notOriginal Research Article

MODULATORY EFFECT OF AQUEOUS GINGER (ZINGIBER OFFICINALE) EXTRACT ON GENTAMICIN SULFATE INDUCED OXIDATIVE STRESS AND NEPHROTOXICITY IN FEMALE ALBINO RATS.

ABSTRACT

Acute renal failure is a common complication of gentamicin, an aminoglycoside antibiotic widely used against gram-negative infections, often mediated by oxidative stress. This study investigates the potential protective effects of aqueous ginger extract against gentamicin-induced nephrotoxicity in female albino rats. Rats were divided into four groups: control (distilled water and feed), ginger extract (2.14 ml/kg), gentamicin (100 mg/kg), and combined ginger extract with gentamicin. Biochemical assays measured blood urea nitrogen (BUN), creatinine, cholesterol, triglycerides, albumin, white blood cell count, and packed cell volume. Kidney homogenates were analyzed for catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), glutathione transferase (GST) activities, and malondialdehyde (MDA) levels. Histopathological evaluations of kidney tissues were also performed. Gentamicin administration significantly increased BUN and creatinine levels, decreased GSH levels, and increased MDA levels, indicating severe nephrotoxicity and oxidative stress. Ginger extract administration did not alleviate gentamicin-induced renal damage or oxidative stress, despite reducing serum cholesterol levels. The study also observed a reduction in CAT activity with ginger extract administration. Histopathological analysis revealed clear nephritis in the gentamicin-treated groups, particularly exacerbated in the group receiving both gentamicin and ginger extract. These outcomes suggest that while aqueous ginger extract did not mitigate gentamicin-induced renal injury in this study, its cholesterol-lowering effects highlight potential benefits in managing atherosclerosis. Further research is necessary to explore under varying conditions which ginger extract may discuss renal protection.

Keywords:*Ginger, Glutathione transferase (GST), Albumin, Histopathological analysis, therosclerosis `*

1.0 INTRODUCTION

Nephrology is the branch of medicine that focuses on the kidneys and kidney-related diseases. The kidneys are organs responsible for filtering waste products (such as urea) from the bloodstream and excreting them, along with water, as urine. In both humans

and dogs, the kidneys are situated in the rear portion of the abdomen. Positioned above each kidney is the adrenal gland (also known as the suprarenal gland), and each kidney is enveloped by two layers of fat (the perirenal and pararenal fat), which serve to cushion it. The congenital absence of one or both kidneys, referred to as unilateral or bilateral renal agenesis, can occur [1]. The fundamental functional unit of the kidney is the nephron, of which there are more than a million within the cortex and medulla of each normal adult kidney. It regulates water and soluble substances (especially electrolytes) in the body by first filtering the blood under pressure, then reabsorbing some necessary fluid and molecules back into the blood while eliminating those that are not needed. Reabsorption and secretion are accomplished with other co-transport and counter-transport mechanisms established in the nephrons and are associated with collecting ducts. This segment of the nephron is crucial to the process of water conservation by the organism. In the presence of antidiuretic hormone (ADH; also called vasopressin), these ducts become less permeable to water, facilitating its reabsorption, thus concentrating the urine and reducing its volume. When the organism must eliminate water, such as after excess fluid intake, the reduction of ADH is decreased, and the collecting tubule becomes less permeable to water, rendering the urine dilute and abundant. Failure of the organism to decrease ADH production appropriately, a condition known as syndrome of inappropriate ADH (SIADH), may lead to water retention and dangerous dilution of the body fluid, which may cause severe neurological damage. Failure to produce ADH (or inability of the collecting ducts to respond to it) may cause excessive urination, called diabetes insipidus (DI). The functions of the kidney include excretion of a variety of waste products produced by metabolism, including the nitrogenous wastes, urea (from protein catabolism) and uric acid (from

nucleic acid metabolism) and water. Homeostatic functions such as acid-base balance, regulation of electrolyte concentration, control of blood volume, and regulation of blood pressure. Plasma Volume, Any significant rise or drop in plasma osmolality is detected by the hypothalamus, which communicates directly with the posterior pituitary gland. A rise in osmolarity causes the gland to secrete antidiuretic hormone, resulting in water reabsorption by the kidney and an increase in urine concentration. The two factors work together to return the plasma osmolality to its normal levels. The kidneys also secrete a variety of hormones, including erythropoietin, urodilatin, renin, and vitamin D. Kidney diseases and disorders can be either congenital or acquired. The congenital conditions include congenital hydronephrosis, congenital obstruction of the urinary tract, duplicated ureter, horseshoe kidney, polycystic kidney disease, renal dysplasia, unilateral small kidney, and medullary sponge kidney. Acquired conditions include diabetic nephropathy, glomerulonephritis, hydronephrosis, interstitial nephritis, kidney tumors (Wilms tumor, renal cell carcinoma), lupus nephritis, minimal change disease, pyelonephritis, acute or chronic renal failure, and kidney stones. Renal calculi, also known as kidney stones, are solid concretions (crystal aggregations) of dissolved minerals in urine, which typically form inside the kidneys or ureters. The term nephrolithiasis refers to the presence of calculi in the kidneys and urinary tracts, respectively [2]. Kidney stones can be attributed to underlying metabolic conditions, such as renal tubular acidosis, Dent's disease, and medullary sponge kidney, and many healthcare facilities will screen for such disorders in patients with recurrent kidney stones, although most stones arise spontaneously. Symptoms of kidney stones include colicky pain radiating from the flank to the groin, hematuria due to damage to the wall of the ureter or urethra, dysuria when passing stones, oliguria due to obstruction of the

bladder or urethra by a stone, or extremely rarely, simultaneous obstruction of both ureters by a stone, and nausea or vomiting due to an embryological link with the intestine. Preventive strategies involve dietary modification and sometimes drug therapy, with the goal of reducing the excretory load on the kidneys [3]. Ginger (Zingiber officinale) is a versatile plant with a long history of traditional medicinal use. Its rhizome has been employed to alleviate various ailments, including joint inflammation, cold symptoms, sore throats, fever, cramps, and constipation [4]. Additionally, ginger exhibits antioxidant properties and cytoprotective effects, making it a potential treatment for microbial infections, inflammatory conditions, and emesis [5]. The bioactive constituents of Zingiber officinale, such as zingerone, gingerdiol, zingibrene, gingerols, and shogaols, are responsible for its antioxidant activities [6]. Interestingly, ginger has been found to play a preventive role in ischemia/reperfusion (I/R) injury in rat kidneys, suggesting its potential in protecting against kidney damage caused by reduced blood flow and subsequent restoration [7]. Furthermore, ginger has demonstrated an inhibitory effect on cisplatin-induced nephrotoxicity in rats, indicating its possible therapeutic application in mitigating drug-induced kidney injury [7]. While ginger is commonly used for managing morning sickness during the first trimester of pregnancy, recent literature reviews have concluded that its safety during pregnancy has not been definitively established [8].Ginger rhizomes are also extensively utilized in foods, especially in Asian cuisines, due to their medicinal benefits. The primary bioactive constituents in Zingiber officinale are the pungent vanilloids, -gingerol and -paradol, along with other phenolic compounds such as shogaols and zingerone. Ginger rhizomes are also rich in essential minerals, including high amounts of iron (54-62 mg/100g) and calcium (1.0%-1.5%). The antioxidant, antitumor, and anti-inflammatory

properties of ginger are primarily attributed to its pungent constituents, particularly -gingerol. The antioxidant effects of ginger have been demonstrated in various studies, including one where ginger was shown to overcome the oxidative stress induced by the pesticide malathion in rats. Ginger is also recognized for its potent antitumor activities, as evidenced by in vitro experiments, with the vanilloids -gingerol and -paradol playing a key role in inhibiting cellular proliferation and inducing apoptosis in cancer cells. Furthermore, the anti-inflammatory effects of -gingerol have been studied in mouse models, where it was found to suppress the inflammatory response induced by the phorbol ester TPA. Similarly, ginger oil has been shown to significantly reduce joint swelling in a rat model of severe arthritis, with the oleoresin constituents and phenolic compounds, such as paradol and shogaol, exhibiting inhibitory effects on the cyclooxygenase-2 (COX-2) enzyme, a crucial mechanism in the regulation of the inflammatory process.

2.0 MATERIALS AND METHODS

2.1 Sample Preparation

Fresh samples of ginger (Zingiber officinale), belonging to the Zingiberaceae family, were procured from Mushin Market, Mushin, Lagos State, Nigeria. Prior to extraction, the rhizomes were washed, the scaly skin was peeled off, and they were re-washed with distilled water to remove unwanted materials such as sand, debris, and other contaminants. The peeled ginger rhizomes were reduced to smaller particles by grating, and the grated ginger was subjected to hot water (boiled) for 10 minutes, using a ratio of 50g of ginger in 200ml of distilled water. The solution was filtered using glass wool, and the resulting extract was concentrated by direct heat and finally dried in an oven at 80°C

to determine the concentration of the active ingredients in the ginger extract, yielding 10.5g of dried extract per 50g of wet ginger rhizome.

2.2 Experimental Design

Twenty-nine female albino rats weighing between 100-180 g were procured from the University of Ibadan for the purpose of this research. Based on their intended uses, the animals were redistributed into four different groups (Groups A, B, C, and D) and housed in well-ventilated iron cages at room temperature. They were fed a standard rat diet and provided with drinking water. The room and the cages were regularly cleaned and disinfected to avoid infections. The acclimatization period lasted for two weeks, during which all the groups were fed the rat feed and had access to drinking water throughout the day.

Group A: Received rat feed and distilled water.

Group B: Received rat feed, distilled water, and ginger aqueous extract (2.14 mL/kg or 146 mg/kg).

Group C: Received rat feed, distilled water, and gentamicin (100 mg/kg) without ginger extract.

Group D: Received rat feed, distilled water, ginger extract (2.14 mL/kg), and gentamicin (100 g/kg).

(a) (b)

Figure 1.0 female albino rats weighing between 100-180 g

2.3 Blood Collection For Hematological and Biochemical Analysis

Under light ketamine hydrochloride (5 mg/kg) anesthesia, blood was obtained by cardiac puncture. Serum was prepared from the collected blood by centrifuging the sample at 3000 rpm for 20 minutes and was subjected to biochemical assays, including blood urea nitrogen (BUN), creatinine, cholesterol, triglycerides, and albumin. A portion of the blood was collected in a heparinized bottle for white blood cell count and packed cell volume.

2.4 Kidney Homogenate Preparation

From the sacrificed rats, the kidneys were dissected out, blotted on filter paper, washed in 0.9% saline, and weighed immediately. Kidney homogenate was prepared by homogenizing 0.5 g of kidney tissue in phosphate buffer. The prepared homogenate was centrifuged at 3000 g for 20 minutes, and the supernatant was collected and stored in a refrigerator for further use, including assays for catalase, superoxide dismutase, lipid

peroxidation (thiobarbituric acid), reduced glutathione (GSH), and glutathione-S-transferase, which were performed within two weeks.

2.5 Histopathological Evaluation

A kidney was taken from a rat (two from each group) for histopathological evaluation. The histopathological evaluation involved collecting kidney samples from four groups of rats. Group A (GA) served as the control, receiving distilled water and standard rat feed. Group B (GB) was given ginger extract and rat feed. Group C (GC) received rat feed and an overdose of gentamicin, and Group D (GD) was administered feed, ginger extract, and an excessive dose of gentamicin. The collected samples were fixed in 10% formalin, processed, and sectioned at 4 microns. The tissue sections were then stained using the conventional hematoxylin and eosin method, and the slides were thoroughly interpreted and summarized according to the respective groups.

2.6 Hematological Analysis

2.6.1 Determination of Packed Cell Volume (PCV) [Hematocrit]

The rat blood collected in a heparinized container was drawn into a hematocrit tube, sealed at one end with Plasticine, and centrifuged for 20 minutes at 3000 rpm. A hematocrit reader was used to determine the volume of the red cells relative to the whole column, which is known as the hematocrit reading or packed cell volume.

2.6.2 White Blood Cell Count

The method described by [9] was used. The rat blood in the heparinized bottle was drawn to the 0.5 mark on the stem of a white cell pipette and diluted with a diluting fluid to the 11 mark immediately above the bulb. Using an improved Neubauer Chamber, the

cells in the four corner squares and those in the central square millimeter of the ruled area were counted.

2.6.3 Biochemical Analysis

Catalase activity was assessed using the method by [10], measured spectrophotometrically by the decrease in absorbance at 240 nm (A240) as hydrogen peroxide (H_2O_2) decomposed, with a Beckman spectrophotometer (USA) and a molar extinction coefficient of 40.0 m^{-1} cm^{-1}. The materials used included a phosphate buffer (50 mM, pH 7.0) prepared from KH_2PO_4 and $Na_2HPO_4 \cdot 2H_2O$, and hydrogen peroxide (30 mM). In the procedure, 0.2 mL of the sample was mixed with 1.8 mL of 30 mM H_2O_2, or 0.1 mL of kidney homogenate supernatant, with phosphate buffer as the blank. Absorbance was recorded at 240 nm every 30 seconds for 1 minute.

2.6.4 Superoxide Dismutase (SOD) Assay

Superoxide dismutase (SOD) activity was assayed according to the method of [11]. 3 mL of 50 mM Na_2CO_3 buffer + 0.02 mL (20 µL) of the sample (kidney homogenate supernatant) + 0.03 mL (30 µL) of epinephrine, and the absorbance was measured at 480 nm for 3-5 minutes. The blank used was 3 mL of buffer + 0.02 mL of water + 0.03 mL of the substrate (sample).

Enzyme Activity = $\frac{\frac{\Delta OD}{min480} * Vt}{\sum x * Vs}$ = Amount of O_2, produced per ml

Where:

Vt = Total volume of reaching sample

Vs = Volume of sample

$\sum x$ = $4020.0 m^{-1} cm^{-1}$

The superoxide anion radical (O_{2-}) spontaneously dismutes to O_2 and H_2O_2 rapidly. (Ω 105 m^{-1} 5^{-1} at PH7). SOD catalyses the dismutation of superoxide into oxygen and hydrogen peroxide.

2.6.5 Thiobarbituric Acid Assay (TBARS)

The method according to [12] was used. Malondialdehyde, formed from the breakdown of polyunsaturated fatty acids, serves as a convenient index for determining the extent of the peroxidation reaction.1.0 mL of the kidney homogenate supernatant was mixed with 2.0 mL of the TCA-TBA-HCl reagent and thoroughly mixed. The solution was heated for 15 minutes in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1000 rpm for 10 minutes. The absorbance of the sample was determined at 535 nm against a blank that contained all the reagents minus the kidney homogenate supernatant. The malondialdehyde concentration in the sample was calculated using an extinction coefficient of 1.56 × 105 m^{-1} cm^{-1} [13].

$$\text{Malondialdehyde (nmol)} = \frac{OD * Vt * F}{\sum Vs}$$

Where:

OD = Optical density (absorbance reading)

Vt = Total volume of reaction mixture

V = Volume of the sample

E = Standard coefficient (1.56 x105m^{-1} cm^{-1})

F = dilution factor (Optional)

2.6.6 Glutathione-S-Transferase (GST) Assay

The method of Habig (1974) was used. The reaction was measured by observing the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) with reduced glutathione (GSH). This was done by monitoring the increase in absorbance at 340 nm. One unit of the enzyme would conjugate 10 nanomoles of CDNB with reduced glutathione per minute at 25℃ [15]. Glutathione-S-Transferase catalyzed the reaction (binding) of GSH (reduced glutathione) and CDNB.1 mL of phosphate buffer (0.2 M phosphate buffer, pH 6.5), 0.1 mL of CDNB (20 mM CDNB) and 1.7 mL of distilled water were added, and the mixture was incubated at 37℃ for 5 minutes. Then, 0.1 mL of the kidney supernatant homogenate and 0.1 mL (20 mM GSH) were added, and the absorbance was taken for 5 minutes at 340 nm.

2.6.7 Reduced Glutathione (GSH) Assay

The procedure of [16] was used for this analysis. GSH is known as a substrate in both conjugation and reduction reactions. The level of GSH was determined spectrophotometrically. 100 µL of the kidney supernatant homogenate was added to 1 mL of 0.2 M Tris-EDTA buffer (pH 8.2), 0.9 mL of 20 mM EDTA (pH 4.7), and 20 µL of 10 mM DTNB. After 30 minutes of incubation at room temperature, the mixture was centrifuged, and the absorbance of the supernatant was read against distilled water at 412 nm.

$$\text{GSH} = \frac{OD * Vt}{\Sigma\ Vs}$$

Where:

OD = Absorbance

Vt = Total volume of reaction mixture

Vs = Volume of sample in reaction mixture

$\sum$ = Extinction coefficient =13,600mcm.

2.6.8 Triglyceride Assay

Serum triglycerides were analyzed using a standard enzymatic procedure from the Hirnen Gesellschaft for Biochemical and Diagnostic mbH (Germany). The triglycerides were quantified after enzymatic hydrolysis with lipases. The indicator is quinoneimine, which is formed from hydrogen peroxide, 4-amino-antipyrine, and 4-chlorophenol under the catalytic influence of peroxidase. The procedure was outlined by [17]. The absorbance of the sample (AA sample) and the standard ΔA (STD) against the reagent blank at 500nm was taken within 60 minutes. The Triglycerides Concentration (C) was calculated using.

$$C = \frac{2.28 * \Delta A\ sample\ (\frac{mmol}{L})}{\Delta A(STD)}$$

2.6.9 Total Cholesterol Assay

The serum total cholesterol was analyzed using standard enzymatic procedures (Human Biochemical, Germany) outlined by [18]. The assay principle involved the

determination of cholesterol after enzymatic hydrolysis and oxidation. For the assay, the following reagents were mixed: phosphate buffer (100 mmol/L, pH 6.5), 4-amino phenazone (0.3 mmol/L), phenol (5 mmol/L), peroxidase (>5 ku/L), cholesterol oxidase (>100 u/L), and sodium oxide (0.05%). The standard used was 3 mL of cholesterol at 5.17 mmol/L. In the pipetting scheme, 10 µL of the sample or standard was added to 1000 µL of reagent (RGT), while the reagent blank consisted of 1000 µL of reagent alone. The mixture was incubated at 37°C, and absorbance was measured at 500 nm within 60 minutes, comparing the sample or standard against the reagent blank.The serum total cholesterol was calculated as follows

$$\text{Total serum cholesterol} = \frac{5.17 * \Delta A \text{ sample}}{\Delta A \text{ (Standard)}} (\text{mmol/L})$$

2.6.10 Albumin Assay

Serum albumin was analyzed using a standard procedure from Human Gesellschaft for Biochemical and Diagnostic mbH (Germany) involving a colorimetric method, as outlined by [19]. In this method, albumin is determined after it forms a colored complex with bromocresol green in a citrate buffer. For the assay, the following reagents were used: citrate buffer (30 mmol/L), bromocresol green (260 µmol/L), albumin standard (4 g/dL or 40 g/L), and sodium azide (0.05%). In the pipetting scheme, 10 µL of the sample or standard was added to 1000 µL of reagent (RGT), while the reagent blank consisted of 1000 µL of reagent alone. The absorbance of the sample and the standard was

measured against the reagent blank at 578 nm within 30 minutes (ΔA).Albumin Concentration was calculated using:C= $\frac{40*\Delta A \text{ sample}}{\Delta A \text{ (Standard)}}(g/L)$

2.6.11 Test for Creatinine

Serum creatinine was analyzed using the standard procedure from Randox Laboratory Ltd (United Kingdom) based on the colorimetric method described by [20], where creatinine reacts with picrate in an alkaline solution to form a colored complex. The reagents used included a standard at 177 µmol/L, picric acid at 35 mmol/L, sodium hydroxide at 1.6 mmol/L, and trichloroacetic acid (TCA) at 1.2 mol/L. For the assay, 1.0 mL of TCA and 1.0 mL of serum were pipetted into centrifuge tubes, mixed with a glass rod to evenly disperse the precipitate, and centrifuged at 2,500 rpm for 10 minutes. The supernatant was collected for further analysis. In the assay procedure, 0.5 mL of distilled water was used for the blank, 0.5 mL of TCA for the standard, and 1.0 mL of supernatant for the sample, all mixed with 1.0 mL of reagent mixture. Each tube was incubated for 20 minutes at 37°C, and the absorbance of the sample (A_sample) and standard (A_standard) was measured against the blank at 520 nm.

Concentration of creatinine in serum is given as = $\frac{A\,sample*177}{A\,standard}\mu mol/L$

2.8.10 Blood Urea Nitrogen (BUN)

Blood urea nitrogen was analyzed using the standard procedure from Randox Laboratory Ltd (United Kingdom), based on the colorimetric method outlined by [21].

Urea in the serum is hydrolyzed to ammonia in the presence of urease, and the ammonia is then measured photometrically by Berthelot's reaction. Sodium nitroprusside and urease were combined as solution 2, phenol was diluted with 660 mL of distilled water as solution 3, and sodium hypochlorite was diluted with 750 mL of distilled water as solution 4. 10 µL of distilled water was used for the blank, 10 µL of the standard solution for the standard, and 10 µL of the sample for the sample, all mixed with 100 µL of solution 2. This mixture was incubated at 37°C for 10 minutes. Subsequently, 2.50 mL of solution 3 and 2.50 mL of solution 4 were added to each tube, mixed, and incubated again at 37°C for 15 minutes. The absorbance of the sample (A sample) and standard (A standard) was measured against the blank at 546 nm.The urea concentration was calculated using the following method.

Urea Concentration $= \frac{A\ sample * 80}{A\ standard} (mg/dl)$

Where 1 mg of urea corresponds to 0.467mg of urea nitrogen.

3.0 RESULTS AND DISCUSSION

3.1 Physical Observations

The following were observed during the course of this project

I. Reduced weight gain, excessive thirst, hypo activity, lack of coordination, lethargy, difficulty breathing, emaciation, and prostration in groups C and D. Three rats died in group C and two in group D.

II. During the process of sacrifice, fat deposits were seen around the kidneys in the rats given aqueous ginger extract, with more deposits observed in group B compared to group D.

3.2 Histopathological Evaluation

H/E = Hematoxylin and eosin staining

Kidney GA (X400 Magnification)

H/E staining shows normal renal glomeruli and tubules. There is congestion of the cortical vessels and tubules.

Kidney GB (X400 Magnification)

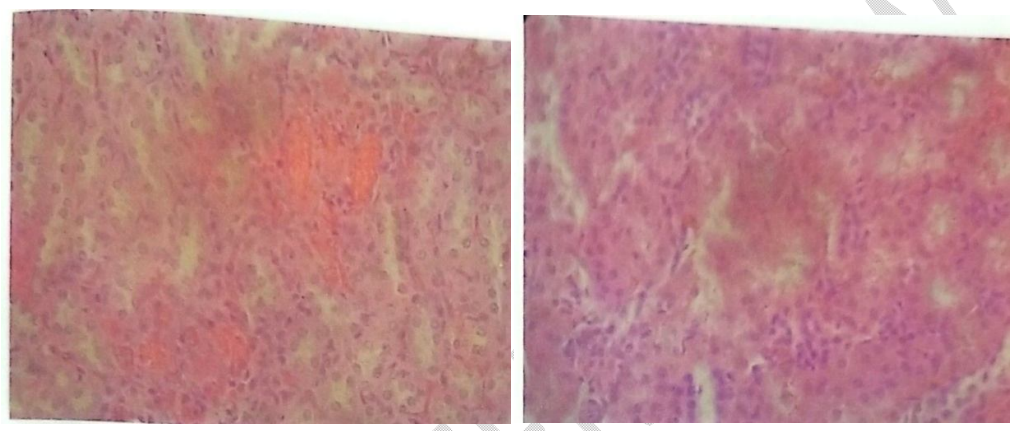
H/E staining shows normal renal glomeruli and tubules. There is moderate congestion of the medullary vessels.

Kidney GC (X400 Magnification)

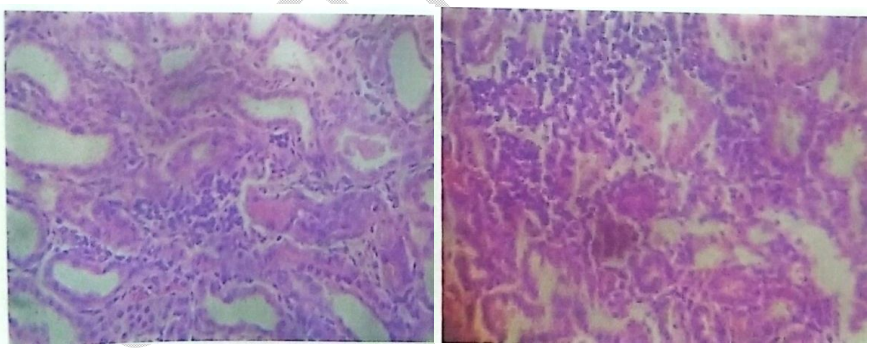
H/E staining shows preserved renal tissue, with widened Bowman's spaces and collecting ducts. The collecting ducts are frequently distorted, seen in both longitudinal and transverse sections. Some of the ducts contain eosinophilic secretions.

Kidney Gd (X400 Magnification)

H/E staining shows severe architectural distortion. There is diffuse cortical and medullary congestion extending to involve the glomeruli. There is also severe interstitial nephritis.



Group A **Group B**



Group C **Group D**

Figure 2.0 Plates of Histopathological Evaluation

3.4 Summary of Histopathological Analysis

Table 1: Summary of Hispathological Analysis of the Rat Kidneys

Groups	Renal Architecture	Cortex	Medulla	Glomerulli	Tubules	Interstitium
Group A	Preserved	Congested		-	-	-
Group B	Preserved	-	Congested	-	-	-
Group C	ivlild distortion			WideBM spaces	Distorted	**Severe nephritis**
Group D	Severe distortion	Congested	Congested	Congested	Distorted	**Severe nephritis**

Table 2: Mortality Effect of Treatment on Rats

Groups	No of Rats	Dosage of Gentamacin administration	Dosage of Ginger Administration	No of Death
Group A	6	100mg/kg	-	-
Group B	6	100mg/kg	2.14ml/kg	-
Group C	9		-	**3**
Group D	8		2.14ml/kg	**2**

3.5 Biochemical Evaluation

Table 3: Effect of Treatment on PCV and WBC Count

FEMALE ALBINO RATS	Mortality (N)	Packed cell Volume (PCV)%	White blood cell (WBC/L)x10°
Group A	NIL	41.8	**1.1**
Group B	NIL	44.4[a]	**1.03[a]**
Group C	3	35[ab]	**0.96[a,b]**
Group D	2	33.5[abx]	**0.7.8[a,b,c]**

Table 4: Effect of Treatment on Serum Albumin Creatinine and Bun

FEMALE ALBINO RATS	Serum Creatinine (mg/L)	Serum Albumin (g/L)	Blood Urea Nitrogen level (BUN). (g/l)
Group A	0.74	51.2	**17.82**
Group B	1.11[a]	37.29a	**12.95[a]**
Group C	1.6[a,b]	35.96[a,n]	**122.52[a,b]**
Group D	1.9[a,b,c]	35.65[a,n,x]	**45.33[ab,c]**

Table 5: Effect of Treatment on Total Cholesterol and Triglyceride

FEMALE ALBINO RATS	Serum total cholesterol mg/dL	Serum Triglyceride
Group A	189.1	**163.4**
Group B	168.4[a]	**108.6[a]**
Group C	69.8[a,b]	**182.8[n,b]**
Group D	39.5[a,b,c]	**79.9[a,nb,c]**

Table 6: Effect of Treatment on Some Antioxidant Enzymes and Oxidative Stress Parameters.

FEMALE ALBINO RATS	GSH Level (U/L) x 10^{-4}	GST Activity (U/L) X 10^{-3}	SOD Activity (U/L) x 10^{-4}	CATALASE Activity (U/L) x 10^{-2}	MDA Level (n mol/Ml) x 10^{-5}
Group A	3.31	11.7	2.2	.732	**1.7**
Group B	3.0^{na}	5.0^{a}	$2.4^{na.}$	4.3^{a}	$\mathbf{1.98^{n}}$
Group C	$2.2^{a,b}$	$5.21^{a,nb}$	$O^{a,b,}$	$5.5^{a,n}$	$\mathbf{2.4^{a,b}}$
Group D	$2.9^{a,nb,c}$	$5.5^{a,nb,nc}$	$O^{a,b,nc}$	$3.4^{a,n,x}$	$\mathbf{2.5^{a,b,nc}}$

3.6 Graphical Representation of Biochemical Evaluation

Figure 3. Graph Showing Effect of Treatment of Packed Cell Volume (PCV) of Rats Blood

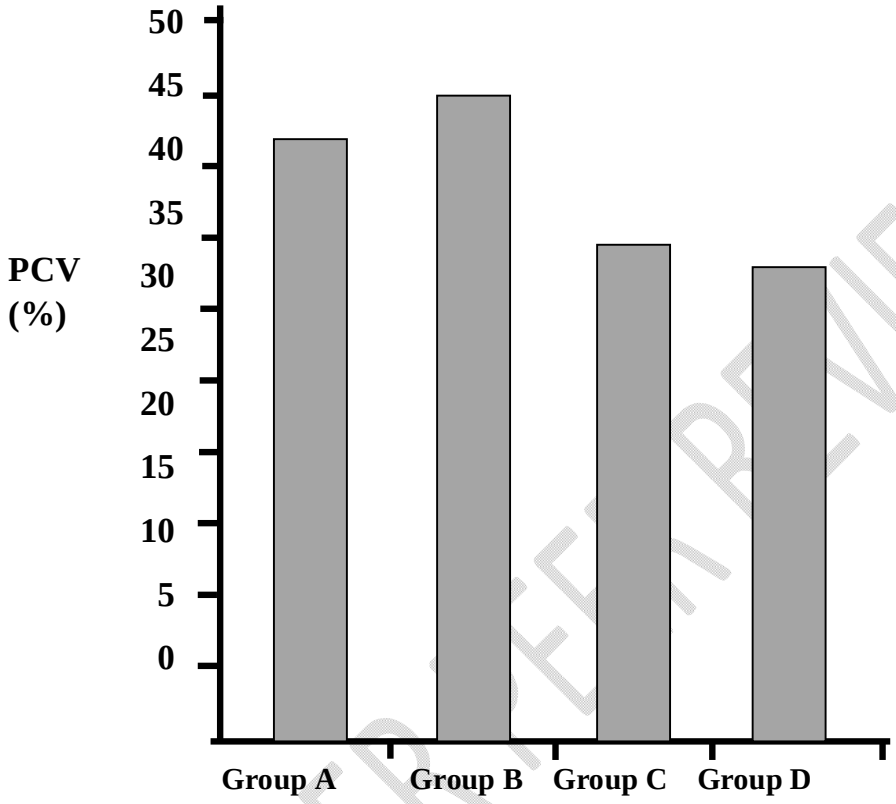


Figure 4 Graph Showing Effect of Treatment on White Blood Cell (WBC) of Rats Blood (X10^9 per Litre)

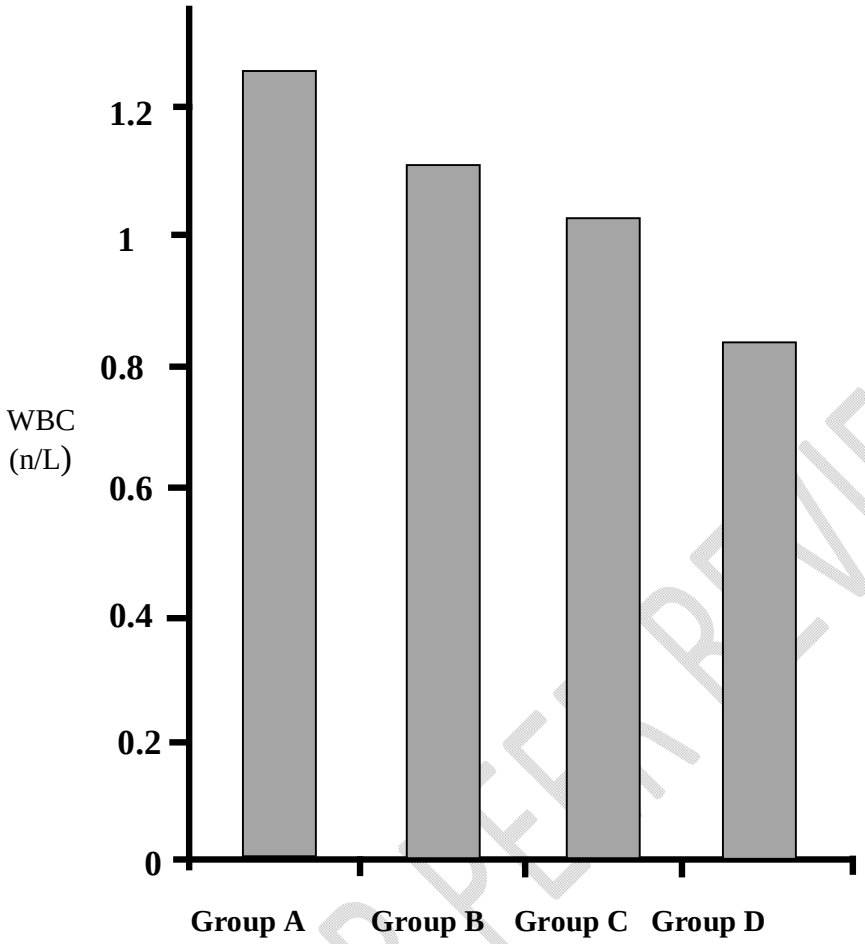


Figure 5 Graph Showing Effect of Treatment on Creatinine Level of Blood Serum of Rat (Mg/L)

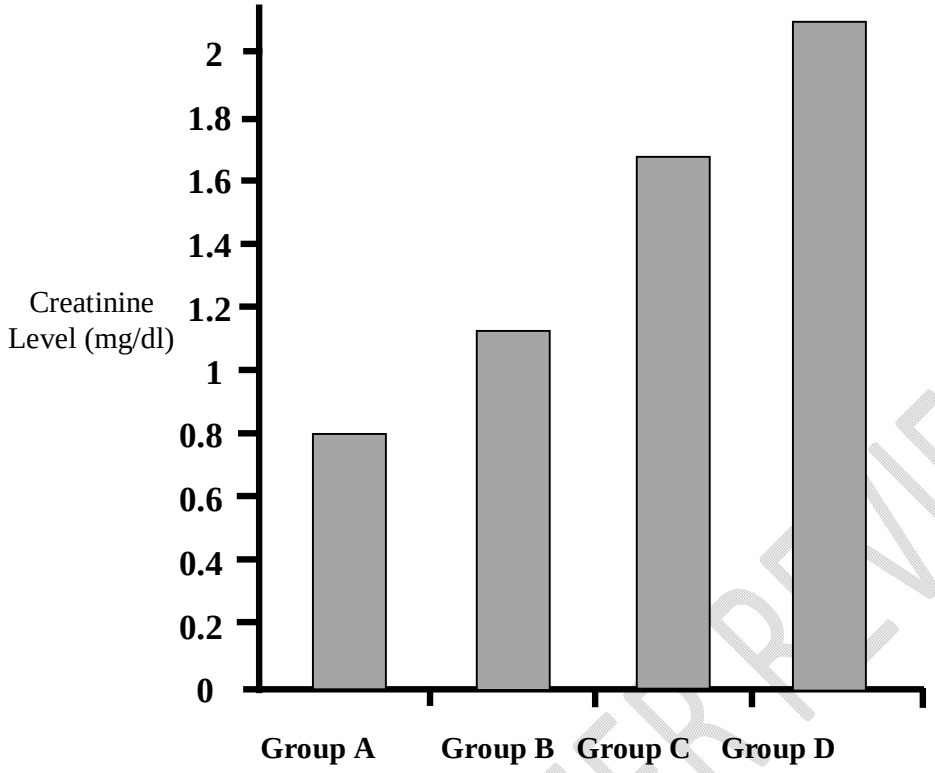


Figure 6. Graph Showing Effect of Treatment on Serum Albumin of Rat (g/L)

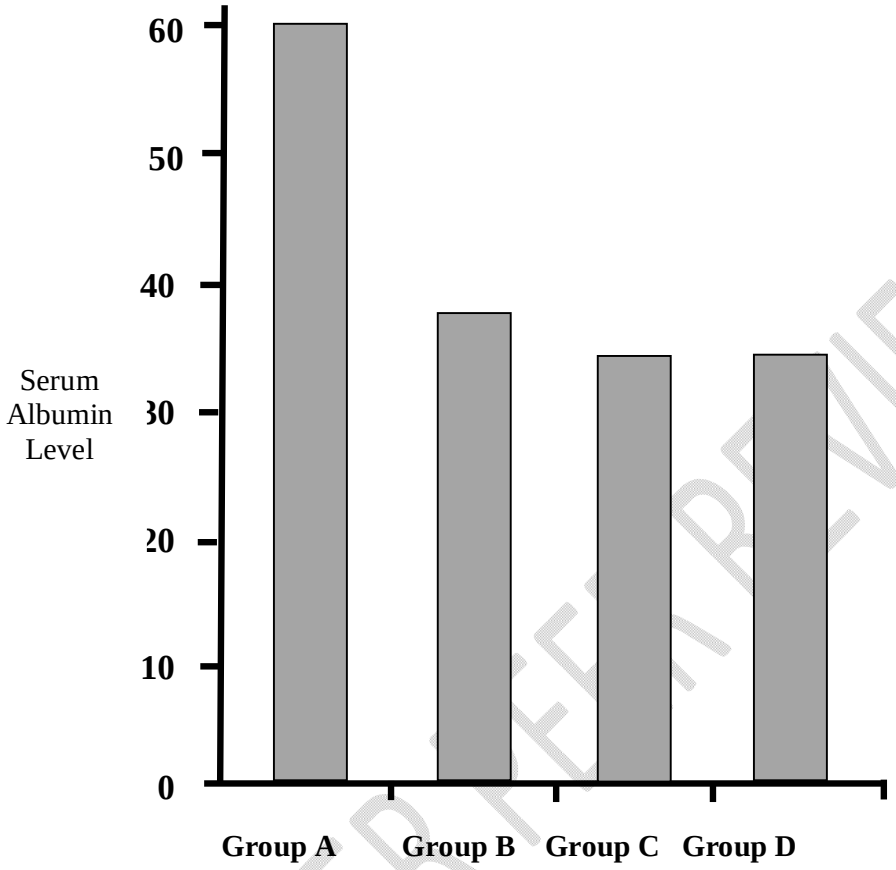


Figure 7 Graph Showing Effect of Treatment on the Blood Urine Nitrogen (BUN) Level in the Serum of Rate (g/L)

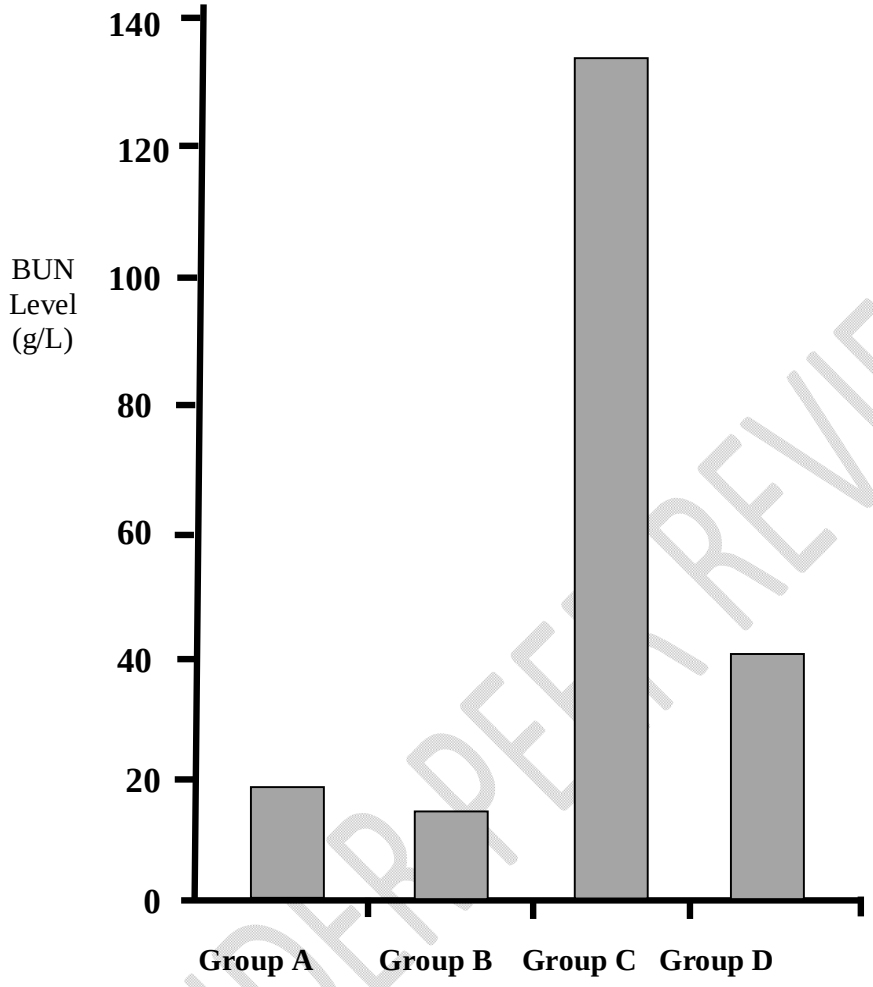


Figure 8Graph Showing Effect of Treatment on Total Cholesterol Rat Serum (mg/L)

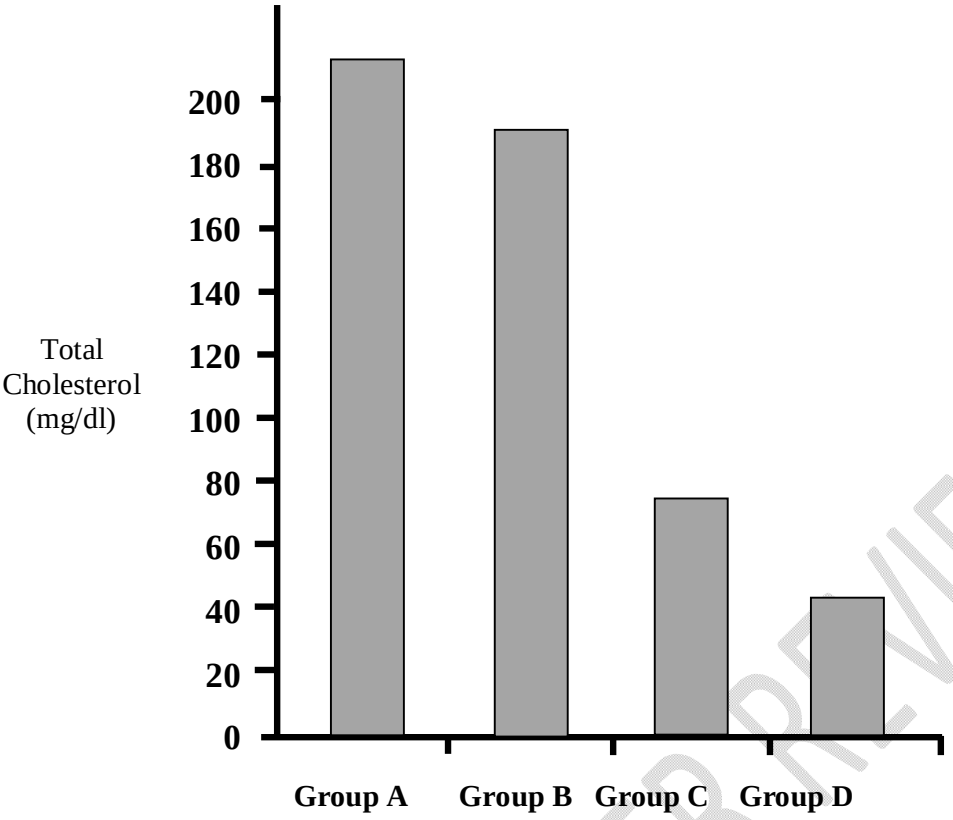


Figure 9 Graph Showing Effect of Treatment on Triglycerides of Rat Blood Serum (mg/dl)

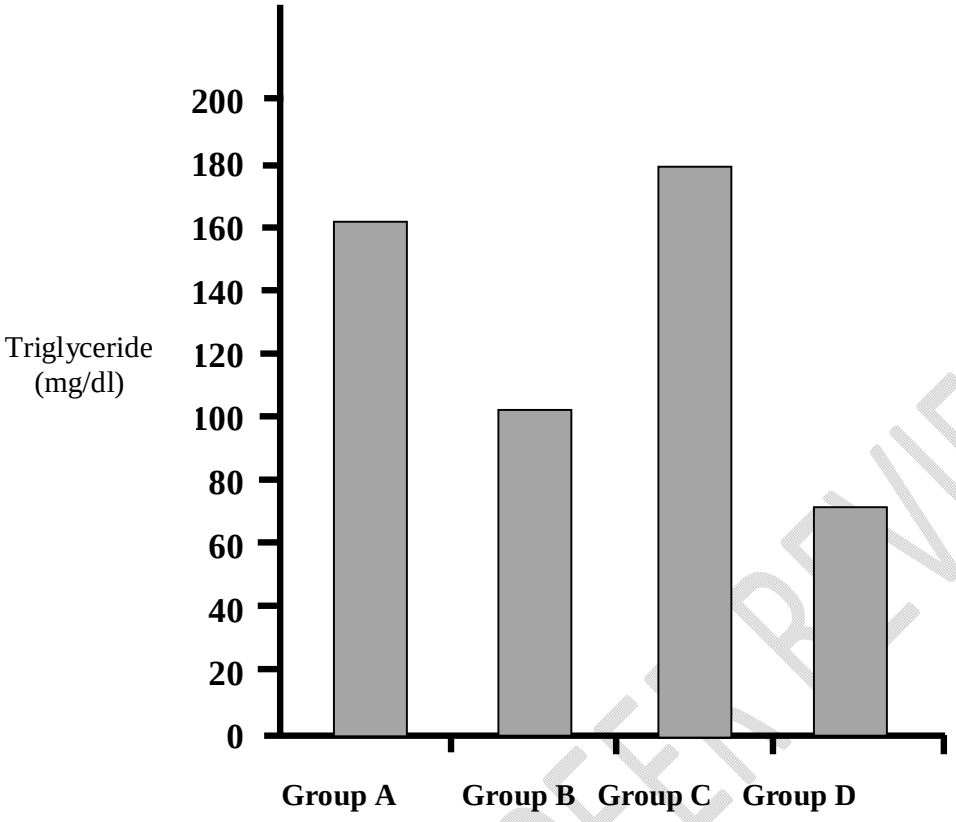


Figure 10 Graph Showing Effect of Treatment on Reduction Glutathione (GSH) In Rat Kidney (Homogenate) (µ/L)

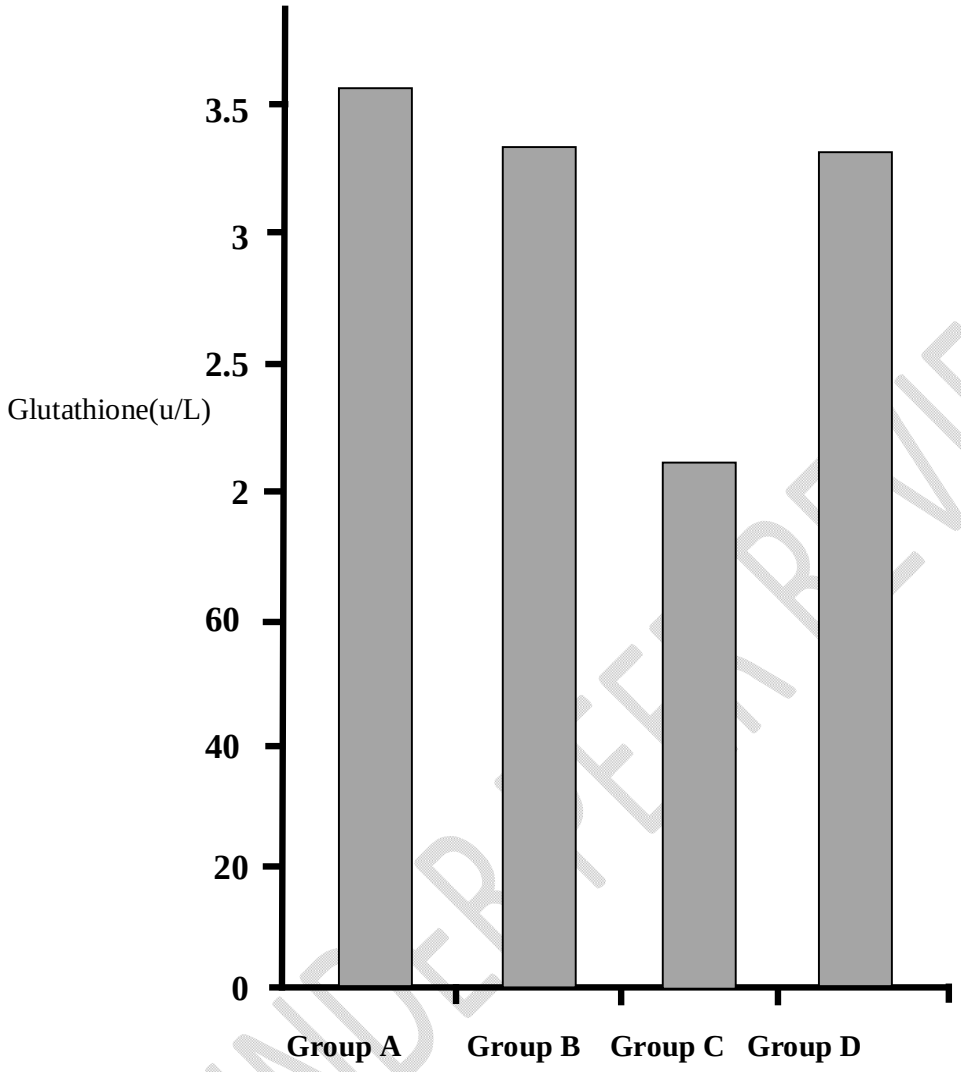


Figure 11 Graph Showing Effect of Treatment on Glutathione-S-Transferase (GST) In Rat Kidney (Homogenate) (µ/L)

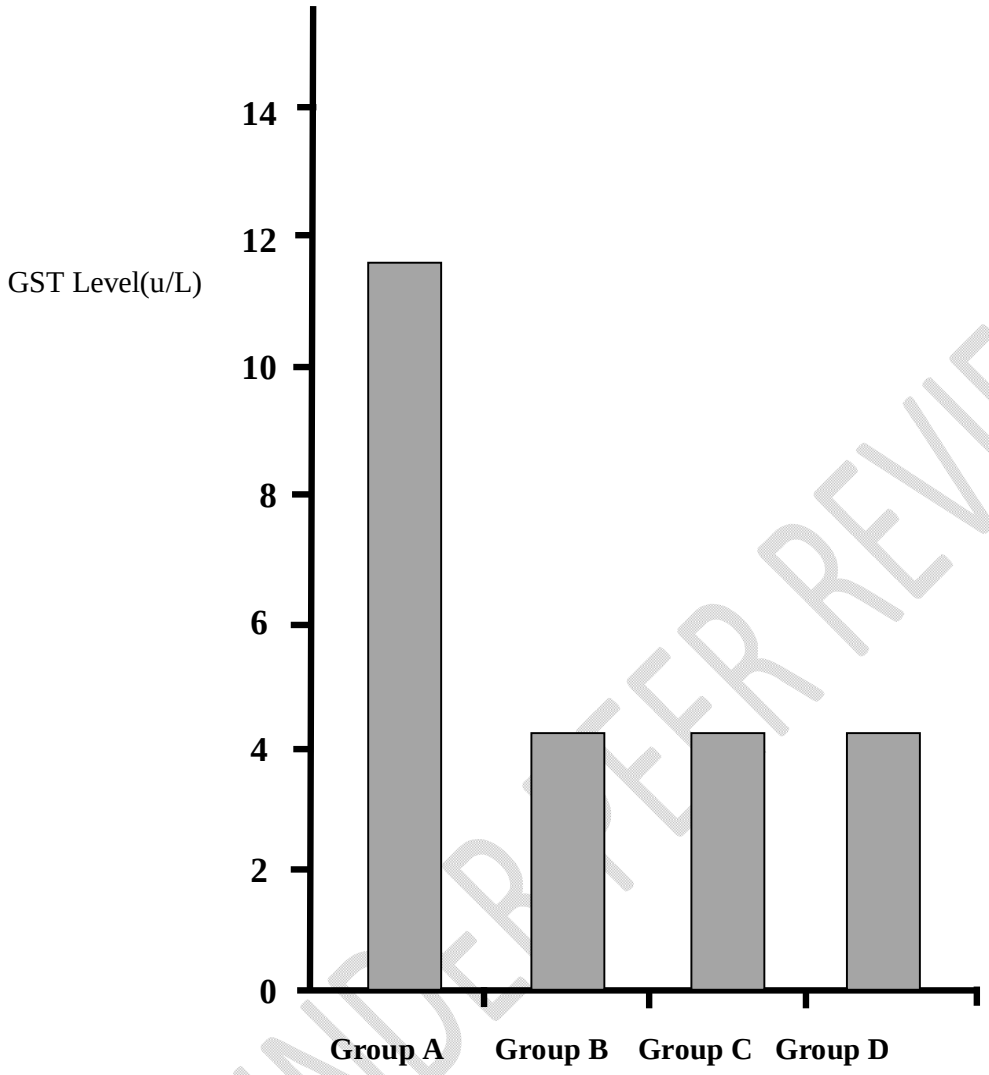


Figure12 Graph Showing Effect of Treatment on the Activity of Superoxide Dismutase (SOD) In Rat Kidney Homogenate (µ/L)

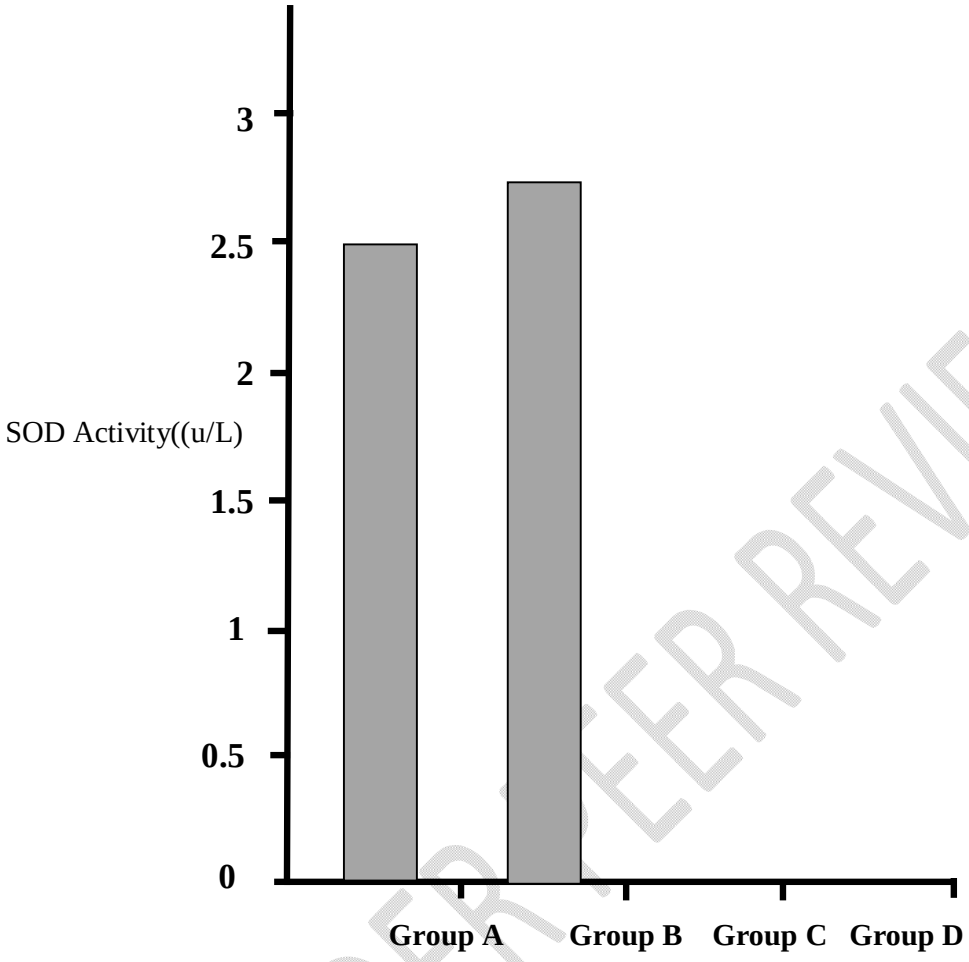


Figure 13 Graph Showing Effect of Treatment on Theiatalase Activity in Rate Kidney (Homogenate) (µ/L)

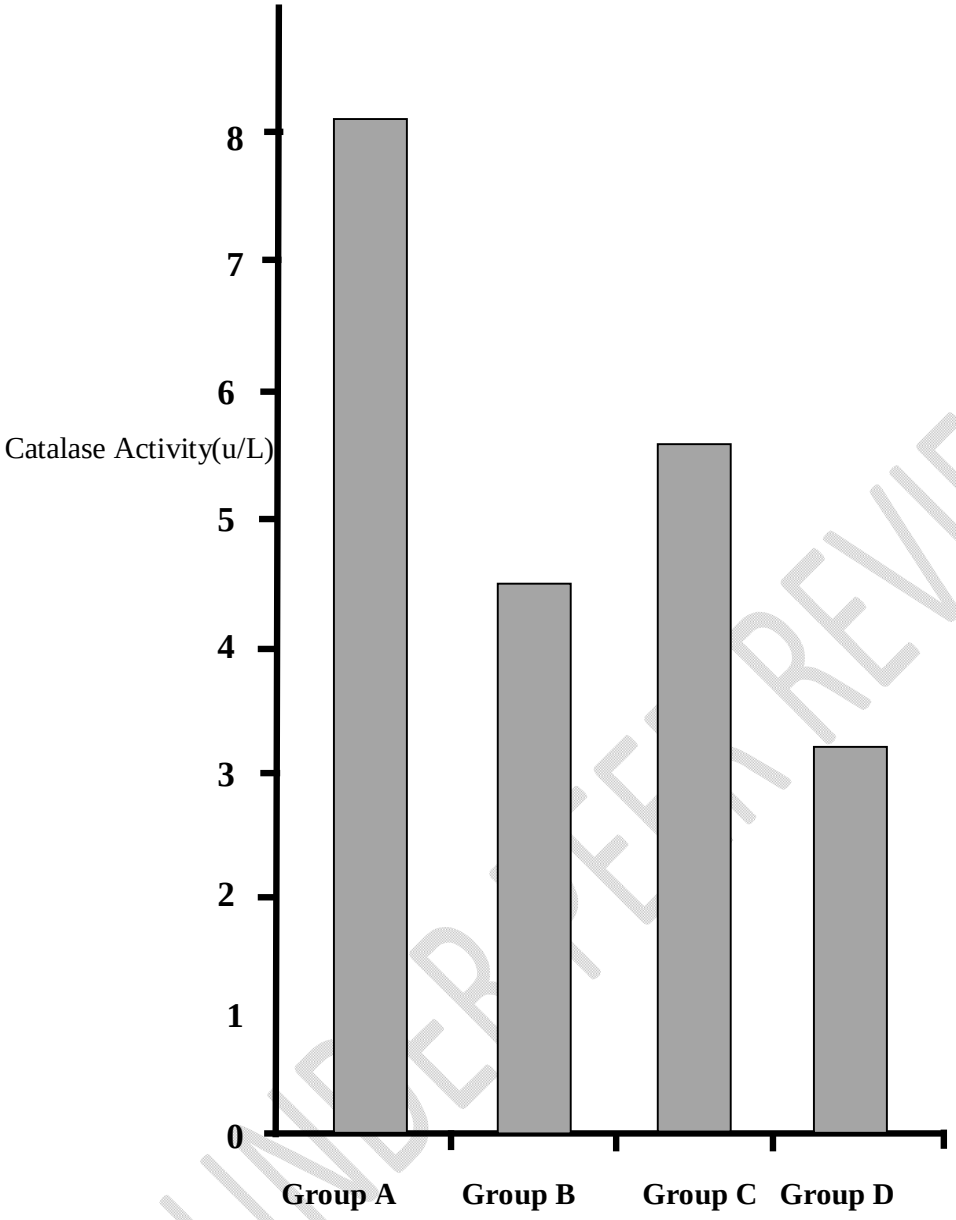
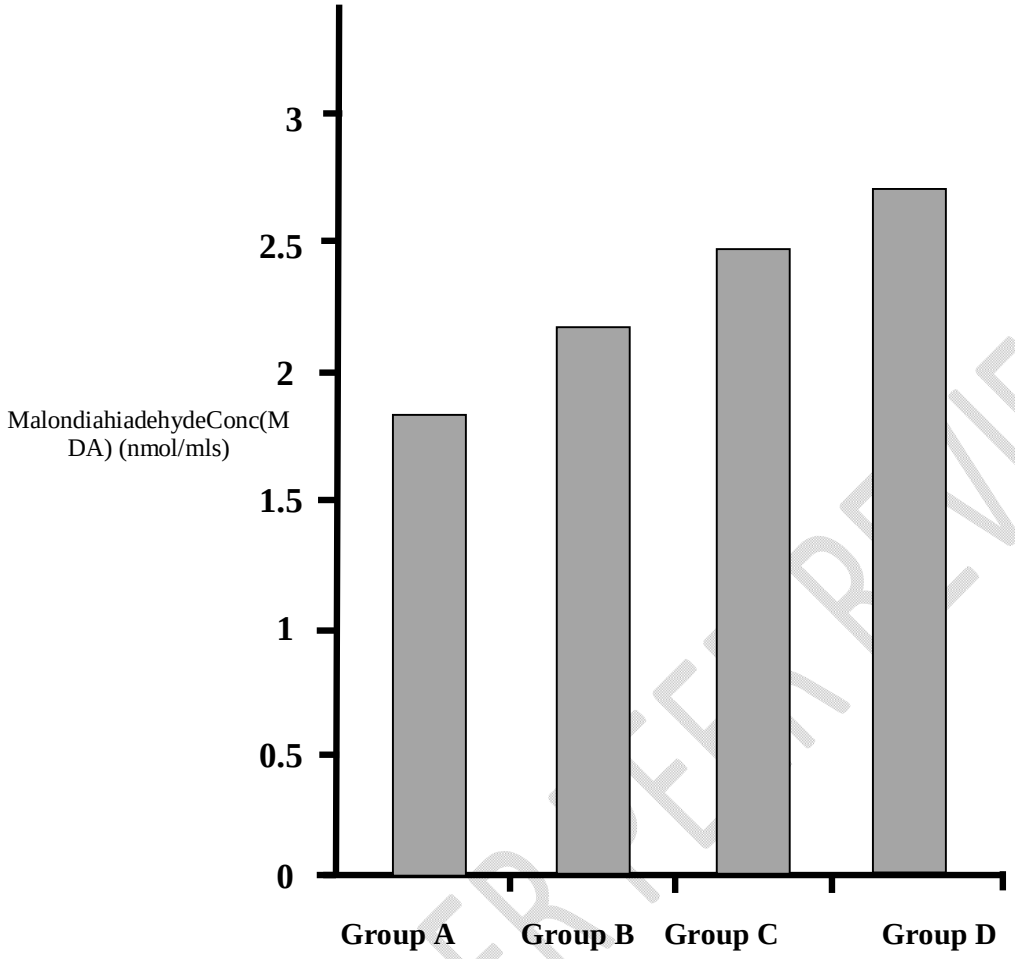


Figure 14 Graph Showing Effect of Treatment on Malondialdehyde (MDA) (Nmol/ml)



3.6 Summary of Results

3.6.1 Packed Cell Volume (PCV)

From Table 3, there is a significant difference in PCV between groups B, C, D and the normal control group A. PCV is significantly lower ($p < 0.05$) in group A compared to group B, and significantly higher compared to groups C and D. PCV is significantly higher in group B compared to groups C and D, and significantly higher in group C compared to group B.

3.6.2 White Blood Cell Count (WBC)

As shown in Table 3, the white blood cell levels were observed to significantly ($p < 0.05$) decrease in groups B, C, and D compared to group A. WBC is significantly higher in group B compared to groups C and D, and significantly higher in group C compared to group D.

3.6.3 Serum Creatinine

The effect of treatment on creatinine level was observed in all the groups, as shown in Table 4. The creatinine level significantly increased in groups B, C, and D compared to group A. The creatinine levels in groups C and D significantly increased compared to group B. The creatinine level in group A also significantly increased compared to group C.

3.6.4 Serum Albumin

The effect of treatment on serum albumin was observed in all the groups. There was a significant decrease in serum albumin in groups B, C, and D compared to group A. There was no significant difference in serum albumin between group C and group B, nor was there any significant difference between group D and group C.

3.6.5 Blood Urea Nitrogen (BUN)

The level of blood urea nitrogen (BUN) was measured in the different groups, as summarized in Table 4. The BUN level was significantly ($p < 0.05$) higher in group A compared to group B, and lower in group A compared to groups C and D. The BUN level significantly ($p < 0.05$) decreased in group D compared to group C.

3.6.6 Total Serum Cholesterol

The level of total serum cholesterol was measured in the different groups, as shown in Table 5. The cholesterol level in group A was significantly ($p < 0.05$) higher compared to groups B, C, and D. The cholesterol level in group B was significantly ($p < 0.05$) higher compared to groups C and D, while group C was significantly ($p < 0.05$) higher compared to group D.

3.6.7 Triglycerides

The level of triglycerides in all groups was also measured, as shown in Table 5. There was a significant decrease in the triglyceride level in groups B (108.8) and D (79.9) compared to group A (163.4), while group C (182.8) showed a significant ($p < 0.05$) increase compared to group A. Group B showed a significant ($p < 0.05$) decrease compared to group C, with no significant difference compared to group D. Group D showed a significant decrease compared to group C.

3.6.8 Catalase Activity

The catalase activity was measured in all groups, as shown in Table 6. The level of catalase activity significantly ($p < 0.05$) decreased in group A (0.007) compared to groups B (0.044), C (0.055), and D (0.034). There was no significant ($p > 0.05$) difference in catalase activity between group B and groups C and D, while group D showed a significant ($p > 0.05$) decrease in catalase activity compared to group C.

3.6.9 Glutathione Level (GSH)

The level of GSH was measured in all the groups, as shown in Table 6. The observations showed no significant difference in the GSH level between group A and group B. There was a significant decrease in GSH level in groups C and D compared to group A. The GSH level significantly decreased in groups C and D compared to group B. The GSH level significantly increased in group B compared to group C.

3.6.10 Glutathione-S-Transferase (GST)

The activity of GST was measured in all the groups, as shown in Table 6. The observation shows a significant ($p < 0.05$) decrease in groups B, C, and D compared to group A. There was no significant difference in GST activity between group B and groups C and B, nor was there any significant difference between group C and group D.

3.6.11 Malondialdehyde Assay (MDA)

The level of malondialdehyde was measured in all the groups, as shown in Table 6. The MDA level increased in groups B, C, and D. There was no significant increase in MDA in group B compared to group A, while the MDA level increased in groups C and D compared to group B. When compared to group B, there was a significant difference in the MDA level in groups C and D. However, there was no significant difference between group C and group D.

3.6.12 Superoxide Dismutase (SOD)

The activity of SOD was measured, as shown in Table 6. The statistical analysis shows no significant ($p > 0.05$) difference between group A (normal control) and group B. The SOD activity significantly decreased in groups C and D compared to group A. It also shows a significant decrease in SOD activity in groups C and D compared- to group B. SOD activity significantly ($p < 0.05$) decreased in group C compared to group B.

4.0 DISCUSSION

Gentamicin, a widely used antibiotic, can cause acute kidney injury as a significant adverse effect. Extensive research indicates that reactive oxygen species (ROS) are key contributors to gentamicin-induced nephrotoxicity [22] [23]. Gentamicin administration has been shown to cause substantial nephrotoxicity, evidenced by increased blood urea nitrogen (BUN) and creatinine levels, along with a marked reduction in glutathione (GSH) and an increase in malondialdehyde (MDA), indicating that the tissue damage induced by gentamicin is mediated through oxidative mechanisms[22][23]. Ginger has demonstrated strong antioxidant properties, with its component gingerone inhibiting lipid peroxidation and effectively scavenging free radicals [24] [25]. Animal studies have suggested that ginger can reduce cholesterol levels and positively impact plasma lipid composition, potentially preventing atherosclerosis [24] [25]. However, the effects of ginger on gentamicin-induced nephrotoxicity are mixed. While ginger has antioxidant properties, some studies found that concurrent administration of ginger and gentamicin may have cumulative negative effects, reducing immunity and exacerbating kidney damage[23]. Ginger administration, either separately or simultaneously with gentamicin, significantly increased MDA levels, suggesting that ginger extract may worsen gentamicin-induced nephrotoxicity [24]. Histopathological analysis showed more severe architectural distortion and interstitial nephritis in rats receiving both gentamicin and ginger compared to gentamicin alone [22] [24]. Creatinine levels, a major marker of kidney dysfunction, were significantly increased in groups receiving ginger, indicating that ginger may enhance gentamicin-induced nephrotoxicity rather than mitigate it [22].while ginger has antioxidant properties and potential benefits for reducing cholesterol and preventing atherosclerosis, its effects on gentamicin-induced nephrotoxicity are unclear. Some studies suggest ginger may exacerbate kidney damage caused by gentamicin, while others indicate potential protective effects. Further research is needed to clarify the relationship between ginger and gentamicin-induced nephrotoxicity.

5.1 CONCLUSION

In conclusion, this research indicates that the aqueous extract of ginger did not offer a protective benefit against oxidative stress and nephrotoxicity induced by gentamicin, this lack of protection could be linked to dosage levels or the detrimental effects of the iron content's catalytic activity. Nonetheless, ginger extract significantly lowers serum cholesterol levels, which may make it a useful recommendation for preventing atherosclerotic conditions

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