

Original Research Article

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

Abstract

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. Thirty male Wistar rats weighing 100-170g were divided into five (5) groups; with 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 (6) days; C received chloroform extract of *Abrus precatorius* at 100mg/kg administered orally three (3) days prior and then concurrently with gentamicin for six days, D received 200 mg/kg of the extract and was administered orally for three (3) days prior and concurrently with gentamicin for six days and E received gentamicin administered intraperitoneally for six (6) days followed by administration of 200 mg/kg chloroform extract of *Abrus precatorius* for three (3) days. Subsequently, body weight and organ weight were determined. Blood, serum and kidney homogenate were obtained. Creatinine, Xanthine oxidase, Myeloperoxidase and Nitric oxide were assayed for in the blood and serum respectively. Advanced oxidative protein product (AOPP), 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 of the kidney was done to check for structural changes while immunohistochemistry was also carried out to check for protein expressions using Bcl2, CRP and NFkB. 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, 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 NFκB in the toxicant group, however, the reverse was seen in the extract treated groups. From these results, it can be concluded that the chloroform extract of *Abrus precatorius* stem bark has nephroprotective properties.

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

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 have reported it to possess 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 more common these days when compared with before, because the patients presents with more predisposing factors (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 to 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).

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

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

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

Haematology: Blood was obtained via the retro-orbital route, collected into Lithium heparinized bottles and used to check for full blood count (FBC), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet concentration (PLC) and Total leucocyte count (TLC).

Serum Assays: Blood was also collected into plain sample bottles, centrifuged with a macro centrifuge, serum was obtained and used to assay for total protein levels according to Gornal *et al.*, (13). Serum urea was determined using Randox kit (UR2821), while serum creatinine Randox Kit (CR2337) manufactured by (Randox Laboratories Ltd, Crumlin, UK) was used to

measure serum creatinine according to manufacturer's manual. Xanthine oxidase, 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 Griess 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). Glutathione-S- transferase activity was determined according to Habig *et al* (18). Glutathione peroxidase activity was measured according to Buetler *et al.*, (17). 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). Markers of oxidative stress such as Protein carbonyl was determined as described by Reznick and Packer (21), Advanced oxidative protein products (AOPP) was assayed as described by Kayali *et al.*, (22), Hydrogen peroxide generation was measured as described by Woff (23) while lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) according to Varshney and Kale (24).

Histopathology: The kidneys were sectioned longitudinally in two 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 performed after the tissues were obtained 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 NFκB 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, 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 NFκB 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, D= 200 mg/kg chloroform extract, E=200 mg/kg chloroform post treatment). Results expressed in Mean±SD, n=6. Superscripts (a, b) indicate significant difference at $p \leq 0.05$. 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±SD, n=6

167 **Table 2: Effects of the chloroform stem extracts of *Abrus precatorius* on the**
 168 **hematological indices**

	A	B	C	D	E
PCV (%)	45.3±2.5	42.5±2.1	44.2±3.6	43.8±3.1	44.4±2.0
MCV (fl)	60.4±1.6	62.2±1.3	61.0±2.0	60.4±2.1	59.7±1.6
MCH (pg)	20.2±0.7	20.5±0.7	20.3±0.7	20.2±0.7	19.3±0.8
MCHC (g/dl)	33.4±0.6	33.0±0.5	33.2±0.9	33.4±0.6	32.4±0.6

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

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181 **Table 3: Effects of the chloroform stem extract of *Abrus precatorius* on the**
 182 **hematological indices**

	A	B	C	D	E
HB (g/dl)	14.5±0.8	14.7±0.8	14.7±1.2	14.6±2.0	14.4±0.8
RBC (10⁶/μl)	7.2±0.6	7.1±0.2	7.2±0.4	7.2±0.3	7.4±0.2
PLATELETS (10⁵/μl)	2.1±1.0	1.4±0.14	1.2±0.3	1.6±0.3	1.2±0.2

183

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

190

191 Table 4: Effects of chloroform stem extracts of *Abrus precatorius* on White Blood Cell
 192 Profile

	A	B	C	D	E
WBC (10³)	11.8±0.8	8.7±1.7	6.6±0.6	7.1±0.8	6.7±0.7
NEUTRO	28.2±4.0	26.8±4.5	25.4±4.2	30.0±5.9	26±4.6
EOSINO	2.6±1.1	2.0±0.7	3.2±0.8	2.6±0.6	2.0±1.0
MONO	1.8±0.8	2.4±0.9	2.2±0.8	1.4±0.6	1.8±0.8
LYMPHO	67.4±3.4	68.8±4.6	68.6±3.1	66.0±5.9	70.2±4.0

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

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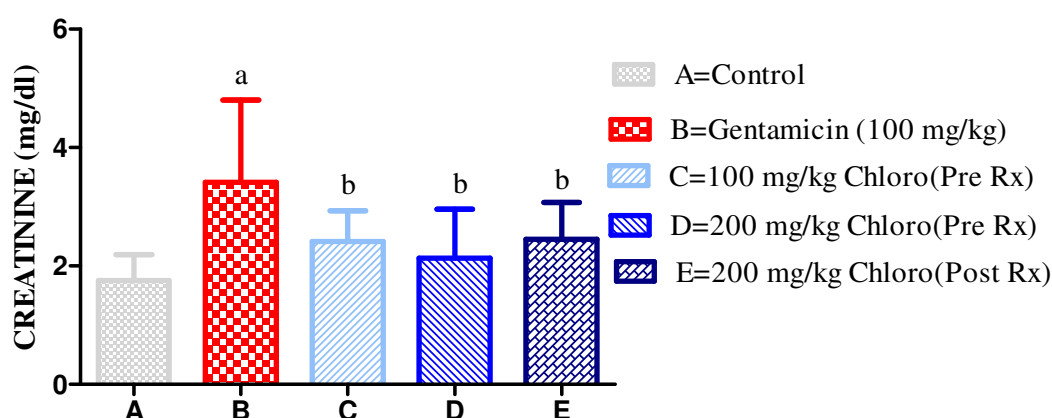
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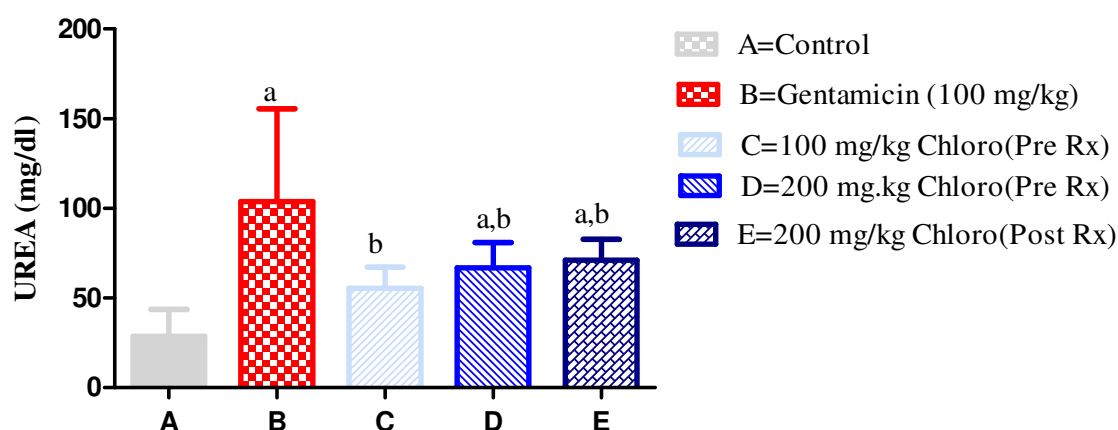
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Figure 1: Effects of the Chloroform Stem extract of *Abrus precatorius* on the serum creatinine levels



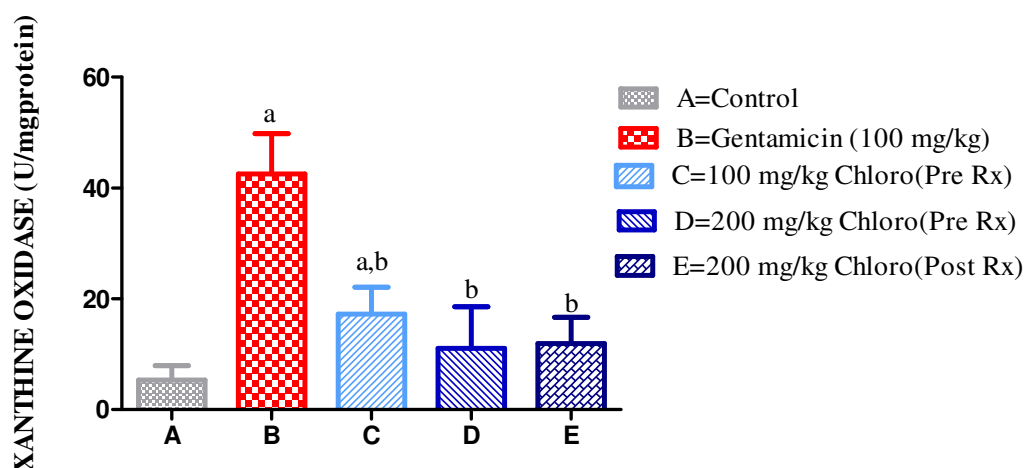
Superscripts (a, b) indicate significant difference at $p < 0.05$. Superscript (a) indicates significant difference when groups B, C, D and E were compared with group A. Superscripts (b) indicates significant difference when groups C, D and E were compared with group B. Results expressed in Mean $\pm$ SD, n=6

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

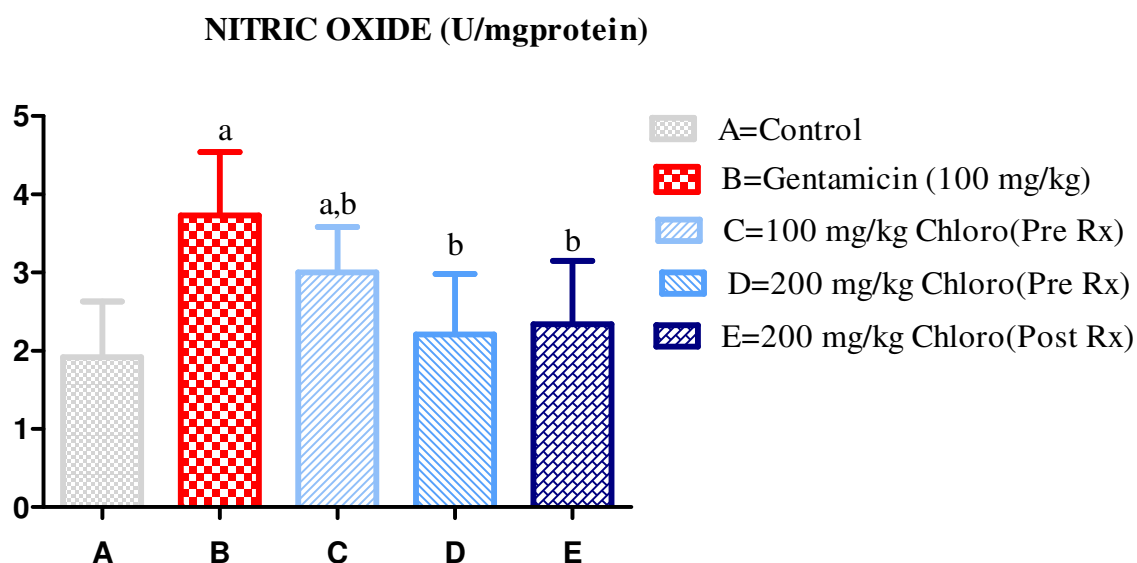
Figure 3: Effects of the Chloroform Stem extract of *Abrus precatorius* on serum Xanthine oxidase levels



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

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



238

239

240 Superscripts (a, b) indicate significant difference at $p < 0.05$. Superscript (a) indicates
 241 significant difference when groups B, C, D and E were compared with group A. Superscripts
 242 (b) indicates significant difference when groups C, D and E were compared with group B.
 243 Results expressed in Mean $\pm$ SD, n=6

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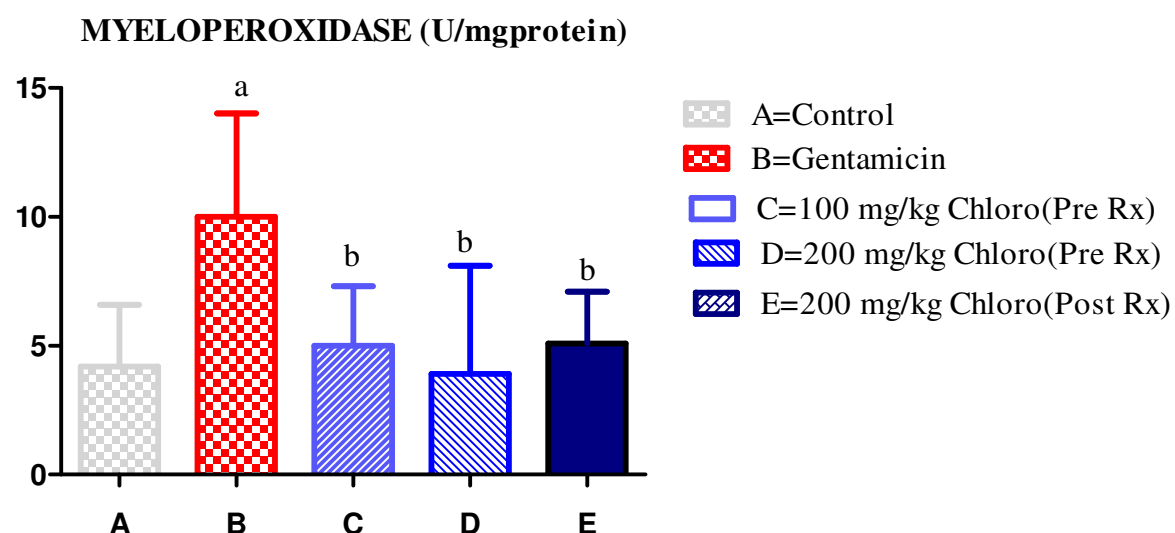
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Figure 5: Effects of the Chloroform Stem extract of *Abrus precatorius* on serum myeloperoxidase levels



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

Table 5: Effects of the chloroform stem extract of *Abrus precatorius* on renal enzymatic antioxidant levels (GST, GPx and SOD)

	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
GPx (units/mgprotein)	8.3±0.8	11.7±2.0 ^b	11.8±1.7	11.9±4.8	8.4±2.6 ^b

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

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

288 **Table 6: Effects of chloroform stem extract of *Abrus precatorius* on reduced GSH, Total**
 289 **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

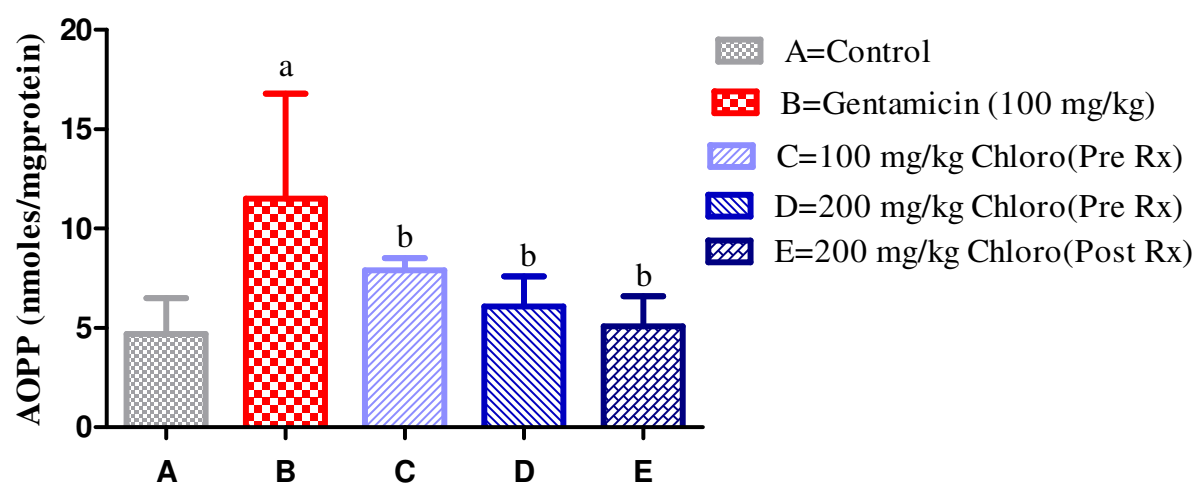
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291 Superscripts (a, b) indicate significant difference at $p < 0.05$. Superscript (a) indicates
 292 significant difference when groups B, C, D, E, F, G and H were compared with group A.
 293 Superscripts (b) indicates significant difference when groups C, D, E, F, G, H were compared
 294 with group B. Results expressed in Mean ± SD, n=6

295 (A=control, B=Gentamicin 100mg/kg, C= 100 mg/kg methanol extract, D= 200 mg/kg
 296 methanol extract, E= 100mg/kg chloroform extract, F= 200 mg/kg chloroform extract, G=
 297 200 mg/kg methanol, extract post treatment, H=200 mg/kg chloroform post treatment)

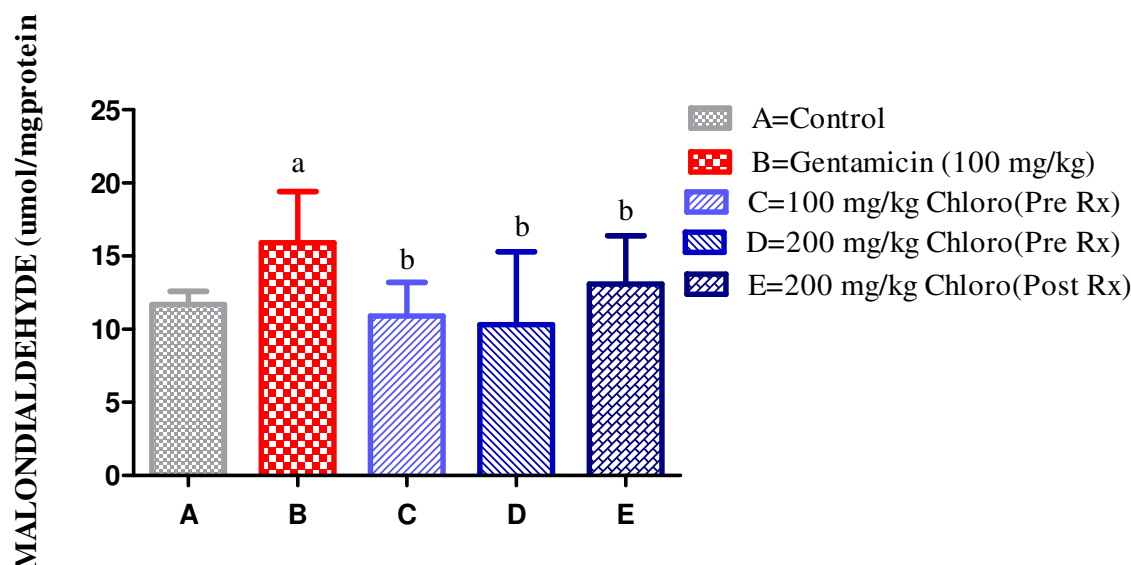
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Figure 6: Effects of chloroform stem extract of *Abrus precatorius* on renal Advanced oxidative protein product (AOPP) levels



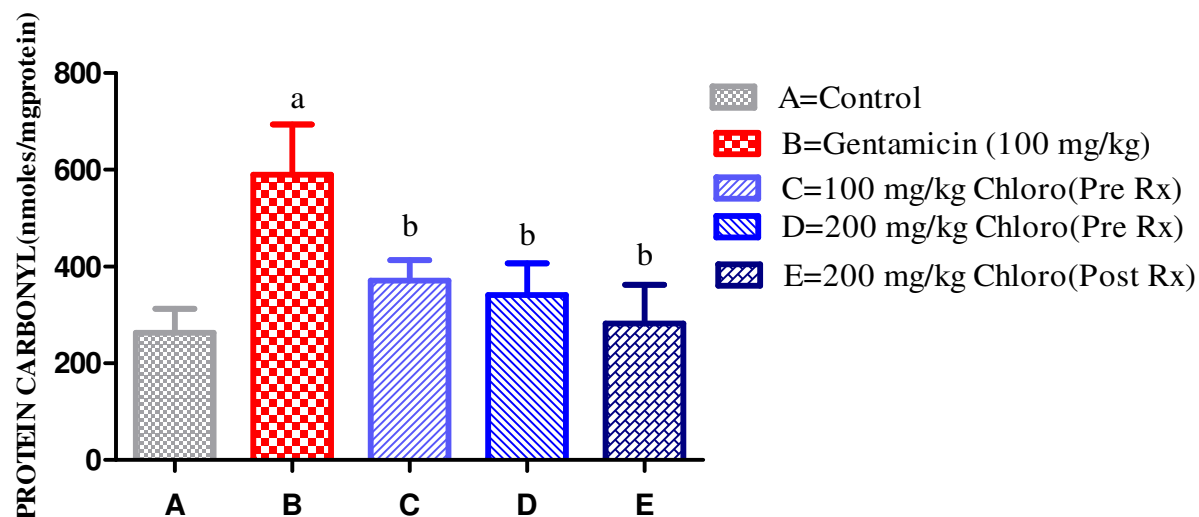
Superscripts (a, b) indicate significant difference at $p < 0.05$. Superscript (a) indicates significant difference when groups B, C, D and E were compared with group A. Superscripts (b) indicates significant difference when groups C, D and E were compared with group B. Results expressed in Mean $\pm$ SD, n=6

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



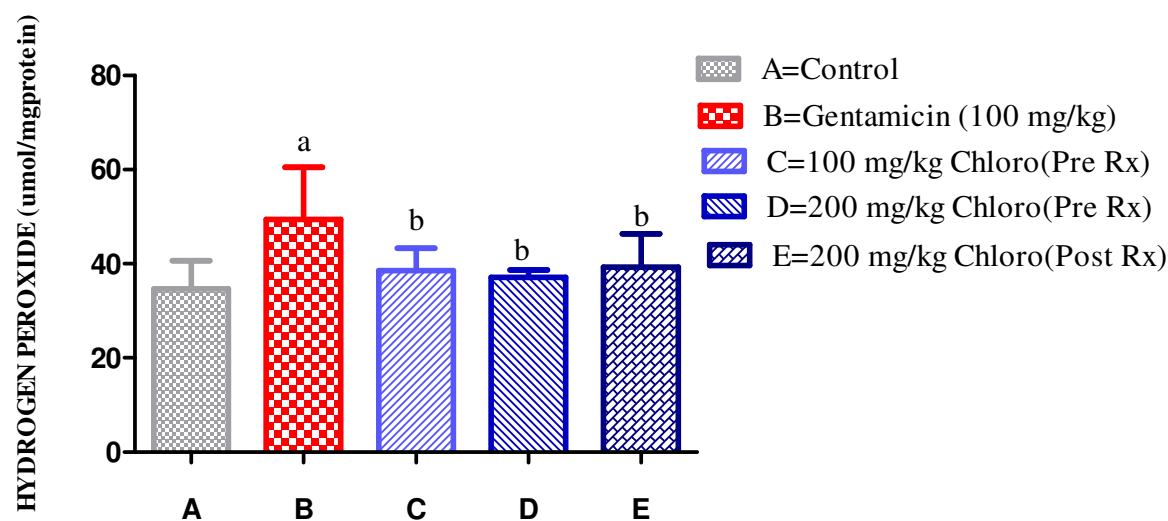
Superscripts (a, b) indicate significant difference at $p < 0.05$. Superscript (a) indicates significant difference when groups B, C, D and E were compared with group A. Superscripts (b) indicates significant difference when groups C, D and E were compared with group B. Results expressed in Mean $\pm$ SD, n=6

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



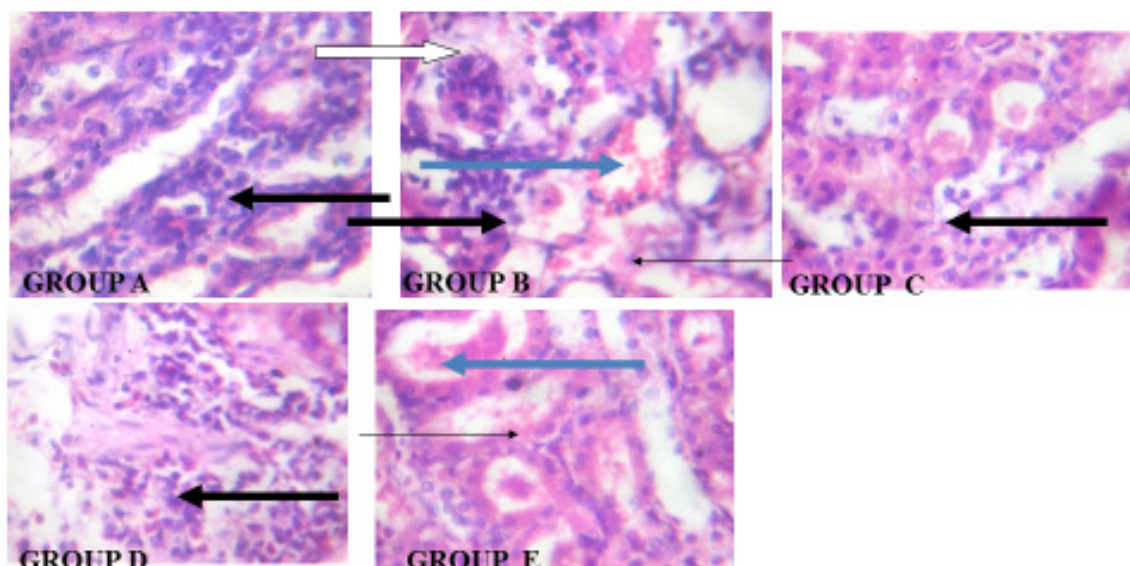
Superscripts (a, b) indicate significant difference at $p < 0.05$. Superscript (a) indicates significant difference when groups B, C, D and E were compared with group A. Superscripts (b) indicates significant difference when groups C, D and E were compared with group B. Results expressed in Mean $\pm$ SD, n=6

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



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

366 HISTOPATHOLOGY



367
 368 Figure 10: Photomicrograph of kidney plates at X400. **Group A** shows normal morphology
 369 of the tubules and no evidence of effacement in the glomerular basement membrane. **Group**
 370 **B** shows congestion of vessels (blue arrow), focal area of inflammation (black arrow), tubular
 371 necrosis (slender arrow), and glomerular atrophy (white arrow). **Group C** shows mild sub
 372 capsular fatty degeneration of the tubules (black arrows). **Group D** shows focal area of
 373 inflammation (black arrows). **Group E** shows presence of red blood cells in the tubule
 374 suggestive of glomerular basement membrane effacement (blue arrow) and a focal area of
 375 tubular necrosis (slender arrow).

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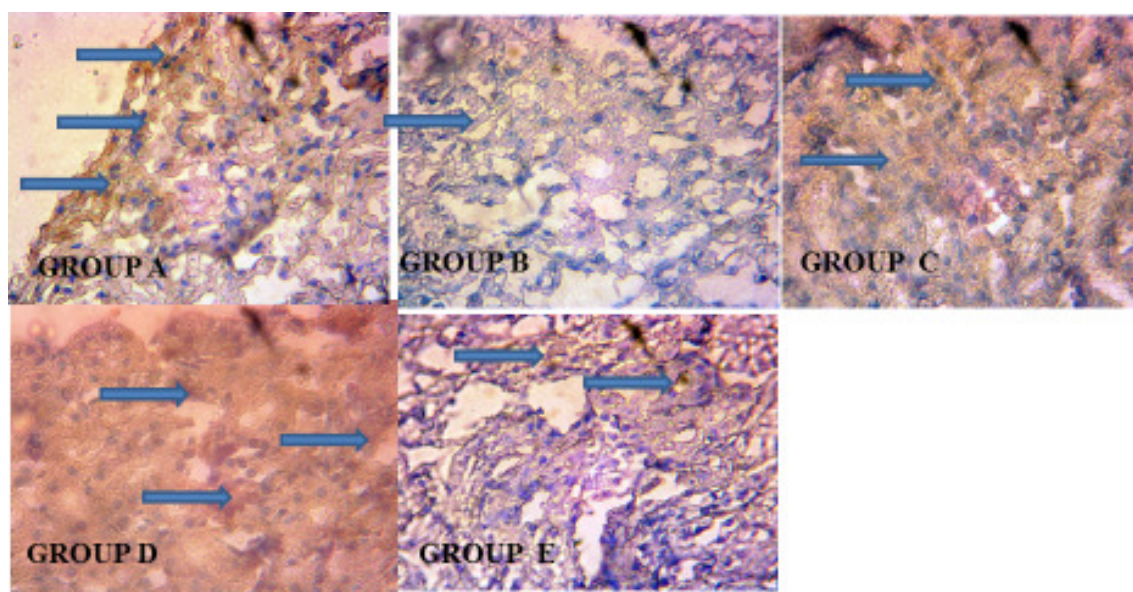
377 (*A=control, B=Gentamicin 100mg/kg, C= 100mg/kg chloroform extract, D= 200 mg/kg*
 378 *chloroform extract, E=200 mg/kg chloroform post treatment*)

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381 IMMUNOHISTOCHEMISTRY

382 Bcl₂ ANTIBODY



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

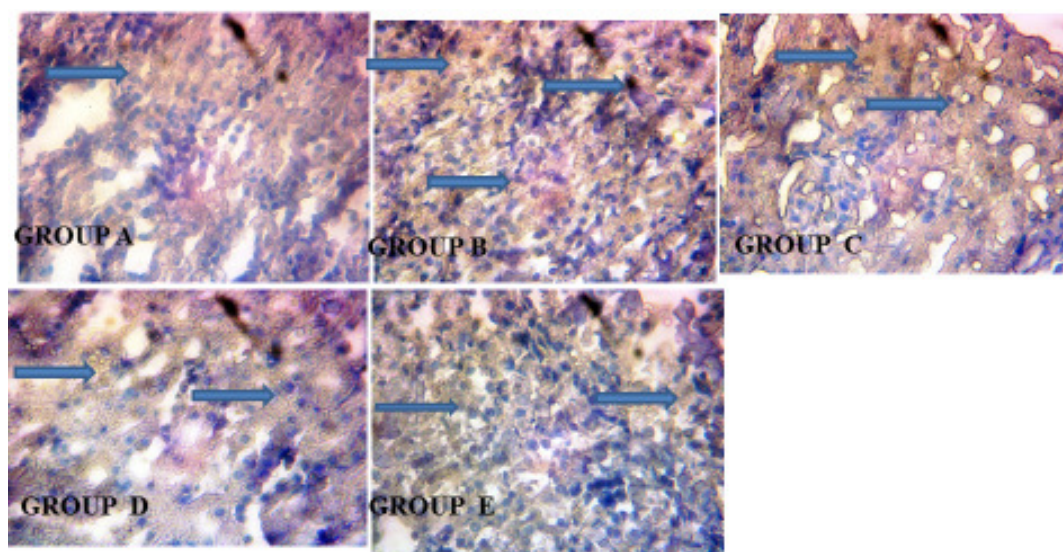
385 **Group B** (Toxicant group/ gentamicin 100 mg/kg) shows least expression of Bcl₂ when
386 compared with **A, C, D and E.**

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

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



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

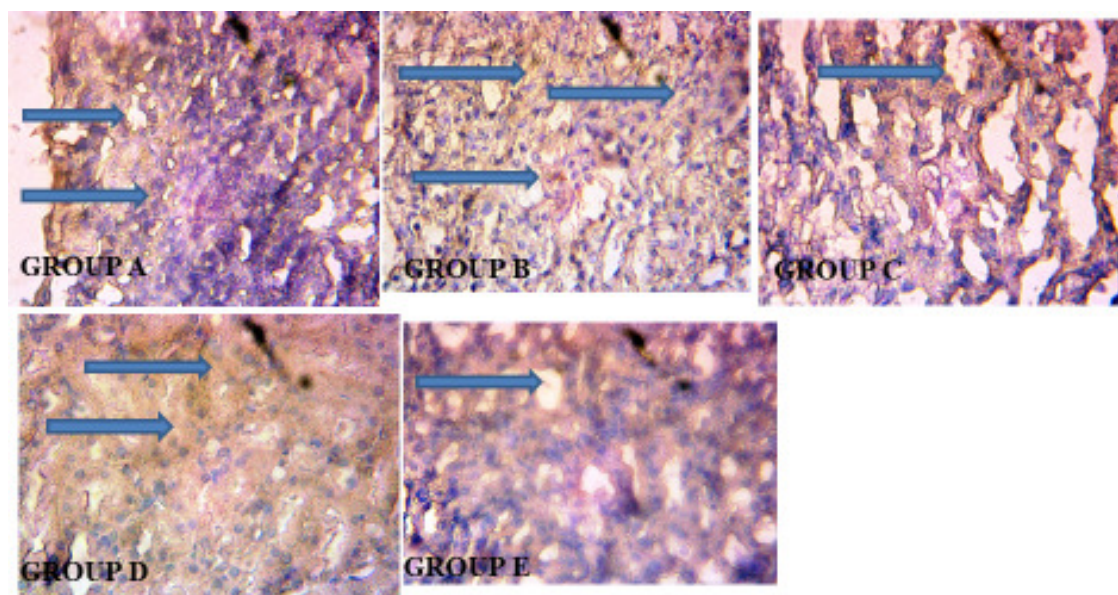
394 **Group B** (Toxicant group/ gentamicin 100 mg/kg) shows highest expression of CRP when
395 compared with **A, C, D and E**.

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

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400 **NF-KB (NUCLEAR FACTOR KAPPA BETA ANTIBODY**



401

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

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

407 **Discussion and Conclusion:**

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

414 There was no statistical significance in all the haematological parameters assayed for,
 415 contrary to the findings of Palani *et al.*, (30) who discovered a significant increase in levels of
 416 Hb, PCV and MCV and a decrease in MCHC and MCH in nephrotoxicity by acetaminophen.

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, similar to the findings of (28,36-38). The extracts however significantly reduced the levels of these markers indicating its ability to protect the kidney from damage due to gentamicin.

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.

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

There was a significant elevation in product of lipid peroxidation in the gentamicin group similar to the findings of Gowda and Swamy (8), Hussain, *et al* (26), Palani *et al.*, (30), and Solanki and Zaveri (40). *Abrus precatorius* was earlier reported to significantly reduce MDA levels in ,4 dinitro benzene sulphonic acid (DNBS) induced inflammatory bowel disease.

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

NFKB proteins functions as dimeric transcription factors that regulate the expression of pro inflammatory genes influencing 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-kB is a transcription factor that is essential in controlling immune and inflammatory responses (48). The down regulation of this protein in this study 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 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 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. In that study, 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.

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