corroborate a debilitated renal antioxidant defence against gentamicin induced oxidative stress and impaired urine LDH with high MDA (high renal-LPO) levels substantiate progressive tissue damage and cell death (s) in the kidneys of test animals.<sup>[29]</sup> Additionally, a monolithic elevation in histopathological score (4+), predominant tubular and glomerular anomalies (observed from T.S of toxic rats) with depressed urine volume output and severe weight loss in toxic group confirms the fatal nephrotoxic effect of gentamicin.

Taken together the results of renal function tests, antioxidant assays and histological features with histopathological scoring exposted herewith, suggests that the VN (200 mg/kg) root, OI (200 mg/kg) whole plant and BA (200 mg/kg) leaves extract treated rats were significantly protected from gentamicin induced acute nephropathy. For instance, attenuation of serum urea, creatinine and urine LDH levels in VN, OI and BA treated rats approves the accretion of glomerular filtration and a comparative elevation in renal antioxidant markers (CAT, GPx and SOD) levels supports that the renal enzyme defence against renal oxidative stress and free radical assaults are augmented. Secondly, a significant diminution in urine and MDA levels endorses the de-escalation of renal lipid peroxidation and cell death in rat kidneys. Finally, restoration of histological signs (alike normal rats) and moderate histopathological scores gained by VN-200 (1+), OI-200 (2+) and BA-200 (2+) groups along with a net increase in final body weights (VN: 146.4%; OI: 154.3% and BA: 130.4%) reassures that the test animals gained normalcy against the gentamicin induced acute renal dysfunction. In this manner, attenuation of major renal parameters levels, reduction in renal LPO, elevation in renal antioxidant markers activities and restoration of normal histological features, following the intervention of VN-200, OI-200 and BA-200 treatment for 7 days, validates the anti-nephrotoxic activities of VN, OI and BA extracts in rats. Moreover, the results obtained in our study are comparable with previously reported nephroprotective activity against gentamicin induced nephrotoxicity of plant species like *Tribulus terrestris* and *Crataeva nurvala*, *Ocimum sanctum*, *Withania somnifera*, *Embllica officinalis*, *Glycyrrhiza glabra*, *Kaloncha piñata*,<sup>[30]</sup> synthetic agents like *fleroxacin*<sup>[31]</sup> and herbo-mineral formulations like *Activit*<sup>[32]</sup> etc.

Phytochemical studies demonstrated presence of vitexin, iso-vitexin, negundin-A and B, vitrofolal-E and F, β-sitosterol, oleanolic acid<sup>[8]</sup> etc., in VN root while oroxylin-A, iso-flavone, prunetin, baicalein, chrysin, β-sitosterol<sup>[33]</sup> as chief phytoconstituents in OI whole plant and barringtogenic acid, tangulic and acutangulic acid<sup>[34]</sup> in BA leaves respectively. It is well documented that

several phytoconstituents (especially flavones, flavonoids, terpenoids and steroidal entities) and plants extracts demonstrated significant anti-oxidant and cyto-protective activities.<sup>[35,36]</sup>

Thus, we presume that the active phytoconstituents present in VN root, OI whole plant and BA leaves might have contributed towards the nephroprotective activities and the modest disparity observed in anti-nephrotoxic potencies of VN, OI and could possibly due to the sparing absorption, altered metabolism and impaired bio-availability of active component(s).

## CONCLUSION

Present investigation was aimed to evaluate and compare the anti-nephrotoxic activity of methanol extract of *Vitex negundo* (Linn.) root, *Oroxylum indicum* (Vent.) whole plant and *Barringtonia acutangula* (Linn.) leaves. The results conceived from the study suggested the nephroprotective of VN, OI and BA extracts against gentamicin induced acute nephrotoxicity in Wistar rats.

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