

Ameliorative properties of the chloroform stem bark extract of *Abrus precatorius* against gentamicin-induced renal damage in rats

Abstract

Aims: This study seeks to evaluate the nephroprotective effects of chloroform stem bark extract of *Abrus precatorius* in a murine model of gentamicin-induced renal damage.

Materials and Methods: Thirty male Wistar rats were divided into five groups; A being the normal control group and given normal saline. B as the toxicant group was given Gentamicin (GM) at 100 mg/kg, intraperitoneally for six days; C received chloroform extract of *Abrus precatorius* at 100mg/kg administered orally three days prior and concurrently with gentamicin for six days, D received 200 mg/kg of the extract and was administered orally for three days prior and concurrently with gentamicin for six days and E received gentamicin administered intraperitoneally for six days followed by administration of 200 mg/kg chloroform extract of *Abrus precatorius* for three days. Body and organ weight were determined. Serum and kidney homogenate were obtained. Creatinine, urea, Xanthine oxidase, Myeloperoxidase and Nitric oxide were assayed for in the serum. Advanced oxidative protein product, Protein carbonyl, Malondialdehyde, Hydrogen peroxide, Superoxide dismutase, Reduced Glutathione, Glutathione-S-transferase, Glutathione peroxidase, Protein thiol, Non-protein thiol were assayed for in the renal homogenate. Histopathological analysis and immunohistochemistry using Bcl2, CRP and NFkB were done to check for structural changes and protein expressions respectively.

Results: Markers of oxidative stress and inflammation were significantly increased in the toxicant group, but a significant reduction of these markers in the extract treated groups at pre and post treatment periods. Both enzymatic and non-enzymatic antioxidant level in the toxicant group were significantly depleted, whereas the levels of these enzymatic and non-enzymatic antioxidant levels were significantly elevated in a dose dependent manner in the extract treated groups. Histopathology revealed tubular necrosis, areas of inflammation, glomerular atrophy, and congestion in the toxicant group. These were ameliorated in the extract treated groups. Immunohistochemistry revealed decreased expression of Bcl₂ and

increase protein expression of CRP and NFkB in the toxicant group; however, the reverse was seen in the extract treated groups.

Conclusions: From these results, it can be concluded that the chloroform extract of *Abrus precatorius* stem bark has nephroprotective properties.

Keywords: *Abrus precatorius*, anti-oxidant, histopathology, immunohistochemistry, Nephroprotective.

Introduction

Abrus precatorius also known as Jequirity is a plant that belongs to the family Fabaceae. It is a woody twinning plant with distinguishing red seeds with black mark at the base, which is very poisonous (1). Various studies reported it to have therapeutic effect against diabetes, migraine, inflammation, pain (2) and bacterial and fungal infections, cause disruption in estrous cycle and block ovulation (3). It has also been reported to be effective in the treatment of wounds, ulcers, sores and throat scratches (4). Drug induced nephrotoxicity is becoming quite common nowadays when compared with previous times; this is probably because the predisposing factors are on the increase (5,6). Gentamicin is a popular aminoglycoside and is used widely in clinical practice for the treatment of gram-negative infections (7). Usually, it causes nephrotoxicity, which is characterized by direct tubular necrosis in glomerular structure in 10-20% of therapeutic case (8). It leads to the generation of hydrogen peroxide and also enhances the generation of reactive oxygen species (ROS) (9,10). Abnormal production of ROS may damage some macromolecules and thus induce cellular injury and necrosis via several mechanisms including peroxidation of membrane lipids, protein denaturation and DNA damage. The change in kidney functions prompted by products of lipid peroxidation is an early occurrence in gentamicin-mediated nephrotoxicity. Gentamicin also acts as an iron chelator and the iron-gentamicin complex is a potent catalyst of radical generation (11, 12). This study was designed to assess the nephroprotective effect of chloroform stem bark extract of *Abrus precatorius* on Gentamicin-induced renal damage in experimental rats' model.

Materials and Methods

Plant Extraction: Fresh stem bark of *Abrus precatorius* was obtained, identified and allotted voucher number UIH-22554 at the Herbarium Unit, Department of Botany, University of Ibadan. It was shade-dried and grinded. This was macerated using analytical grade

63 chloroform, filtered using a Buckner funnel and Whatman No. 1 filter paper. The filtrate was
64 concentrated using a rotary evaporator and water bath.

65 **Experimental animals:** All experiments and protocols described in present study were
66 approved by the UI-ACUREC. Thirty (30) male Wistar rats weighing between 100 and 170g
67 were obtained from the Experimental animal unit of the Faculty of Veterinary Medicine,
68 University of Ibadan for the experiment. They were allowed free access to standard rat pellets
69 and fresh water *ad libitum*. The rats were housed in the animal house unit of the Department
70 of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan with a 12-
71 hour light duration. The rats were acclimatized for two weeks before commencement of the
72 experiment.

73 **Nephroprotective Study:** Thirty (30) male Wistar rats divided into five groups of six
74 animals per group were used in this study. Group A was the control group and received
75 distilled water daily throughout the study. Group B was the toxicant group and received
76 Gentamicin 100mg/kg for six days via intra peritoneal route. Group C animals were
77 pretreated with 100mg of chloroform extract prior to the administration of Gentamicin via
78 oral route for three days and then concurrently with Gentamicin 100mg/kg/i.p. for another six
79 days. Group D animals were pretreated with 200mg of chloroform extract prior to the
80 administration of Gentamicin via oral route for three days and then concurrently with
81 Gentamicin 100mg/kg/i.p. for another six days. Group E animals were treated with
82 Gentamicin 100mg/kg/i.p. for the first six days and then treated with 200mg of chloroform
83 extract alone via oral route for another four days. Subsequently blood was collected, and then
84 the animals were sacrificed using cervical dislocation and the kidneys were obtained, rinsed
85 with normal saline, blotted using filter paper, homogenized using a Teflon homogenizer and
86 0.1M phosphate buffer, pH 7.4 as homogenizing buffer, centrifuged with cold centrifuge to
87 obtain the renal homogenate and stored in the freezer for the assays.

88 **Serum Assays:** Blood was collected into plain sample bottles, centrifuged with a macro
89 centrifuge, serum was obtained and used to assay for total protein levels according to Gornal
90 *et al.*, (13). Serum urea was determined using Randox kit (UR2821), while serum creatinine
91 Randox Kit (CR2337) manufactured by (Randox Laboratories Ltd, Crumlin, UK) was used to
92 measure serum creatinine according to manufacturer's manual. Xanthine oxidase,
93 Myeloperoxidase activities were determined according to methods described by Akaike *et al.*,

(14) and Xia and Zweier (15) respectively. Nitric oxide was also assayed for using Greiss reagent as described by Olaleye *et al.*, (16).

Renal homogenate assays: Total protein levels of the post-mitochondrial fractions of the kidney was determined using Biuret method as described by Gornal *et al.*, (13) with potassium iodide added to the reagent to prevent precipitation of Cu^{2+} ions as cuprous oxide. The method of Beutler *et al.* (17) was followed in estimating the level of reduced glutathione (GSH) in which 0.5 mL of 4% sulfosalicylic acid (precipitating agent) was added to 0.5 mL of sample and centrifuged at 4, 000 rpm for 5 minutes. To 0.5 mL of the resulting supernatant 4.5 mL of Ellman's reagent (0.04 g of DTNB in 100 mL of 0.1M phosphate buffer, pH 7.4) was added. The absorbance was read at 412 nm against distilled water as blank. Glutathione-S- transferase activity was determined according to Habig *et al* (18) using 1-chloro-2, 4-dinitrobenzene as substrate. Glutathione peroxidase activity was measured according to Beutler *et al.*, (17) in which the reaction mixtures contain 0.5 mL of potassium phosphate buffer (pH, 7.4), 0.1 mL of Sodium azide, 0.2 mL of GSH solution, 0.1 mL of H_2O_2 , 0.5 mL of sample and 0.6 mL of distilled water. The mixture was incubated in the water bath at 37°C for 5 min and 0.5 mL of TCA was added and centrifuged at 4,000 rpm for 5 min. A volume of 1 mL of the supernatant was taken and added 2 mL of K_2PHO_4 and 1 mL of Ellman's reagent. The absorbance was read at 412 nm using distilled water as blank. Sulphydryl (thiol) and non-protein thiol levels were determined as described by Sedlak *et al.*, (19). Superoxide dismutase activity was determined according to Misra and Fridovich (20) in which 100 mg of epinephrine was dissolved in 100 mL distilled water and acidified with 0.5 mL concentrated hydrochloric acid. Thirty microliters of post mitochondrial fraction were added to 2.5 mL 0.05 M carbonate buffer (pH 10.2) followed by the addition of 300 μL of 0.3 mM adrenaline. The increase in absorbance at 480 nm was monitored every 30 s for 150 s. The one unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome. Markers of oxidative stress such as Protein carbonyl was determined as described by Reznick and Packer (21). To 100 μL of tissue supernatant were placed in glass tubes, 500 μL of 10mM 2, 4-dinitrophenylhydrazine (DNPH) in 2 N HCl were added and thereafter incubated for 1 hour at room temperature. Reaction mixtures were vortexed every 15 minutes. Then, 500 μL of TCA (20%) were added and the tubes were left for 5 minutes followed by centrifugation at 4, 000 rpm for 10 minutes to collect the protein precipitates. The pellet was then washed twice with ethanol-ethyl acetate (v/v). The final precipitate was dissolved in 600 μL of 6M guanidine hydrochloride solution and

incubated for 15 minutes at 37°C. The absorbance of the sample was measured at 370nm. The carbonyl content was calculated based on the molar extinction coefficient of DNPH ($2.2 \times 10^4 \text{ cm}^2 \text{ M}^{-1}$) and expressed as nmoles/mg protein. Advanced oxidative protein products (AOPP) was assayed as described by Kayali *et al.*, (22). 0.4 ml of renal PMFs were treated with 0.8 ml phosphate buffer (0.1 M; pH 7.4). After 2 minutes, 0.1 ml of 1.16 M potassium iodide (KI) was added to the tube followed by 0.2 ml of acetic acid. The absorbance of the reaction mixture was immediately recorded at 340 nm wavelength. The content of AOPP for each sample was calculated using the extinction coefficient of $261 \text{ cm}^2 \text{ mM}^{-1}$ and the results were expressed as $\mu\text{moles/mg protein}$. Hydrogen peroxide generation was measured as described by Woff (23). To 2.5 mL of 0.1M potassium phosphate buffer (pH 7.4), 250 μL of ammonium ferrous sulphate, 100 μL of sorbitol, 100 μL of xylenol orange (XO), 25 μL of H_2SO_4 and 50 μL of Sample was added. The mixture was mixed thoroughly by vortexed and a light pink color of the reaction mixture was observed. The reaction mixture was subsequently incubated at room temperature for 30 minutes. The mixtures were read at absorbance at 560 nm. The H_2O_2 generated was extrapolated from H_2O_2 standard curve. Lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) according to Varshney and Kale (24). To 1.6 mL of Tris-KCl, 0.5 mL of 30% TCA, 0.4 mL of sample and 0.5 mL of 0.75% TBA prepared in 0.2 M HCl were added. The reaction mixture was incubated in the water bath at 80°C for 45 minutes, cooled on ice and centrifuged at 4,000 rpm for 15 minutes. The absorbance was measured against a blank of distilled water at 532 nm. Lipid peroxidation was calculated with a molar extinction coefficient of $1.56 \times 10^5 \text{ M/cm}$.

Histopathology: The kidneys were sectioned longitudinally into equal halves and were kept in 10% neutral formalin solution; processed and embedded in paraffin wax and sections were taken using a microtome. The sections were stained with haematoxylin and eosin and were observed under a light microscope. The sections were evaluated for the pathological symptoms of nephrotoxicity such as necrosis, fatty infiltration, fibrosis, lymphocyte infiltration in kidneys (25).

Immunohistochemistry: Immunohistochemistry of paraffin embedded tissue of the kidney was done from buffered formalin perfused rats. Paraffin sections were melted at 60 °C in the oven. Dewaxing of the samples in xylene was followed by passage through ethanol of decreasing concentration (100-80%). Peroxidase quenching in 3% H_2O_2 /methanol was carried out with subsequent antigen retrieval performed by microwave heating in 0.01 M

citrate buffer (pH 6.0) to boil. All the sections were blocked in normal goat serum (10%, HistoMark[®], KPL, Gaithersburg MD, USA) and probed with CRP antibody, NFkB antibody and Bcl-2 antibody (Abclonal[®]), 1:375 for 16 h in a refrigerator. Detection of bound antibody was carried out using biotinylated (goat anti-rabbit, 2.0µg/ml) secondary antibody and subsequently, streptavidin peroxidase (Horse Radish Peroxidase- streptavidin) according to manufacturer's protocol (HistoMark[®], KPL, Gaithersburg MD, USA). Reaction product was enhanced with diaminobenzidine (DAB, Amresco[®], USA) for 6 – 10 min and counterstained with high definition haematoxylin (Enzo[®], NY - USA), with subsequent dehydration in ethanol. The slides were covered with cover slips and sealed with resinous solution. The immunoreactive positive expression of CRP, Bcl-2 and NFkB intensive regions were viewed starting from low magnification on each slice then with 400 × magnifications using a photo microscope (Olympus) and a digital camera (Toupcam[®], ToupTek Photonics, Zhejiang, China).

Statistics: Results are expressed as mean ± standard deviation (SD). All results were analyzed using student's t- test and one-way ANOVA with Tukey's post-hoc test using Graph prism 5 and p-values < 0.05 considered to be statistically significant

Results

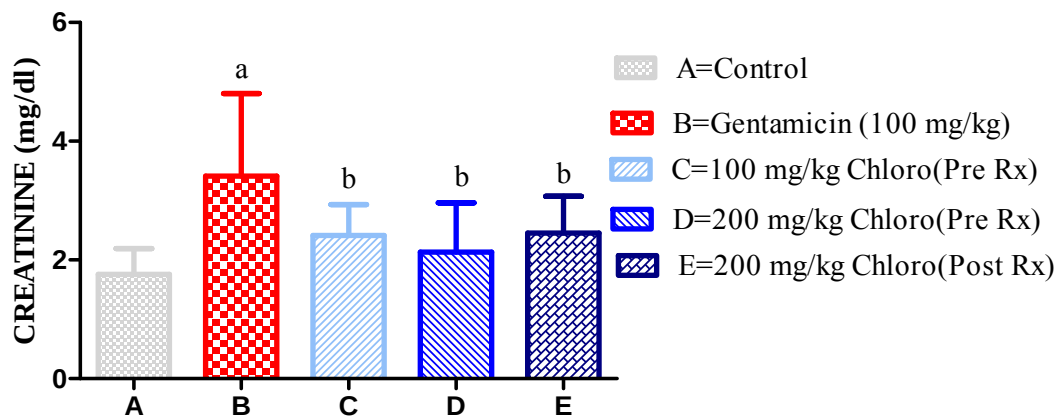
The results at P<0.05 showed a significant increase in markers of oxidative stress in the serum and renal homogenate of the toxicant group, but a significant reduction of these markers in the extract treated groups at pre and post treatment periods. The result also showed significant depletions of both enzymatic and non-enzymatic antioxidant level in the toxicant group whereas the levels of these enzymatic and non-enzymatic antioxidant levels were significantly elevated in a dose dependent manner in the extract treated groups. Histopathology revealed tubular necrosis, areas of inflammation, glomerular atrophy, and congestion of blood vessels in the toxicant group. These lesions however were ameliorated in the extract treated groups. Immunohistochemistry also revealed decrease expression in protein of Bcl₂ and increase protein expression of CRP and NFkB in the toxicant group, however, the reverse was seen in the extract treated groups.

Table 1: Effects of the chloroform stem extracts of *Abrus precatorius* on the organo-somatic indices

	A	B	C	D	E
%WEIGHT GAIN	17.2±4.1	15.2±2.4	20±7.0 ^b	26.4±8.2 ^b	22.3±10.7 ^b
BODY WEIGHT	127±10.6	162.8±7.5	135.6±9.4	139.7±24.9	148±13.0
ORGAN WEIGHT	0.7±0.2	0.8±0.3	0.8±0.2	0.7±0.3	1.0±0.4
KIDNEY/BODY WEIGHT	0.5±0.2	0.4±0.2	0.6±0.2	0.5±0.2	0.6±0.2

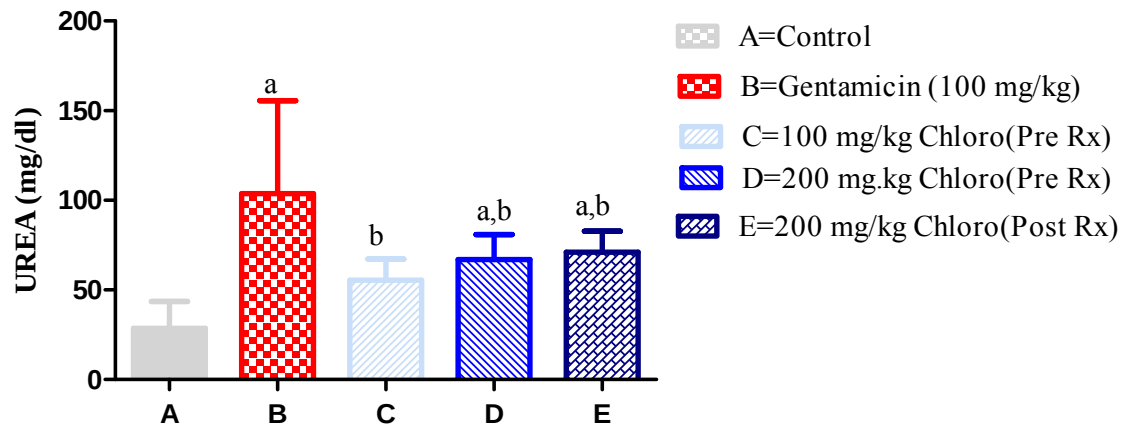
(A=control, B=Gentamicin 100mg/kg, C= 100mg/kg chloroform extract + Gentamicin 100mg/kg , D= 200 mg/kg chloroform extract + Gentamicin 100mg/kg , E= Gentamicin 100mg/kg + 200 mg/kg chloroform post treatment). Results expressed in Mean± SD, n=6. Superscripts (a, b) indicate significant difference at $P=0.05$. Superscript (a) indicates significant difference when groups B, C, D and E were compared with group A. Superscripts (b) indicates significant difference when groups C, D and E were compared with group. Results expressed in Mean± SD, n=6

Figure 1: Effects of the Chloroform Stem extract of *Abrus precatorius* on the serum creatinine levels



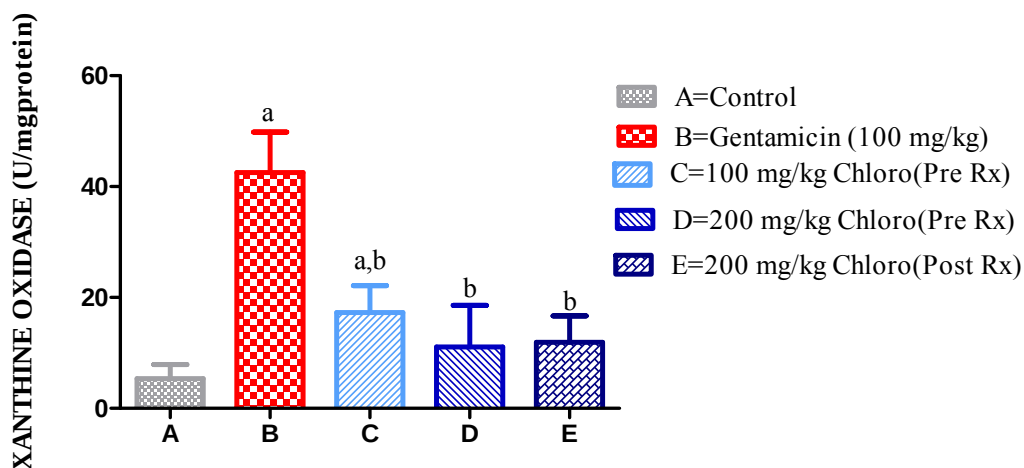
There was significant elevation in serum creatinine level in the toxicant group when compared with the control, while *Abrus precatorius* significantly ameliorated it. Superscripts (a, b) indicate significant difference at $P=0.05$. Superscript (a) indicates significant difference when groups B, C, D and E were compared with group A. Superscripts (b) indicates significant difference when groups C, D and E were compared with group B. Results expressed in Mean $\pm$ SD, n=6

Figure 2: Effects of the Chloroform Stem extract of *Abrus precatorius* on the serum urea levels.



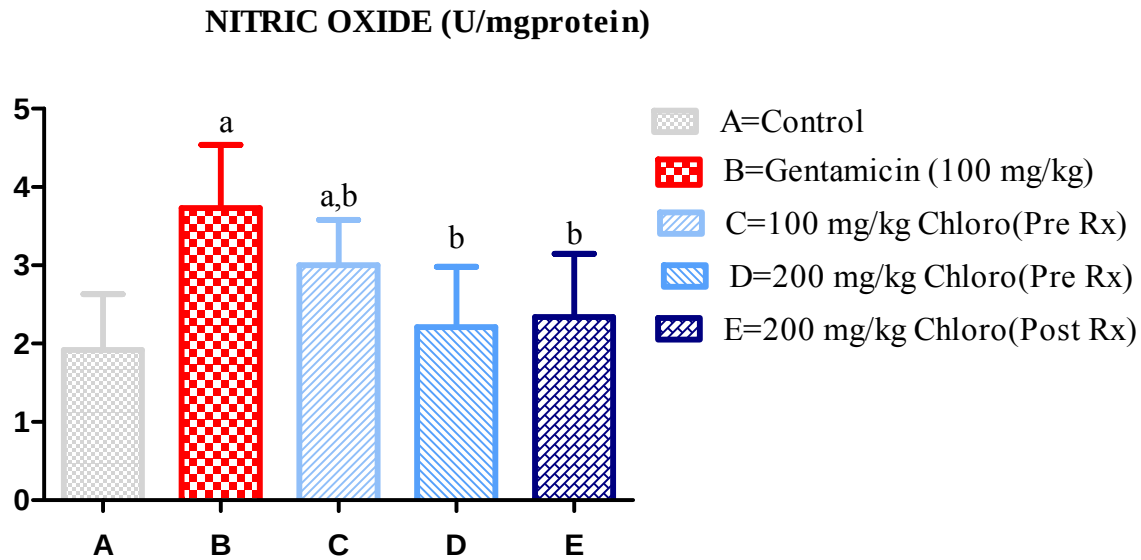
There was significant elevation in serum urea level in the toxicant group when compared with the control, while *Abrus precatorius* significantly ameliorated it. Superscripts (a, b) indicate significant difference at $P=0.05$. Superscript (a) indicates significant difference when groups B, C, D and E were compared with group A. Superscripts (b) indicates significant difference when groups C, D and E were compared with group B. Results expressed in Mean $\pm$ SD, n=6

Figure 3: Effects of the Chloroform Stem extract of *Abrus precatorius* on serum Xanthine oxidase levels



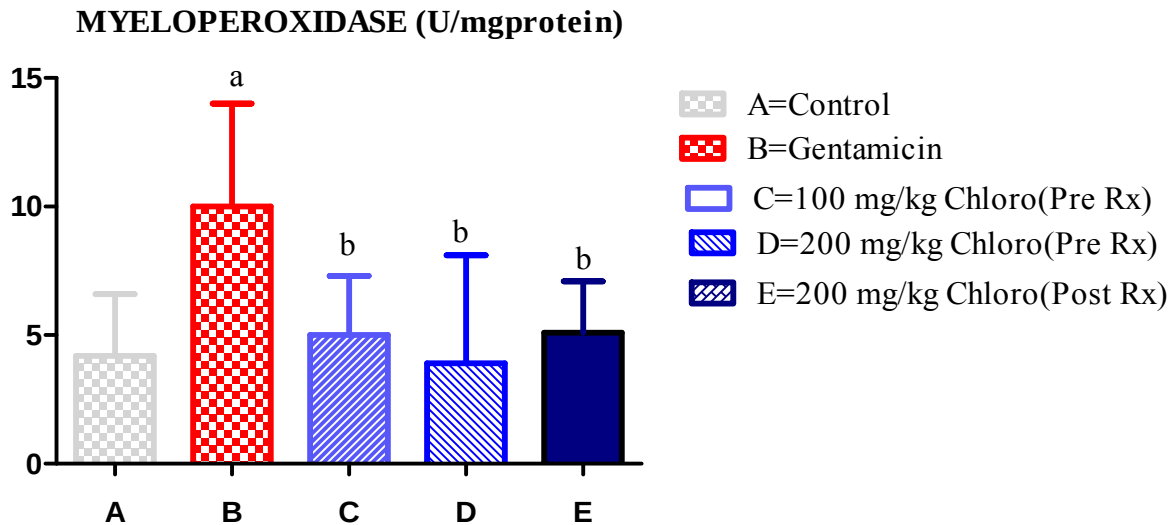
There was significant elevation in serum xanthine oxidase level in the toxicant group when compared with the control, while *Abrus precatorius* significantly ameliorated it. Superscripts (a, b) indicate significant difference at $P=0.05$. Superscript (a) indicates significant difference when groups B, C, D and E were compared with group A. Superscripts (b) indicates significant difference when groups C, D and E were compared with group B. Results expressed in Mean $\pm$ SD, n=6

Figure 4: Effects of the Chloroform Stem extract of *Abrus precatorius* on serum nitric oxide levels



There was significant elevation in serum nitric oxide level in the toxicant group when compared with the control, while *Abrus precatorius* significantly ameliorated it. Superscripts (a, b) indicate significant difference at $P=0.05$. Superscript (a) indicates significant difference when groups B, C, D and E were compared with group A. Superscripts (b) indicates significant difference when groups C, D and E were compared with group B. Results expressed in Mean $\pm$ SD, n=6

Figure 5: Effects of the Chloroform Stem extract of *Abrus precatorius* on serum myeloperoxidase levels



There was significant elevation in serum myeloperoxidase level in the toxicant group when compared with the control, while *Abrus precatorius* significantly ameliorated it. Superscripts (a, b) indicate significant difference at $P=0.05$. Superscript (a) indicates significant difference when groups B, C, D and E were compared with group A. Superscripts (b) indicates significant difference when groups C, D and E were compared with group B. Results expressed in Mean $\pm$ SD, n=6

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	A	B	C	D	E
SOD (units/mgprotein)	5.3±2.0	2.9±0.1 ^a	3.3±0.5 ^{a,b}	3.6±1.2 ^b	4.5±1.0 ^b
GST (umol/min/mgprotein)	18.0±5.0	5.6±2.6 ^a	17.0±5.8 ^b	15.2±4.8 ^b	11.6±3.2 ^b

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Table 2: Effects of the chloroform stem extract of *Abrus precatorius* on renal enzymatic antioxidant levels (GST, GPx and SOD)

GPx (units/mgprotein)	8.3±0.8	11.7±2.0 ^a	11.8±1.7	11.9±4.8	8.4±2.6 ^b
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315 Gentamicin significantly depleted renal SOD and GST levels while *Abrus precatorius*
316 improved the levels, GPx levels were elevated in the toxicant group when compared with
317 control. Superscripts (a, b) indicate significant difference at $P=0.05$. Superscript (a) indicates
318 significant difference when groups B, C, D and E were compared with group A. Superscripts
319 (b) indicates significant difference when groups C, D and E were compared with group B.
320 Results expressed in Mean± SD, n=6

321 (A=control, B=Gentamicin 100mg/kg, C= 100mg/kg chloroform extract + Gentamicin
322 100mg/kg, D= 200 mg/kg chloroform extract + Gentamicin 100mg/kg, E= Gentamicin
323 100mg/kg + 200 mg/kg chloroform post treatment)

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Table 3: Effects of chloroform stem extract of *Abrus precatorius* on Renal reduced GSH, Total thiol, and Non protein thiol

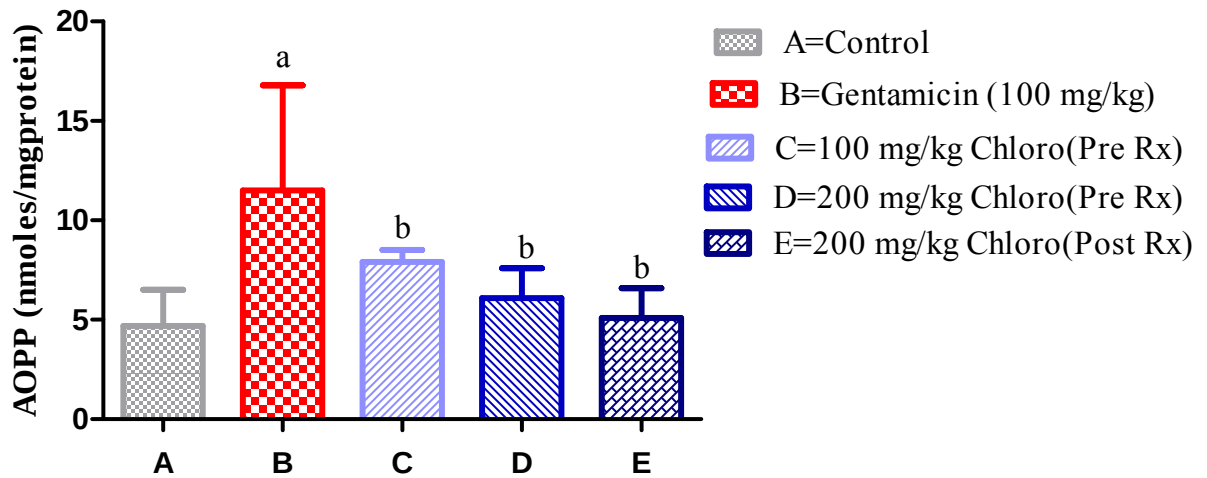
	A	B	C	D	E
Reduced GSH	82.7±2.4	75.3±1.8^a	80.6±3.3^b	81.3±6.4^b	77.6±3.9^b
Total Thiol (U/mgprotein)	2.9±0.4	2.3±0.3^a	2.8±0.6^b	3.0±0.9^b	3.0±0.8^b
Non-Protein Thiol (U/mgprotein)	3.5±0.4	2.1±1.3^a	3.1±0.6^b	3.3±1.1^b	3.0±0.5^b
Total Protein (mgprotein)	26.1±2.5	16.8±0.7^a	20.3±2.0^b	24.1±5.4^b	25.1±5.3^b

Reduced glutathione, total thiols, non-protein thiols and total protein levels were significantly increased in the toxicant group, however, groups treated with chloroform stem extract of *Abrus precatorius* showed values similar to those of control. Superscripts (a, b) indicate

341 significant difference at $P=0.05$. Superscript (a) indicates significant difference when groups
342 B, C, D, and E were compared with group A. Superscripts (b) indicates significant difference
343 when groups C, D, and E were compared with group B. Results expressed in Mean $\pm$ SD, n=6
344 (*A=control, B=Gentamicin 100mg/kg, C= 100mg/kg chloroform extract + Gentamicin*
345 *100mg/kg, D= 200 mg/kg chloroform extract + Gentamicin 100mg/kg , E= Gentamicin*
346 *100mg/kg + 200 mg/kg chloroform post treatment*)

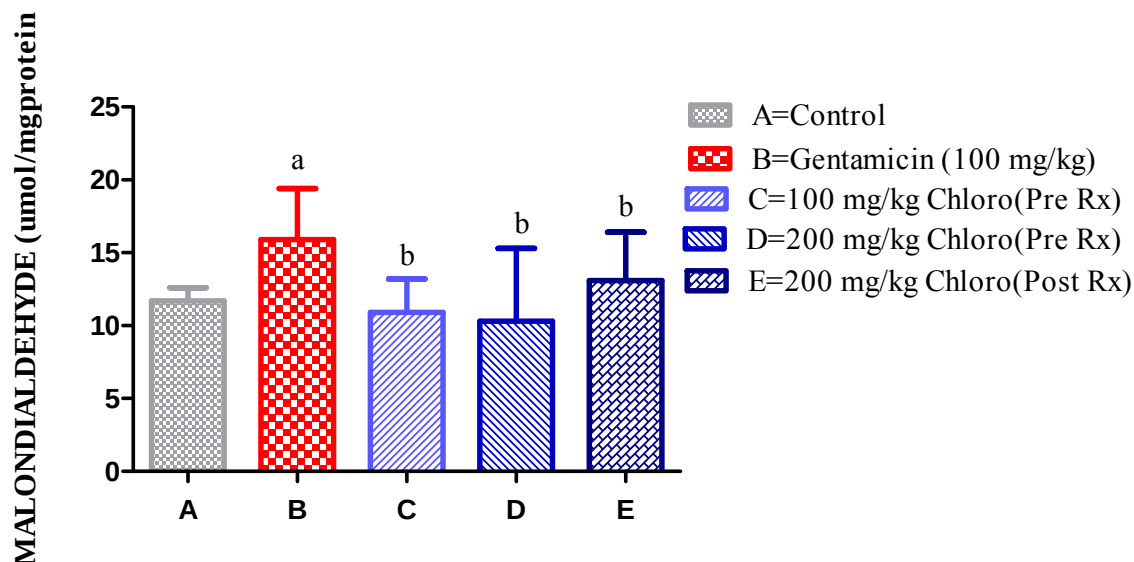
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Figure 6: Effects of chloroform stem extract of *Abrus precatorius* on renal advanced oxidative protein product (AOPP) levels



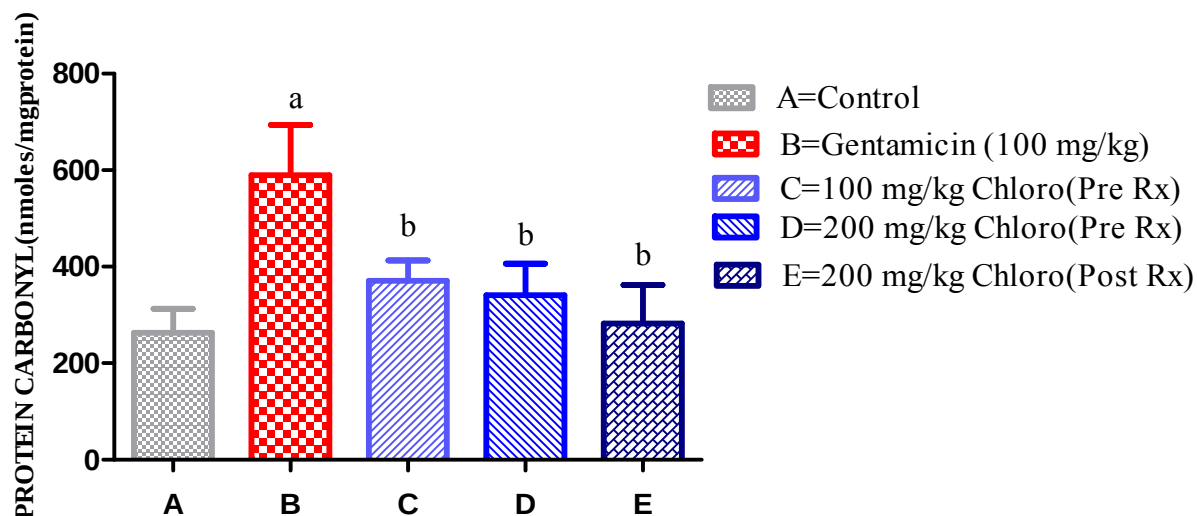
Gentamicin significantly elevated renal levels of AOPP, however treatment with *Abrus precatorius* significantly reduced AOPP levels. Superscripts (a, b) indicate significant difference at $P=0.05$. Superscript (a) indicates significant difference when groups B, C, D and E were compared with group A. Superscripts (b) indicates significant difference when groups C, D and E were compared with group B. Results expressed in Mean $\pm$ SD, n=6

Figure 7: Effects of chloroform stem extract of *Abrus precatorius* on renal lipid peroxidation products level (malondialdehyde MDA)



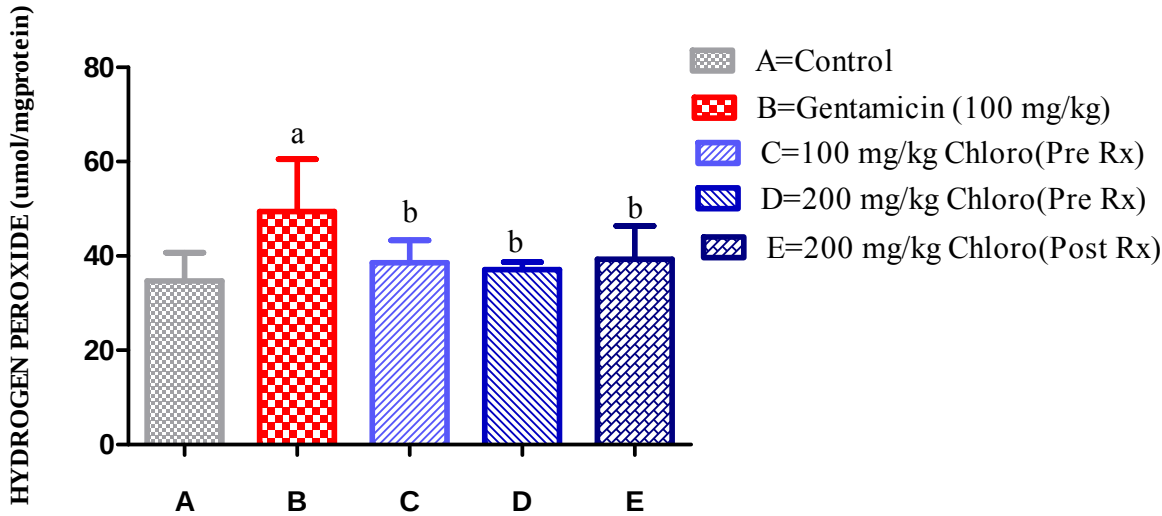
Gentamicin significantly elevated renal levels of MDA, however treatment with *Abrus precatorius* significantly reduced MDA levels. Superscripts (a, b) indicate significant difference at $P=0.05$. Superscript (a) indicates significant difference when groups B, C, D and E were compared with group A. Superscripts (b) indicates significant difference when groups C, D and E were compared with group B. Results expressed in Mean $\pm$ SD, $n=6$.

Figure 8: Effects of chloroform stem extract of *Abrus precatorius* on renal protein carbonyl levels



Gentamicin significantly elevated renal levels of protein carbonyl, however treatment with *Abrus precatorius* significantly reduced protein carbonyl levels. Superscripts (a, b) indicate significant difference at $P=0.05$. Superscript (a) indicates significant difference when groups B, C, D and E were compared with group A. Superscripts (b) indicates significant difference when groups C, D and E were compared with group B. Results expressed in Mean $\pm$ SD, $n=6$

Figure 9: Effects of chloroform stem extract of *Abrus precatorius* on renal Hydrogen peroxide level



Gentamicin significantly elevated renal levels of hydrogen peroxide levels, however treatment with *Abrus precatorius* significantly reduced hydrogen peroxide levels. Superscripts (a, b) indicate significant difference at $P=0.05$. Superscript (a) indicates significant difference when groups B, C, D and E were compared with group A. Superscripts (b) indicates significant difference when groups C, D and E were compared with group B. Results expressed in Mean $\pm$ SD, n=6

HISTOPATHOLOGY

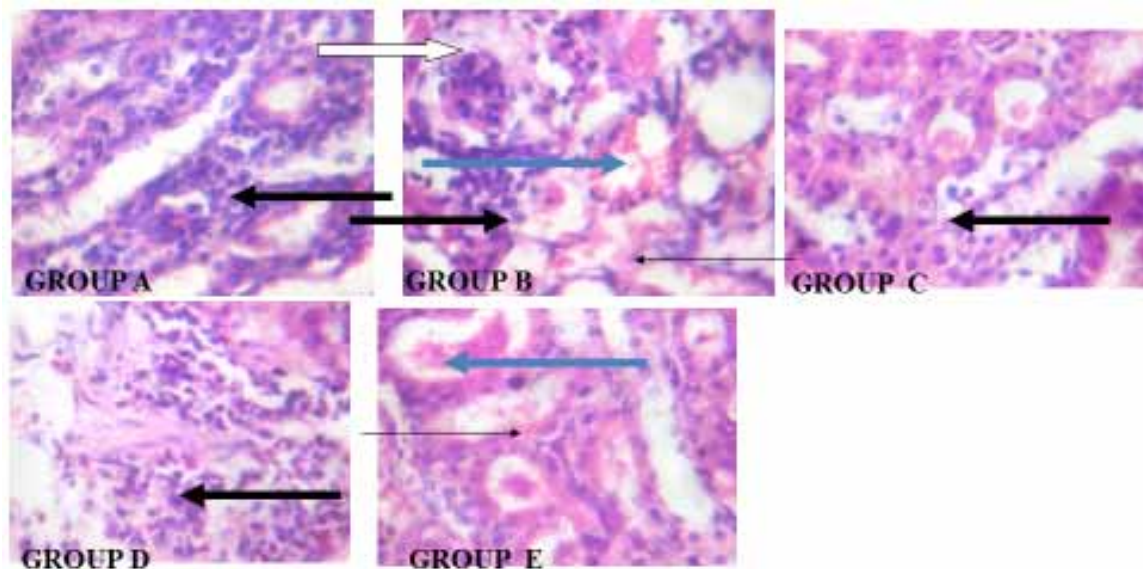
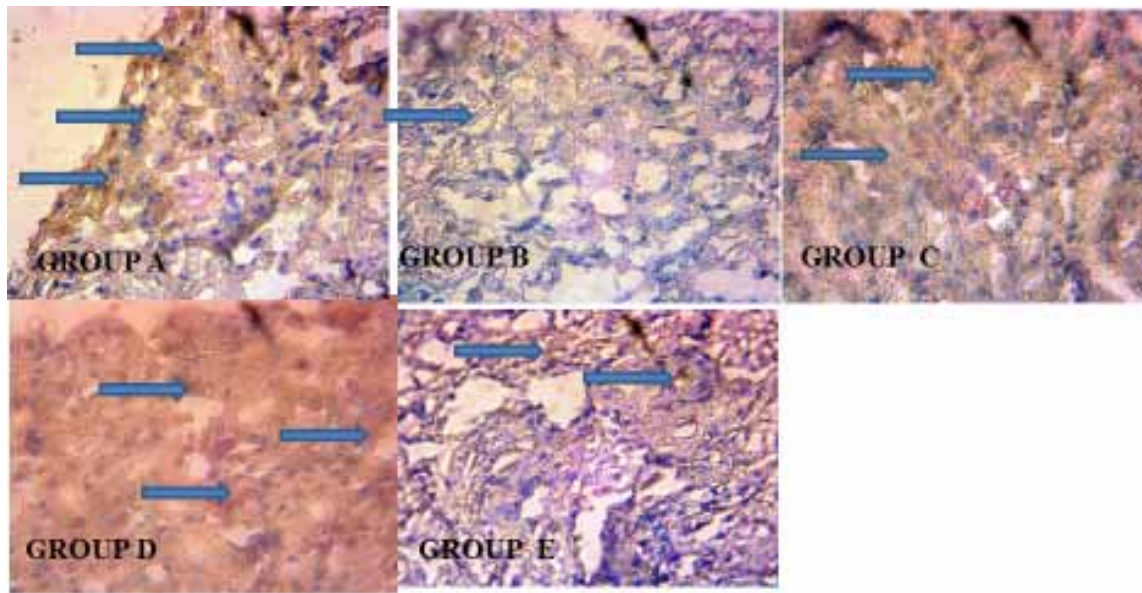


Figure 10: Photomicrograph of kidney plates at X400. **Group A** shows normal morphology of the tubules and no evidence of effacement in the glomerular basement membrane. **Group B** shows congestion of vessels (blue arrow), focal area of inflammation (black arrow), tubular necrosis (slender arrow), and glomerular atrophy (white arrow). **Group C** shows mild sub capsular fatty degeneration of the tubules (black arrows). **Group D** shows focal area of inflammation (black arrows). **Group E** shows presence of red blood cells in the tubule suggestive of glomerular basement membrane effacement (blue arrow) and a focal area of tubular necrosis (slender arrow).

(A=control, B=Gentamicin 100mg/kg, C= 100mg/kg chloroform extract + Gentamicin 100mg/kg, D= 200 mg/kg chloroform extract + Gentamicin 100mg/kg , E= Gentamicin 100mg/kg + 200 mg/kg chloroform post treatment)

436 IMMUNOHISTOCHEMISTRY

437 Bcl₂ ANTIBODY



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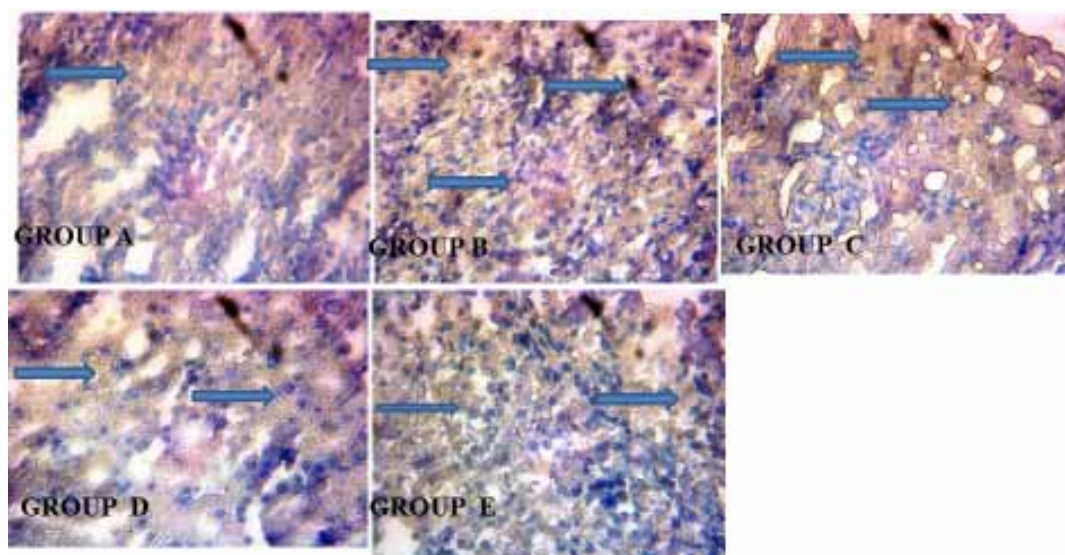
439 **Figure 11 Group A** (normal control) shows highest expression of Bcl₂ in the kidney section.

440 **Group B** (Toxicant group/ gentamicin 100 mg/kg) shows least expression of Bcl₂ when
441 compared with **A, C, D and E**.

442 (*A=control, B=Gentamicin 100mg/kg, C= 100mg/kg chloroform extract + Gentamicin*
443 *100mg/kg, D= 200 mg/kg chloroform extract + Gentamicin 100mg/kg, E= Gentamicin*
444 *100mg/kg + 200 mg/kg chloroform post treatment*)

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446 **CRP (C-REACTIVE PROTEIN) ANTIBODY**



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448 **Figure 12: Group A** (normal control) shows least expression of CRP in the kidney section.

449 **Group B** (Toxicant group/ gentamicin 100 mg/kg) shows highest expression of CRP when
450 compared with **A, C, D and E**.

451 (*A=control, B=Gentamicin 100mg/kg, C= 100mg/kg chloroform extract + Gentamicin*
452 *100mg/kg, D= 200 mg/kg chloroform extract + Gentamicin 100mg/kg, E= Gentamicin*
453 *100mg/kg + 200 mg/kg chloroform post treatment*)

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NF-KB (NUCLEAR FACTOR KAPPA BETA ANTIBODY)

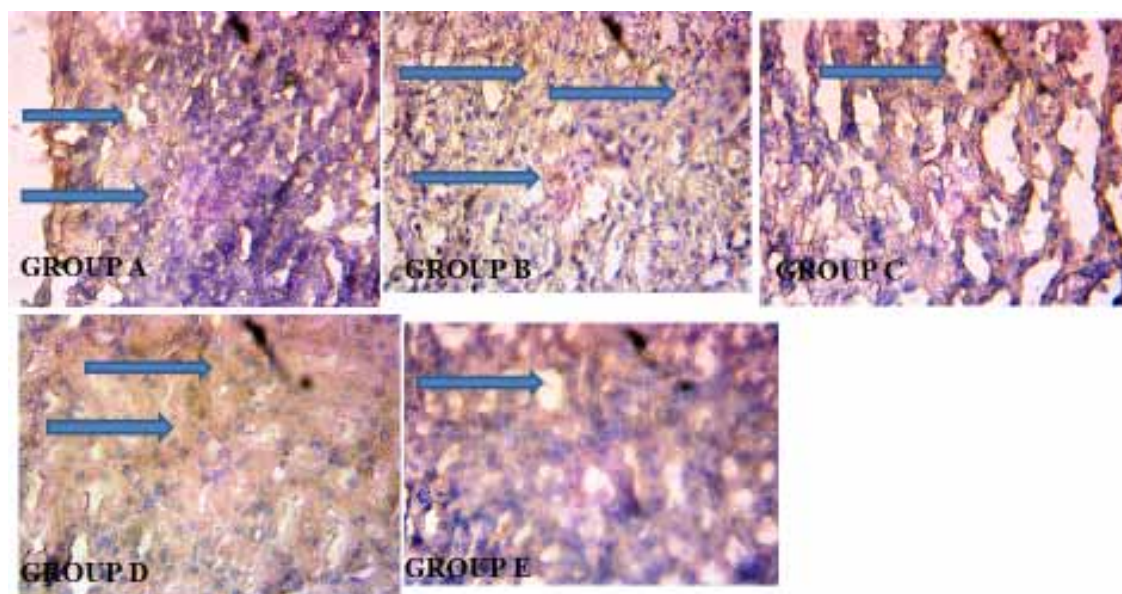


Figure 13: Group A (normal control) shows least expression of NF-KB in the kidney section. **Group B** (Toxicant group/ gentamicin 100 mg/kg) shows highest expression of NF-KB when compared with **A, C, D and E**.

(A=control, B=Gentamicin 100mg/kg, C= 100mg/kg chloroform extract + Gentamicin 100mg/kg, D= 200 mg/kg chloroform extract + Gentamicin 100mg/kg, E= Gentamicin 100mg/kg + 200 mg/kg chloroform post treatment).

Results

In this present study, we discovered no significant changes in organosomatic indices across the groups (Table 1). Serum creatinine, urea and xanthine oxidase levels were significantly increased in the Gentamicin alone group whereas the extracts at both pre and post treatment brought it down to values similar to control values (Figures 1-3), serum nitric oxide and myeloperoxidase levels were also significantly elevated in the gentamicin alone group and the extracts at both pre and post treatment significantly ameliorated it (Figures 4-5).

On renal enzymatic antioxidant level, SOD, and GST were significantly reduced in the gentamicin alone group when compared with the control and other groups (Table 2). Reduced glutathione, total thiol, non protein thiol and total protein levels were significantly depleted in the gentamicin alone group when compared with the control and the extract treated groups (Table 3). Renal AOPP, MDA, Protein Carbonyl and hydrogen peroxide levels were

significantly elevated in the gentamicin alone group when compared with the control and the extract treated groups (Figures 6-9).

On histopathology, the gentamicin alone group exhibited various pathological signs such as congestion, focal areas of inflammation, tubular necrosis, and glomerular atrophy, the extract treated groups however showed milder lesions (Figure 10). On immunohistochemistry, the gentamicin alone group exhibited least expression of Bcl2 when compared with the control and extract treated groups (Figure 11), C-reactive protein and NF-KB were highly expressed in the gentamicin alone group when compared with the control and the extract treated groups (Figures 12-13).

Discussion:

There was no significant difference in organ weight, kidney/body ratio across the groups (Table 1), this is similar to the findings of Gowda and Swamy (8), but contrary to that of Hussain *et al*, (26) and Debnath *et al.*, (27) who recorded an increase in organ weight in gentamicin induced nephrotoxicity. However, there was a significant difference in the percentage weight gain in toxicant group when compared with the extract treated groups. Body weight loss as a result of GM induced damage has been reported (27-29).

The renal glomerulus easily filters creatinine and reabsorption by the tubules does not happen, thus increased creatinine levels in the serum is as a result of kidney damage leading to decrease in glomerular filtration rate (GFR) and creatinine filtration (8). The increase in serum creatinine level is therefore a result of damage induced by gentamicin (31-33). There is usually an increase in serum urea level when its rate of production surpasses its rate of elimination (34, 35). The increase in urea is therefore an indication of renal damage. This study showed a statistical significant elevation in serum urea, creatinine (Figures 1 and 2), in the gentamicin alone group, similar to the findings of (28, 36-38). The extracts of *Abrus precatorius* at both doses however significantly reduced the levels of these markers indicating its ability to protect the kidney from damage due to gentamicin possibly by improving the ability of the glomerulus to filter urea and creatinine.

Nitric oxide levels Asante *et al.*, (39) reported that ethanol leaf extract of *Abrus precatorius* scavenges NO in inflammation. The aqueous leaf extract of *Abrus precatorius* was also found to reduce NO oxide levels in DNBS induced inflammatory bowel disease (40). This study has thus corroborated these earlier findings.

506 The levels of reduced glutathione, glutathione peroxidase, glutathione-s-transferase have
507 been shown to be reduced in gentamicin-induced nephrotoxicity (26, 30, 41). Superoxide
508 dismutase has also been shown in previous to be significantly depleted in nephrotoxicity (8,
509 30, 38). This causes increase in activity of superoxide radical thus aggravating
510 nephrotoxicity. SOD is said to be one of the most important enzymes involved in
511 improvement of toxicity due to reactive oxygen species (42, 43). Solanki and Zaveri (40)
512 earlier reported aqueous leaf extract of *Abrus precatorius* increased the levels of catalase,
513 superoxide dismutase and reduced glutathione in an anti-inflammatory study. In this study
514 also, there was a significant reduction in renal SOD, GSH, GST, total protein, thiols and non-
515 protein thiols levels in the toxicant group, but the extract significantly elevated these levels in
516 the extract treated group (Tables 2 and 3). However, there was a significant elevation in the
517 GPx levels in the toxicant and extract treated groups when compared with the normal group
518 (Table 2). This is contrary to the findings of Palani *et al.*, (30) and Farombi and Ekor (41)
519 who both reported significant depletions in GPx levels in the gentamicin induced toxicity.
520 This contrary finding might be the body system's way of fighting the toxicity before
521 crashing. Gentamicin induced nephrotoxicity has been reported to produce a depletion in total
522 protein level (27, 44).

523 There was a significant elevation in malondialdehyde, a product of lipid peroxidation in the
524 gentamicin group (Figure 7) similar to the findings of Gowda and Swamy (8), Hussain, *et al*
525 (26), Palaniet *al.*, (30), and Solanki and Zaveri (40). Gentamicin induces oxidative stress via
526 lipid peroxidation, leading to further destruction of macromolecules. *Abrus precatorius* was
527 earlier reported to significantly reduce MDA levels in 4dinitro benzene sulphonic acid
528 (DNBS)-induced inflammatory bowel disease.

529 According to Luft *et al.*, (45), tubular damage does not affect glomerular function, however
530 Solez (46) posited that raised creatinine levels as a result of tubular necrosis could cause
531 tubular obstruction and the casts could further aggravate renal damage. Gentamicin has been
532 shown to induce structural changes in the kidney (27, 28). Pedraza-Chaverri *et al.*, (47)
533 reported tubular necrosis and glomerular atrophy. Gowda and Swamy (8) reported tubular
534 casts, necrosis and dilatation. In this study, it was discovered that gentamicin caused tubular
535 necrosis, inflammation, glomerular atrophy and congestion of blood vessels (Figure 10).
536 Chloroform extract of *Abrus precatorius* however ameliorated these lesions in the treated
537 groups. These observations further showed that the plant extract has nephroprotective effect
538 on gentamicin-induced renal damage. The mechanism of action of *Abrus precatorius* renal

protection is therefore explained by the inhibition of hydrogen peroxidation, lipid peroxidation, protein carbonylation and enhancement of both enzymatic and non-enzymatic antioxidant defense status.

NFkB proteins influence a broad range of biological processes including innate and adaptive immunity, inflammation and stress responses. This pathway is activated upon appropriate cellular stimulation, most often by signals related to pathogens or stress. NF- κ B is a transcription factor that is essential in controlling immune and inflammatory responses (48). The down regulation of this protein in this study (Figure 13) is an indication that the plant has anti-inflammatory property and thus corroborated the findings of Asante *et al.*, (39) and Solanki and Zaveri (40).

CRP is classified as an acute phase reactant, which means that its levels will rise in response to inflammation (49) and it is thus used mainly as a marker of inflammation. In several diseases, CRP reveals ongoing inflammation and tissue damage much more accurately than do other indicators of the acute-phase response (50). It's down regulation in this study (Figure 12) also attested to the anti-inflammatory effect of the chloroform extract of *A. precatorius*.

Bcl2 primarily neutralizes certain stress signals that is activated by proapoptotic pathway, and downregulation of Bcl2 results in increased apoptosis (51). The up regulation of Bcl2 seen in this study (Figure 11) indicates the antiapoptotic effect of this plant. In this study, with Immunohistochemistry, there was increased expression of NFkB and CRP in the toxicant group, and a decreased expression of Bcl2; however, the reverse was seen with the extract treated groups.

Normal doses of Gentamicin can result in nephrotoxicity according to Kosek *et al.*, (52). Bennett *et al.*, (53) however has a contrary opinion, where the report says that nephrotoxicity can only occur when 5-10 times the normal dose is taken. GM-induced toxicity is believed to be via the generation of reactive oxygen species (54, 55). In our study, a daily dose of 100mg/kg of gentamicin was used to induce renal damage but chloroform extract of *Abrus precatorius* however ameliorated the nephrotoxicity induced by the gentamicin possibly because of its anti-inflammatory, anti-oxidative and anti-apoptotic properties. Alkaloids and flavonoids, which have been reported to be present in *Abrus precatorius*, have antioxidant properties (56, 57). These phytoconstituents present in the plant could therefore be responsible for the protection offered by the plant extract.

Conclusion:

We thus conclude that chloroform stem bark extracts of *Abrus precatorius* ameliorates Gentamicin Induced renal damage, making it a potential drug candidate in the treatment of renal damage.

Ethical Disclaimer:

As per international standard or university standard written ethical permission has been collected and preserved by the authors.

Consent: NA

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Conflict of interest

The authors have no conflict of interest to declare

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