

NEPHROPROTECTIVE ACTIVITIES OF THE METHANOL LEAF EXTRACT AND FRACTIONS OF *MOMORDICA BALSAMINA* LINN. IN ALBINO RATS

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ABSTRACT

Background: Previous studies have shown that methanol leaf extract of *Momordica balsamina* Linn. (*Cucurbitaceae*) can ameliorate renal diseases and injuries in gentamicin-induced nephrotoxicity albino rats. The aim of the study was to investigate the nephroprotective activities of methanol leaf extract and fractions of *Momordica balsamina* Linn. (*Cucurbitaceae*) in albino rats. **Methods:** Fifty-four male albino rats weighing between 100 ± 10 g were randomly divided into nine groups of six rats per group. Group I served as a normal control while the other eight groups were administered intra-peritoneally with gentamicin (80 mg/kg body weight per day) for seven days to induce nephrotoxicity. Group II served as nephrotoxic (experimental) control. Group III was subsequently treated with the standard drug (Furosemide, 80 mg/kg body weight per day). Methanol leaf extract, fractions I and II of the leaf extract of *M. balsamina* Linn. at a daily

dose of 200 and 400 mg/kg body weight respectively were used to treat group IV and V, VI and VII and VIII and IX separately. All treatments were carried out by oral gavages for fourteen (14) consecutive days. Assays of renal function biomarkers such as serum electrolytes profile (Na^+ , K^+ , Cl^- and HCO_3^-), Urea, Creatinine, Blood Urea Nitrogen (BUN), as well as Total Protein and histopathological examination were also carried out. **Results:** Results obtained showed that gentamicin administration induced nephrotoxicity in albino rats which were characterized by significant ($P < 0.05$) elevation of all renal injury biomarkers (Urea, Creatinine and BUN concentrations), increased serum electrolytes (Na^+ , K^+ and Cl^-) and renal tubular necrosis when compared with the normal control group. However, oral

post-treatment with leaf extract, fractions I and II of *M. balsamina* Linn. in a dose-dependent manner compared to the experimental control restored all the indices with significant ($P < 0.05$) restoration observed in fraction II. **Conclusion:** *M. balsamina* Linn. leaf exhibits nephroprotective activity through the amelioration of renal injuries in albino rats.

KEYWORDS: Nephroprotective, *Momordica balsamina* Linn., Gentamicin, Renal injury.

INTRODUCTION

Kidney disease is of epidemic proportions and its prevalence will double in the next twenty-five years, particularly in the developing countries.^[1] There are now over one million dialysis patients worldwide with an incidence of about 500,000 new patients each year.^[2,3,4] When the kidneys are exposed to toxic agents, either accidentally or intentionally, alteration in morphology may occur which will directly affect the glomerulus and renal tubules and subsequently result to kidney problem.^[5] According to World Kidney Day^[1], the major causes of kidney disease include diabetes, high blood pressure, urinary tract infection, obesity, glomerulonephritis, and polycystic with diabetes accounting for nearly 44%. Furthermore, World Health Organization^[3] statistics revealed that more than 36.8 million Nigerians are suffering from various forms of kidney disease which suggest that one in ten Nigerians is suffering from some form of kidney disorder or another with the majority of them die every day due to poverty, ignorance, cost and inaccessibility to treatment. It has been revealed that, kidney damage and kidney related diseases cause more death than malaria and HIV/AIDS in Nigeria.^[6,3]

Gentamicin has been widely used for inducing acute renal failure in experimental animals and the evaluation of reno-protective agents. The pathological mechanisms involved in gentamicin-induced nephrotoxicity include induction of oxidative stress, increase serum creatinine, blood urea nitrogen and decrease in glomerular filtration rate.^[7] However, usage of antioxidants improved histological injuries such as tubular necrosis, tubular cell edema and apoptosis in gentamicin-injected rats.^[8,9,10]

M. balsamina Linn., Balsam apple (or Balsam pear) and locally called “Garahunia” (Hausa Language), “Ejinrin” (Yoruba Language), belongs to the family *Cucurbitaceae*.^[11] In the Northern part of Nigeria and Republic of Niger, the leaf was reportedly cooked and used as blood tonic as part of green vegetables soup for lactating mother.^[12] The leaf of the plant has also been reported as a medicated soap substitute in treating skin diseases, arrow poison

antidote and in relieving menstrual pain due to its high saponins contents.^[13] The phytochemical screening of the leaf, stem and fruit extracts revealed the presence of saponins, flavonoids, tannins, steroids, lectins and triterpenoids with anti-microbial, anti-viral, anti-septic, anti-bacterial, anti-plasmodial, anti-inflammatory, anti-diarrheal, hypoglycemic, anti-diabetic and analgesic effects.^[14,15,16,17] In view of these considerations, this plant was considered to be interesting for a more detailed study. Hence, this study was undertaken to investigate the nephroprotective efficacies of *M. balsamina* Linn. leaf extract and its fractions in albino rats.

MATERIALS AND METHODS

Materials

Plant material collection

Fresh leaf of *M. balsamina* Linn. was collected in June, 2016 from farms around Modibbo Adama University of Technology, Yola, Adamawa State. The leaf was authenticated by a Botanist and Principal Technologist (Saleh Baba) in the Department of Plant Sciences, School of Life Sciences, Modibbo Adama University of Technology Yola, Adamawa State. After collection, the leaf was washed with distilled water and air dried at room temperature and then powdered with the aid of surface sterilized pestle and mortar and passed through a sterile sieve to obtain the required particles of uniform size.

Plant extraction and fractionation

Powder leaf of *M. balsamina* Linn. (10 g) was macerated in 500 ml (90% v/v) methanol and left in air tight aspirator bottle for 72 hours with occasional stirring with a sterilized glass rod to ensure efficient extraction. The extract was then separated from the sample residue by filtration through Whatman No.1 filter paper (Alade and Irobi^[18] as modified by Karumi *et al.*^[19] and Otimenyin *et al.*^[17] This procedure was repeated three times to ensure complete filtration. The dark green extract obtained was then concentrated under reduced pressure at 40°C-50°C using a rotary evaporator until a dark brown extract was obtained. The fractionation method described by Abbot and Andrews^[20] as modified by Karumi *et al.*^[19] was used to separate the leaf extract into its component fractions. The extract was separated into four clear bands of different colors in the column. Elution of the extract was done with solvent systems of gradually increasing polarity using ethyl acetate, formic acid and water at the combination ratio of 5:4:1 as the mobile phase. The eluted fractions were collected in separate beakers and then poured into wash glasses and evaporated to dryness; scrapped and

poured separately into labeled-airtight containers and then stored in a refrigerator until further used for phytochemicals screening and treatments.

Chemicals and reagents

Gentamicin was sourced from Hi Media, Germany, Creatinine, Urea and Total protein biomedical assay kits were procured from Randox Laboratories Ltd., UK and Greiner Diagnostic GmbH-Bahlingen, Germany methanol (Riedel-deHaen, Germany), hexane, formic acid, ethyl acetate (Sigma Aldrich, USA), silica gel (Sigma Aldrich, USA) were used for the study while all other chemicals and reagents were of analar grade.

Animals

Fifty-four male Albino rats weighing between 100 ± 10 g were obtained from the National Veterinary Research Institute, Vom, Plateau State, Jos, Nigeria. They were housed in stainless metal cages in nine groups of six rats per cage to avoid overcrowding and were kept at room temperature with 12 hours' light and dark cycles. They were allowed to acclimatize for two weeks before the experiment by granting them free access to standard laboratory chow (Vital Feeds, Jos) and water ad libitum. The animals were handled in accordance with the National Institute of Health (NIH) guide for the care and use of laboratory animals.

METHODS

Experimental design

After acclimatization, the animals were randomly divided into nine equal groups of six animals per group and average weight taken and recorded for each group. Group I was injected intra-peritoneally (IP) with sterile normal saline (2 ml) daily for seven days in addition to their normal diet and water and was kept as a normal control group. Group II was injected with 80 mg/kg body weight/day gentamicin intra-peritoneally for seven days to induce nephrotoxicity in addition to their normal diet and water and was kept as a nephrotoxic (experimental) control group while group III was injected with 80 mg/kg body weight/day gentamicin intra-peritoneally for seven days to induce nephrotoxicity and subsequently treated with the standard drug; 80 mg/kg body weight/day furosemide (Lasix) through oral gavages for fourteen days in addition to their normal diet and water and served as a standard control group. Following a modified method described by Karumi^[13] and Abeer^[21]; group IV and V were injected with 80 mg/kg body weight/day gentamicin intra-peritoneally for seven days to induce nephrotoxicity and subsequently received 200 mg/kg body weight/day and 400 mg/kg body weight/day respectively of the *M. balsamina* Linn.

methanol leaf extract through oral gavages for fourteen days in addition to their normal diet and water and served as *M. balsamina* extract treatment I and II respectively. Group VI and VII were injected with 80 mg/kg body weight/day gentamicin intra-peritoneally for seven days to induce nephrotoxicity and subsequently received 200 mg/kg body weight/day and 400 mg/kg body weight/day respectively of the *M. balsamina* methanol leaf extract fraction I through oral gavages for fourteen days in addition to their normal diet and water and served as *M. balsamina* methanol leaf extract fraction I treatment III and IV respectively. Group VIII and IX were injected with 80 mg/kg body weight/day gentamicin intra-peritoneally for seven days to induce nephrotoxicity and subsequently received 200 mg/kg body weight/day and 400 mg/kg body weight/day respectively of the *M. balsamina* methanol leaf extract fraction II through oral gavages for fourteen days in addition to their normal diet and water and served as *M. balsamina* methanol leaf extract fraction II treatment V and VI respectively.

Collection of Samples for Analysis

On the last day of the treatment, the rats were subjected to an overnight fasting after which they were sacrificed using chloroform vapor as an anesthesia. The rib cage was carefully cut-opened and blood was rapidly collected into separate ethylene diaminetetraacetic acid (EDTA) bottles by direct heart puncture using sterile syringe and needle and then centrifuged at 800 x g for 10 minutes to separate the plasma using a bench top centrifuge (MSE England, Model 5391). Sera obtained were carefully removed with the aid of pasteur pipette and put into respective dry plastic specimen bottles labeled accordingly and kept in a refrigerator until further used for the determination of electrolytes profile, urea and creatinine. Similarly, kidney tissues were collected and quickly covered with formalin in sample bottles to preserve them for histological evaluation.

Analytical Procedures

The assay for the renal damage biomarkers such as creatinine, urea and blood urea nitrogen (BUN) were determined according to the manufacturer's procedure of the commercially available kits (Randox Laboratories, UK). Blood urea nitrogen (BUN) was subsequently calculated from the urea concentration. Serum electrolytes profile (Na^+ , K^+ , Cl^- and HCO_3^-) were carried out according to the standard procedure of the commercial kits manufacturers. Total protein was determined according to Lowry *et al.*^[22] Moreover, histopathological evaluation was done according to the method described by Alaadin *et al.*^[23]

Statistical Analysis

The results of replicate experiments were represented as mean \pm standard error of mean (SEM). Statistical analysis was done using Statistical Package for Social Sciences (SPSS) Version 23.0 (SPSS, Incorporation Chicago Illinois, USA). Differences between and within the group means were analyzed using One-way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (MRT) for the Post-hoc treatment. The results were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Results

Table 1 shows the effects of methanol leaf extract and fractions of *M. balsamina* Linn. on some kidney function parameters in gentamicin-induced nephrotoxicity rats (urea, creatinine, blood urea nitrogen (BUN) and total protein) and in normal rats. The result reveals that the concentration of urea significantly ($p < 0.05$) increased in the experimental control group (11.39 mmol/L) when compared with the normal rats (2.84 mmol/L). However, the urea and blood urea nitrogen levels were significantly ($p < 0.05$) increased in gentamicin-induced nephrotoxicity rats treated with fraction II (fraction II treatment VI) of the plant extract (400 mg/kg body weight per day for 14 days) in a dose-dependent manner. Similarly, creatinine level was significantly ($p < 0.05$) increased in gentamicin-induced nephrotoxicity (group II) rats (90.67 μ mol/L) when compared to the normal rats (26.22 μ mol/L). However, the creatinine level was decreased significantly in the treatment groups (I and II) of the extract and fraction I respectively while maximum significant decreased was observed in the groups treated with 200 mg/kg body weight and 400 mg/kg body weight of fraction II (61.67 μ mol/L and 59.33 μ mol/L) respectively. This result shows that fraction II of the extract ameliorate kidney disease in a dose-dependent manner. Furthermore, total protein was decreased in the group administered with gentamicin (55.33 g/L) compared to normal control rats (75.31 g/L) while subsequent treatment with extract, fraction I and fraction II showed a significant ($p < 0.05$) increase in this kidney function indices in a dose-dependent manner.

Table 1: Effects of methanol leaf extract and fractions of *M. balsamina* Linn. on some kidney function parameters in gentamicin-induced nephrotoxicity albino rats.

Group	Urea (mmol/L)	Creatinine (μ mol/L)	BUN (mmol/L)	Total Protein (g/L)
Normal ctrl	2.84 \pm 0.98 ^{bdef}	26.22 \pm 2.31 ^{bdef}	1.33 \pm 0.46 ^{bdef}	75.31 \pm 1.16 ^c
Exp. ctrl	11.39 \pm 0.46 ^{ac}	90.67 \pm 2.60 ^{ac}	5.32 \pm 0.21 ^{ac}	55.33 \pm 2.03 ^{def}
Std ctrl	8.08 \pm 1.98 ^{abc}	77.35 \pm 4.18 ^{abc}	3.77 \pm 0.92 ^a	65.33 \pm 2.73 ^c
Ext Trt I	6.82 \pm 0.37 ^{abd}	68.67 \pm 5.46 ^{abd}	3.19 \pm 0.17 ^{ab}	63.12 \pm 2.65 ^d
Ext Trt II	6.31 \pm 0.37 ^{abd}	63.10 \pm 4.36 ^{abd}	2.95 \pm 0.13 ^{bd}	65.20 \pm 1.53
Fr. I Trt III	5.18 \pm 0.13 ^{abde}	62.33 \pm 9.62 ^{abd}	2.42 \pm 0.06 ^{bd}	67.10 \pm 1.76 ^d
Fr. I Trt IV	5.03 \pm 0.15 ^{abde}	60.10 \pm 2.65 ^{abde}	2.35 \pm 0.07 ^{bd}	66.67 \pm 2.03
Fr. II Trt V	4.96 \pm 0.12 ^{bde}	61.67 \pm 7.86 ^{abde}	2.32 \pm 0.54 ^{bd}	67.90 \pm 1.20
Fr. II Trt VI	4.59 \pm 0.21 ^{bde}	59.33 \pm 9.03 ^{abde}	2.14 \pm 0.10 ^{bd}	70.89 \pm 1.73

KEY: Ctrl = Control; Exp. = Experimental; Std = Standard; Ext = Extract; Trt = Treatment and Fr. = Fraction.

Values are expressed as Mean \pm SEM (n = 6).

^asignificantly (p<0.05) higher compared to the values of normal control in each column.

^bsignificantly (p<0.05) lower compared to the values of experimental control in each column.

^csignificantly (p<0.05) higher compared to the values of extract and fraction I and II.

^dsignificantly (p<0.05) lower compared to the values of standard control in each column.

^esignificantly (p<0.05) lower compared to the values of extract in each column.

^fsignificantly (p<0.05) lower compared to the values of extract and fraction I.

Table 2: Effects of methanol leaf extract and fractions of *M. balsamina* Linn. on serum electrolytes profile in gentamicin-induced nephrotoxicity albino rats.

Group	Na ⁺ (mmol/L)	K ⁺ (mmol/L)	Cl ⁻ (mmol/L)	HCO ₃ ⁻ (mmol/L)
Normal ctrl	113.90 \pm 0.07 ^{def}	11.40 \pm 0.69 ^{bdef}	74.47 \pm 2.26 ^{bde}	21.90 \pm 1.27
Exp. ctrl	144.60 \pm 1.83 ^{ac}	33.67 \pm 0.75 ^{ac}	92.53 \pm 2.20 ^{ac}	16.67 \pm 0.71 ^{def}
Std ctrl	138.48 \pm 1.73 ^{abc}	27.73 \pm 0.29 ^{abc}	83.30 \pm 2.57 ^{abc}	18.43 \pm 0.91 ^{ef}
Ext Trt I	123.33 \pm 0.72 ^{abd}	21.55 \pm 0.55 ^{abd}	79.53 \pm 1.11 ^{bcd}	19.87 \pm 1.13
Ext Trt II	123.11 \pm 0.49 ^{abd}	20.88 \pm 2.26 ^{abd}	77.10 \pm 1.57 ^{bd}	20.80 \pm 0.40
Fr. I Trt III	121.37 \pm 0.73 ^{abd}	22.31 \pm 1.08 ^{abd}	80.27 \pm 5.23 ^{bcd}	20.38 \pm 0.90
Fr. I Trt IV	120.63 \pm 0.54 ^{abd}	19.40 \pm 0.91 ^{abd}	79.93 \pm 5.92 ^{bde}	21.13 \pm 1.09
Fr. II Trt V	119.13 \pm 0.96 ^{bde}	15.04 \pm 0.71 ^{bde}	82.67 \pm 1.50 ^{bc}	21.53 \pm 0.35
Fr. II Trt VI	115.70 \pm 0.90 ^{bde}	12.70 \pm 4.01 ^{bdef}	79.54 \pm 1.42 ^{bd}	21.79 \pm 0.89

KEY: Ctrl = Control; Exp. = Experimental; Std = Standard; Ext = Extract; Trt = Treatment and Fr. = Fraction.

Values are expressed as Mean \pm SEM (n = 6).

^asignificantly (p<0.05) higher compared to the values of normal control in each column.

^bsignificantly ($p < 0.05$) lower compared to the values of experimental control in each column.

^csignificantly ($p < 0.05$) higher compared to the values of extract in each column.

^dsignificantly ($p < 0.05$) lower compared to the values of standard control in each column.

^esignificantly ($p < 0.05$) lower compared to the values of extract in each column.

^fsignificantly ($p < 0.05$) lower compared to the values of extract and fraction I.

Table 2 shows the effects of methanol leaf extract and fractions (I and II) of *M. balsamina* Linn. on serum electrolytic profile (Na^+ , K^+ , Cl^- and HCO_3^-) in gentamicin-induced nephrotoxicity rats. sodium ion (Na^+), potassium ion (K^+) and chloride ion (Cl^-) levels were significantly increased (144.60 mmol/L, 33.67 mmol/L, and 92.53 mmol/L) while HCO_3^- was shown to be significantly decreased (16.67 mmol/L) compared to the normal control group (group I). However, these renal function biomarkers were significantly restored to normal when the rats were treated with both the leaf extract and the fractions (Fractions I and II). The levels of serum sodium, potassium and chloride ions were significantly decreased ($p < 0.05$) in a dose dependent manner across all the extract, fraction I and fraction II treated groups compared to the untreated group. The amelioration was also dose-dependent as the maximum effect was achieved in the group treated with 400 mg/kg body weight of fraction II.

Histological evaluation of the kidney tissues from rats in the normal control group (group I) revealed absolutely normal histological features, illustrated in plate I below. However, there were marked necrotic and dilated tubules, tubular edema, severely damaged glomeruli, high presence of inflammatory infiltrates and epithelial desquamation in gentamicin-induced nephrotoxicity rats (Plate II). The result obtained shows that there was a remarkable attempt at restoration of these histological features in the group of rats treated with fraction II of the leaf extract when compared with the group treated with the methanol leaf extract and fraction I of *M. balsamina* Linn. respectively.

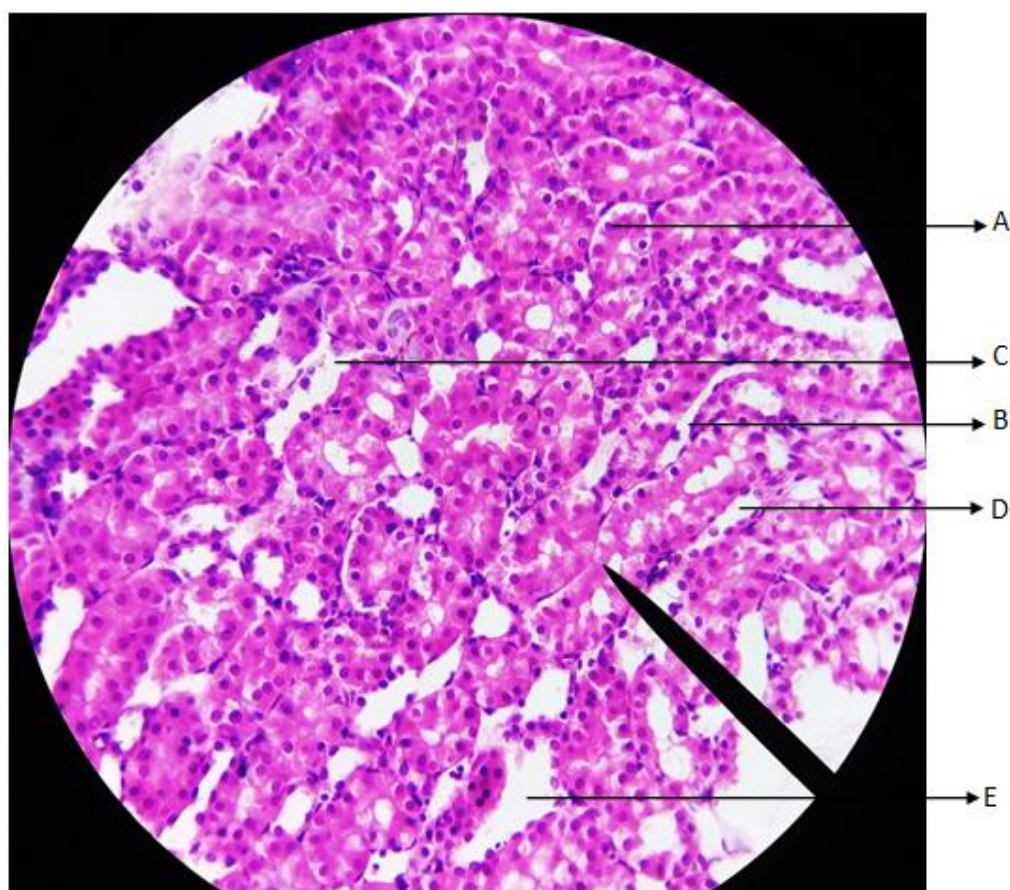


Plate I: Histopathological photomicrograph of renal section from normal control group rats stained with Hematoxylin and Eosin. Section (H and E, $\times 40$) shows normal renal tissue with numerous collecting tubules. Moderate lumen of the renal tubules. Glomeruli were normal with moderate Bowman capsular spaces.

KEY

- A – Bowman's Capsule.
- B – Glomerulus.
- C – Collecting tubules.
- D - Proximal convoluted tubule.
- E – Distal convoluted tubule.

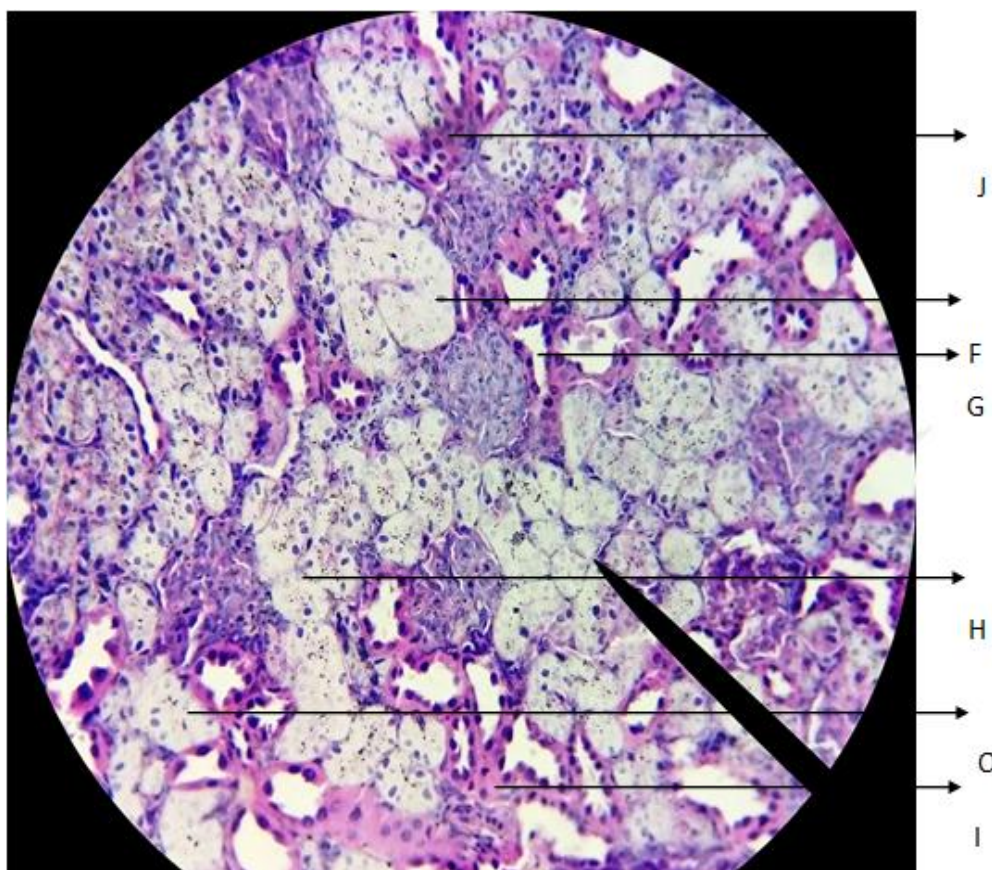


Plate II: Histopathological photomicrograph of renal section from 80 mg/kg body weight gentamicin-induced nephrotoxicity group rats stained with Hematoxylin and Eosin. Section (H and E, $\times 40$) shows most of the tubules were necrotic and dilated. The cortex area shows wide spread tubular edema with severely damaged glomeruli. The Bowman capsular spaces were completely filled up with cells and there was a high presence of infiltrates within the interstitial spaces and glomeruli.

KEY

F - Glomerular congestion.

G – Necrosis.

H – Inflammatory cell.

I – Tubular cast.

O – Interstitial edema.

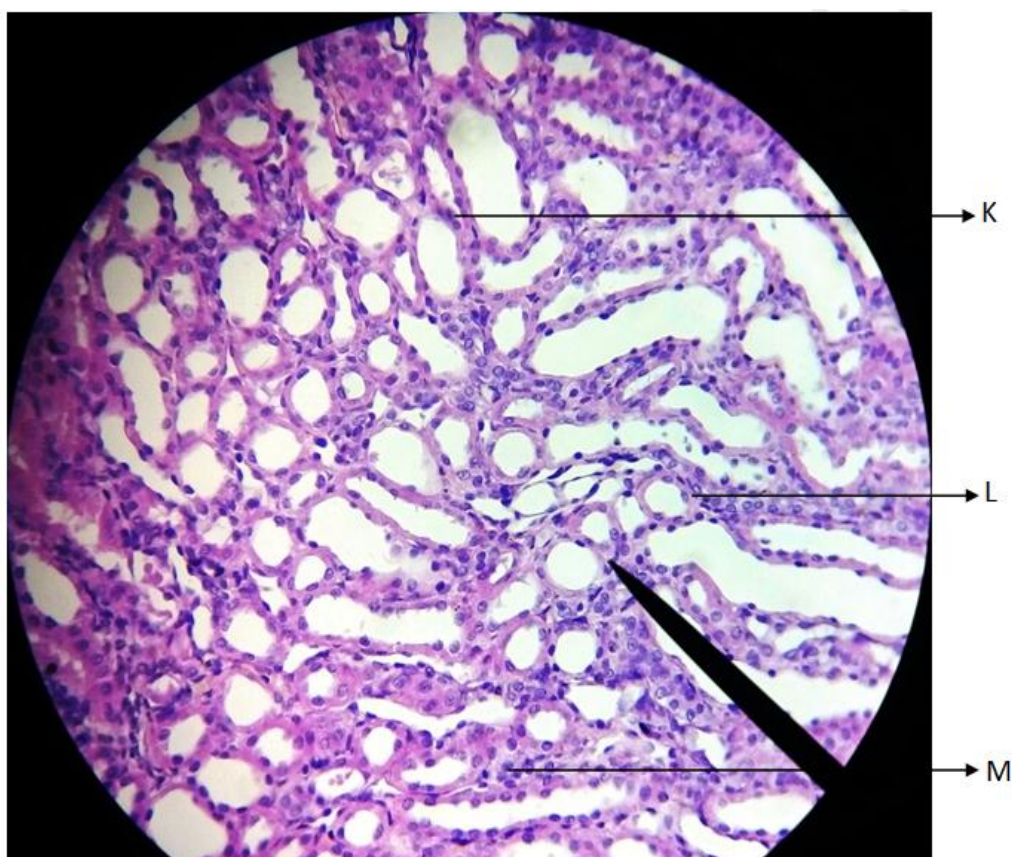


Plate III: Histopathological photomicrograph of renal section from 80 mg/kg body weight gentamicin-induced nephrotoxicity group rats treated with 80 mg/kg body weight furosemide (Lasix) stained with Hematoxylin and Eosin. Section (H and E, $\times 40$) shows little necrotic and dilated tubules. The cortex area shows very little tubular edema with adequate glomeruli that were reparative with adequate Bowman capsular spaces. There was a moderate presence of infiltrates within the interstitial spaces and glomeruli.

KEY

K - Tubular degeneration.

L – Desquamated epithelial cell.

M – Tubular cast.

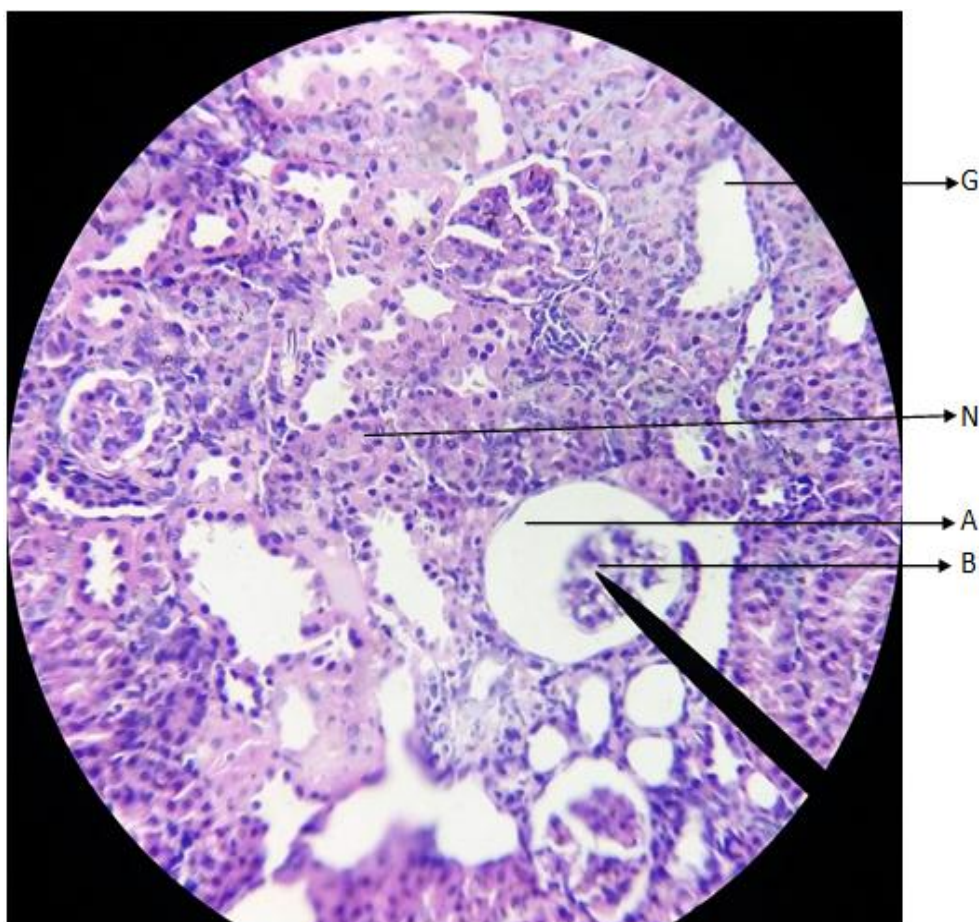


Plate IV: Histopathological photomicrograph of renal section from 80 mg/kg body weight gentamicin-induced nephrotoxicity group rats treated with 200 mg/kg Body weight of methanol leaf extract of *M. balsamina* Linn. stained with Hematoxylin and Eosin. Section (H and E, $\times 40$) shows a weak attempt at restoration of tubular structures amongst moderate necrotic tubules. Mild atrophic and tubular edema with adequate glomeruli Bowman's capsular spaces.

KEY

A – Bowman's capsule.

B – Glomerulus.

G - Necrosis.

N – Blood vessel congestion.

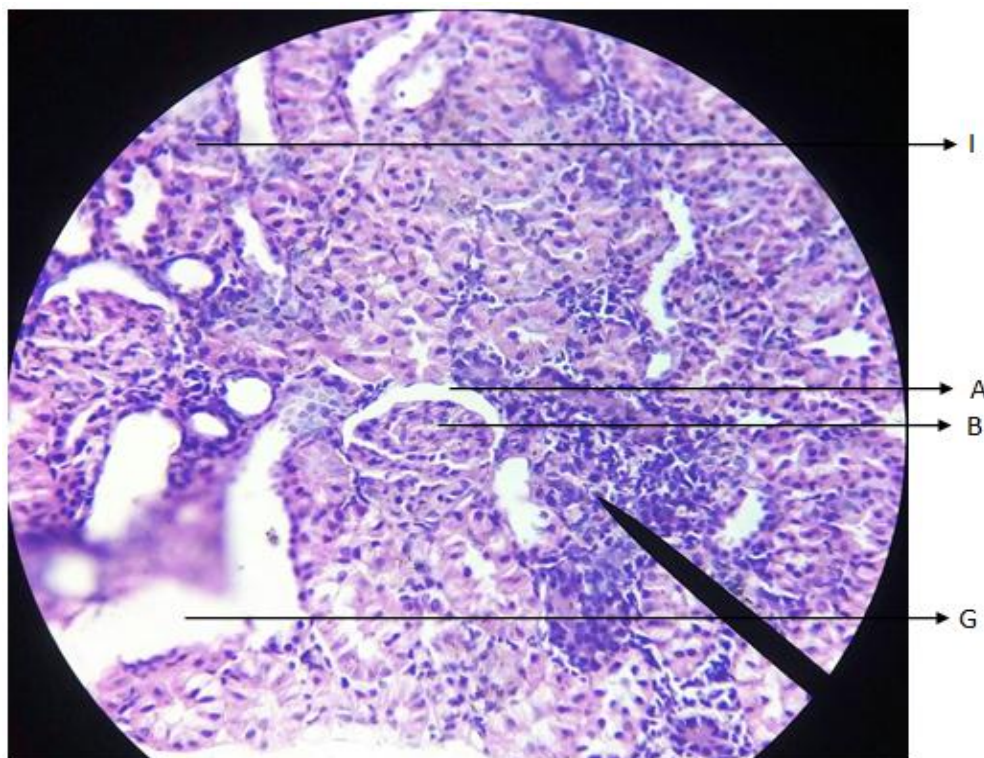


Plate V: Histopathological photomicrograph of renal section from 80 mg/kg body weight gentamicin-induced nephrotoxicity group rats treated with 400 mg/kg body weight of methanol leaf Extract of *M. balsamina* Linn. stained with Hematoxylin and Eosin. Section (H and E, $\times 40$) shows an appreciable attempt at restoration of tubular structures amongst very few necrotic tubules. Mild basement membrane alteration and epithelial desquamation. Weak atrophic and tubular edema with adequate glomeruli Bowman's capsular spaces.

KEY

A – Bowman's capsule.

B – Glomerulus.

G - Necrosis.

I – Tubular cast.

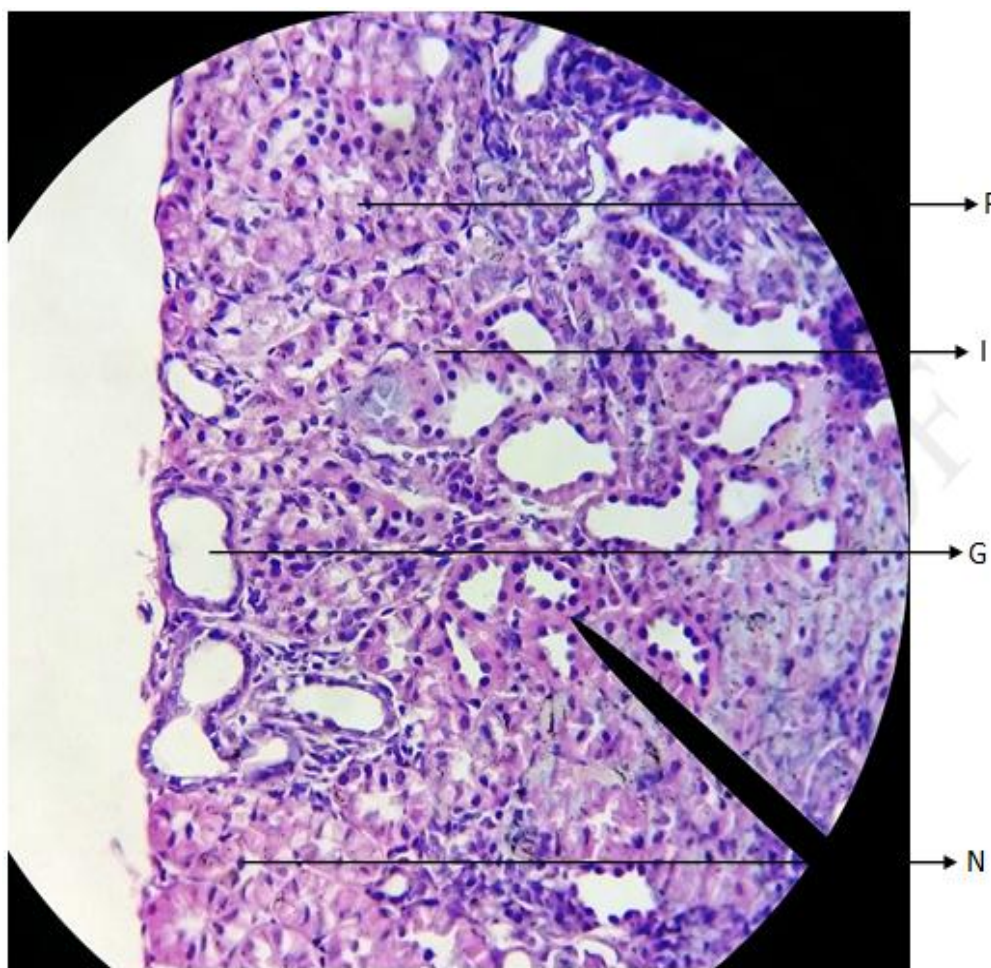


Plate VI: Histopathological photomicrograph of renal section from 80 mg/kg body weight gentamicin-induced nephrotoxicity group rats treated with 200 mg/kg body weight of methanol leaf extract of *M. balsamina* Linn. fraction I stained with Hematoxylin and Eosin. Section (H and E, $\times 40$) shows the cortex area with mild tubular edema and focal basement membrane alteration. Minor necrotic tubules and epithelial desquamation.

KEY

F – Glomerular.

G - Necrosis.

I – Tubular cast.

N – Blood vessel congestion.

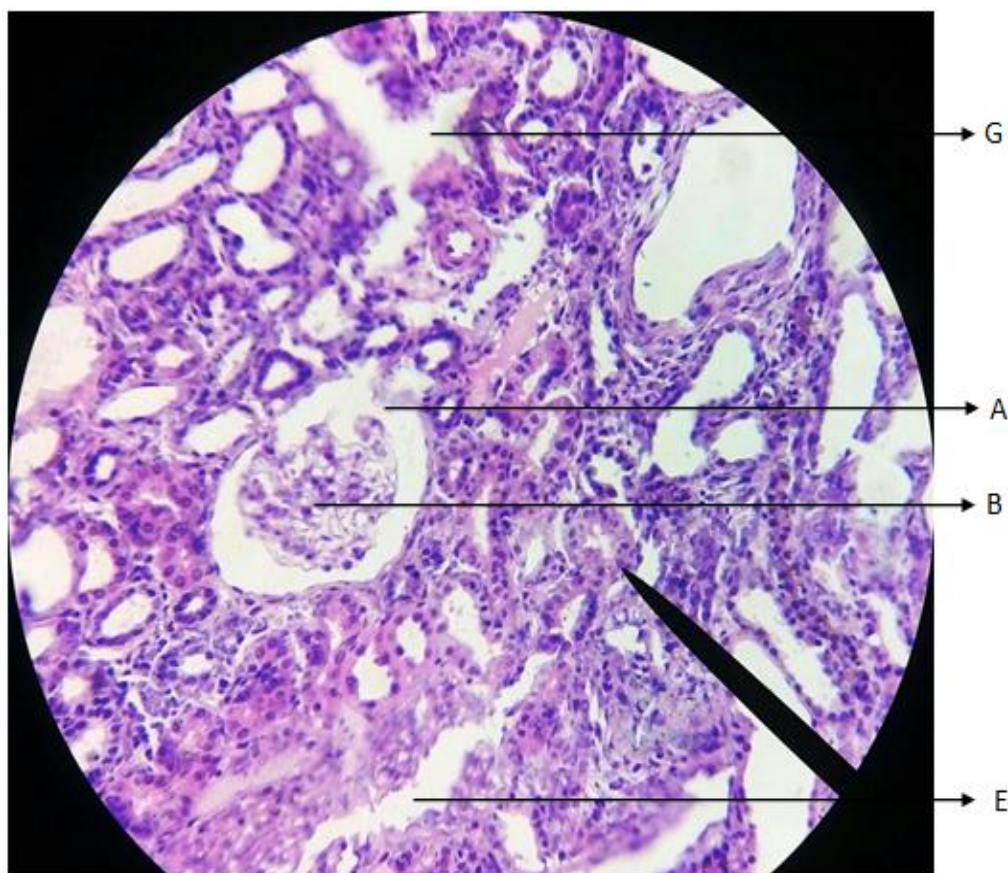


Plate VII: Histopathological photomicrograph of renal section from 80 mg/kg body weight gentamicin-induced nephrotoxicity group rats treated with 400 mg/kg body weight of methanol leaf extract of *M. balsamina* Linn. fraction I stained with Hematoxylin and Eosin. Section (H and E, $\times 40$) shows minor epithelial desquamation and basement membrane alterations. There was a presence of infiltrates within the interstitial spaces and glomeruli.

KEY

A – Bowman's capsule.

B – Glomerulus.

E – Distal convoluted tubule.

G - Necrosis.

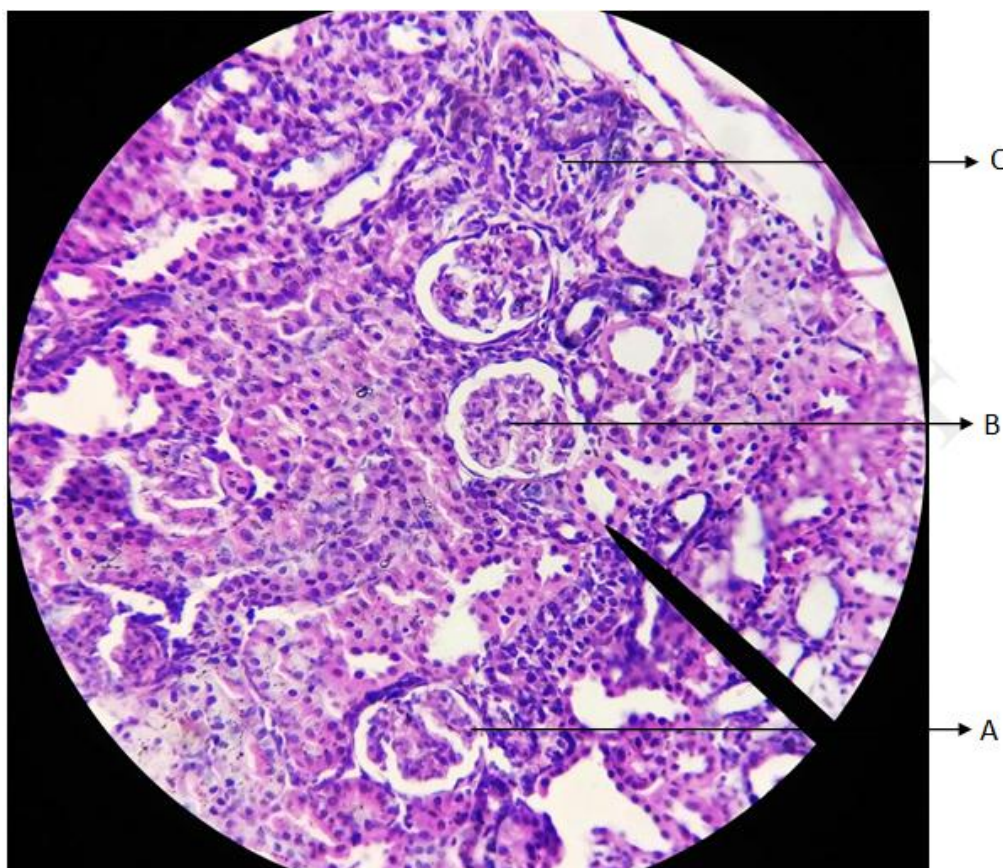


Plate VIII: Histopathological photomicrograph of renal section from 80 mg/kg body weight gentamicin-induced nephrotoxicity group rats treated with 200 mg/kg body weight of methanol leaf extract of *M. balsamina* Linn. fraction II stained with Hematoxylin and Eosin. Section (H and E, $\times 40$) shows minor necrotic tubules. The cortex area shows very little tubular edema with adequate glomeruli and adequate Bowman capsular spaces.

KEY

A – Bowman's capsule.

B – Glomerulus.

C – Collecting tubules.

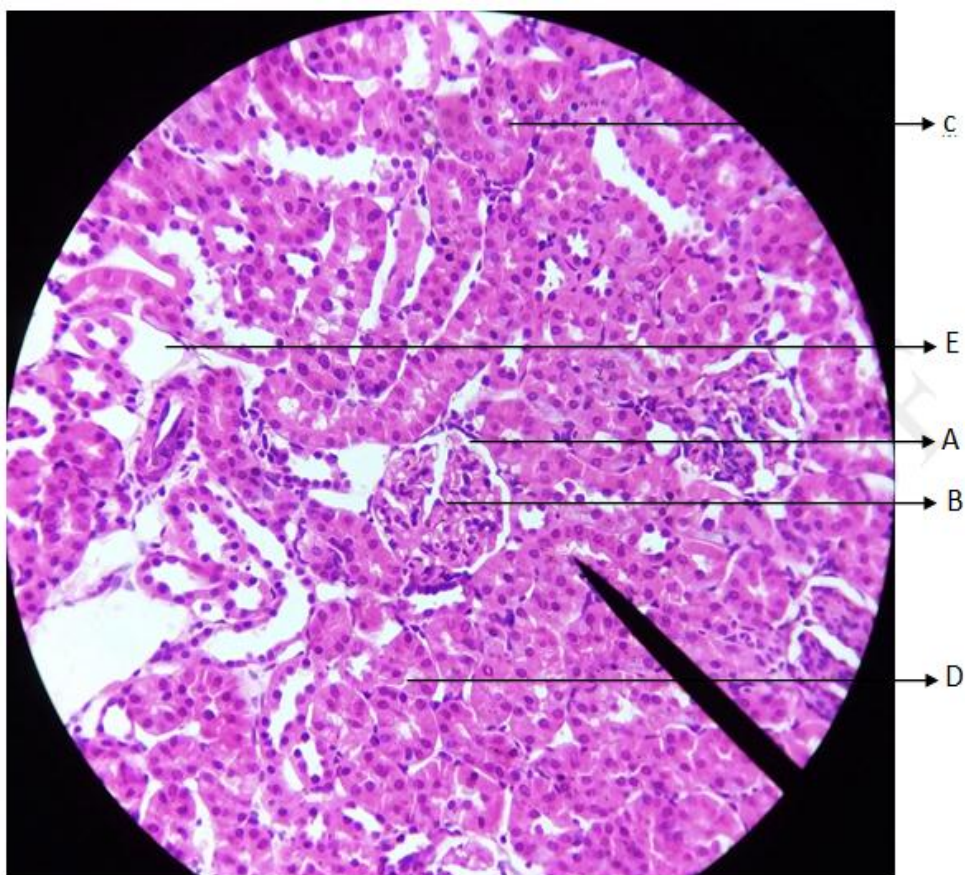


Plate IX: Histopathological photomicrograph of renal section from 80 mg/kg body weight gentamicin-induced nephrotoxicity group rats treated with 400 mg/kg body weight of methanol leaf extract of *M. balsamina* Linn. fraction II stained with Hematoxylin and Eosin. Section (H and E, $\times 40$) shows a remarkable and successful attempt at restoration of tubular structures amongst very minute remnant edematous tubules with adequate glomeruli that were reparative with adequate Bowman's capsular spaces.

KEY

- A – Bowman's capsule.
- B – Glomerulus.
- C – Collecting tubules.
- D – Proximal convoluted tubule.
- E – Distal convoluted tubule.

DISCUSSION

The extract of *M. balsamina* Linn. leaf produced a yield of 22.14% w/w and shown four distinct fractions in silica gel chromatography with each of the fractions provided useful classes of phytochemicals such as alkaloids, phenols, balsams, tannins, flavonoids and cardiac glycosides among others. These phytochemicals were successfully separated, though partially into their relative groups based on their affinity for the stationary phase which in turn was dictated by two properties of the molecules, that is adsorption and solubility. The result of the differential separation of the components of the extract of *M. balsamina* Linn. was in line with the result of Karumi *et al.*^[19] who reported that the extract of *M. balsamina* Linn. gives four clear bands of different colors in the silica gel chromatography column.

Previous studies have revealed the phytochemical constituents of *M. balsamina* Linn. to include triterpenoids, steroids, lectins among others.^[15,16,17] However, in this study, the phytochemical screening of the plant leaf extract shows that the plant has different varieties of phytochemicals that could be considered to play a crucial role in significantly ameliorated the effect of gentamicin – induced nephrotoxicity in rats as compared to the normal control group.^[15] Phenols and alkaloids have been the most significant compounds in the extract as determined through quantitative analysis in our laboratory (unpublished data) have been reported to show multiple activities like antioxidant, anti-inflammatory and anti-cancer among others and also play a significant role in protecting animals against reactive oxygen species.^[24] Phenols, flavonoids, balsams and tannins have been reported in previous studies to have been used against promotion of growth and progression of tumors, anti-inflammatory, antioxidant and hypoglycemic.^[17,24,25] while saponins are potent phytochemical against proteinuria due to its ability to form sparingly digestible saponins-protein complexes in the intestine.^[26] Saponins and tannins could also synergistically enhance kidney functions by promoting enzyme activities.^[27,28] Gentamicin has been widely used for inducing acute renal failure in experimental animals and evaluation of reno-protective agents due to its ability to bind to the cell membranes of proximal tubules and subsequently accumulate in the renal cortex where it gradually damages the cells through increased production of reactive oxygen species.^[29,30,31] In addition to oxidative stress, the pathological mechanisms involved in gentamicin – induced nephrotoxicity include increase serum creatinine, blood urea nitrogen (BUN), and decrease in glomerular filtration rate.^[32,7,10]

The results of this research shown that gentamicin administration in rats led to significant elevation of all the renal damage biomarkers; serum creatinine, urea, and blood urea nitrogen (BUN) (Table 1). This is in consonance with the claim that gentamicin induction results in renal damage in rats and is characterized by elevation of serum creatinine, urea and blood urea nitrogen levels.^[10] Impaired glomerular filtration rate results in increase serum creatinine and urea levels due to significant renal damage such as tubular necrosis and apoptosis.^[33] However, post-treatment of rats with the standard drug, furosemide (Lasix), a loop diuretics drug improved all these renal damage markers while the treatment of rats with the extract of *M. balsamina* Linn. leaf also shows an improvement in the amelioration of the renal injuries when compared with the experimental control group rats. Meanwhile, appreciable and significant ameliorative effects of the plant were observed in the rats treated with 200 mg/kg body weight and 400 mg/kg body weight of both fractions I and II of the extract respectively in all the renal damage biomarkers when compared with the normal control group. These may be due to the phytochemicals present in these fractions, most especially fraction II (saponins, flavonoids and phenols) which may have synergistically scavenged the free radicals generated as a result of gentamicin induction.

Moreover, gentamicin nephrotoxicity characterized by increase in serum electrolytes levels (Na^+ , K^+ , Cl^-) and decrease in bicarbonate (HCO_3^-) level as shown in the result of this study (Table 2) suggested that the site of gentamicin action may be the distal convoluted tubules^[34] which is the point of reabsorption of these electrolytes. Again and interestingly, post-treatment of the plant leaf extract and fraction II reduced serum Na^+ level (115.70 mg/dl) very close to normal control Na^+ level (113.90 mg/dl) at 400 mg/kg body weight. This effect may be due to the mild diuretic property of *M. balsamina* Linn. extract fraction phyto-constituents (saponins, tannins and balsams) mediated through competitive inhibition of water reabsorption in the nephron by blocking the $\text{Na}^+ - \text{K}^+ - \text{Cl}^-$ co-transporter (NKCC2) in the thick ascending limb of the loop of Henle and subsequent reduction in the activities of Na^+ / K^+ pump^[35] which in turn affect the level of bicarbonates in the blood as affirmed by Afzal *et al.*^[36] in Abeer.^[21]

Moreover, in this research, the histopathological analysis of the gentamicin-induced nephrotoxicity in rats kidneys section as compared with the control group indicates marked congestion of the glomeruli, necrotic and dilated tubules, edema, epithelial desquamation, severely damaged glomeruli, and high presence of infiltrates within the interstitial spaces and

glomeruli amongst other kidney tissue histopathological indices which apparently suggested successful renal damage as a result of gentamicin induction.^[8, 37, 21, 25] Interestingly, treatment of the rats in group VIII and group IX with 200 mg/kg body weight and 400 mg/kg body weight respectively of fraction II of *M. balsamina* Linn. leaf extract remarkably ameliorated and restored these histopathological indices to normal (Micrographs VIII and IX / Plates VIII and IX) as the micrograph sections show renal tissue displaying numerous collecting tubules in the medullary areas that were lined by simple cuboidal cells with ovoid nuclei and pink cytoplasm. There was a remarkable attempt at restoration to normal of tubular structures amongst very minute edematous tubules. The context area shows very little remnants of tubular edema with adequate glomeruli that were reparative with adequate Bowman capsular spaces. There was normal presence of infiltrates within the interstitial spaces as shown in the kidney micrograph (Plate IX). This result shows that *M. balsamina* Linn. leaf may contain some of the phytochemicals that could reverse renal tubular injuries and subsequently restore kidney functions to normal.^[21,25]

CONCLUSION

The results obtained from this study have somewhat conclusive suggestion that *Momordica balsamina* Linn. methanol leaf extract and fractions exhibit nephroprotective, anti-inflammatory and mild diuretic properties. Hence, *Momordica balsamina* Linn. leaf is effective against renal dysfunction and oxidative stress which could be due to the presence of bioactive compounds and antioxidants in the plant.

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Conflicts of interest

The authors declared that they have no competing interests as regards the research.

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