Renal Induced Damage Following Mercury Exposure in Adult Wistar Rats

Abstract

This study investigated the histomorphological effect of mercury chloride on the kidney. Heavy metals are hazardous substances that cause serious health risk to ecosystems and organisms due to their high toxicity conferred by nature of their environmental persistence. Mercury is a well-known toxic heavy metal to animals as well as humans. Mercury occurs naturally in the environment in different chemical forms. Elemental mercury is the form used in dental amalgams. Forms more commonly found in nature are inorganic mercury and organic mercury. All mercury forms are considered toxic. It is being widely used in the industrial, medical, agriculture and other fields

Materials and Methods: Thirty six (36) adult wistar rats of both sexes, weighing between 110g-300g were randomly divided into four groups A, B, C, and D with nine animals per group. The animals in groups B, C, and D were administered mercury chloride orally at the concentration of 0.2mg/kg, 0.4mg and 0.5 mg/kg body weights respectively while group (A) served as control and was given distilled water. The administration lasted for a period of 21 days and on the 22nd day all the groups of rats were sacrificed by cervical dislocation, blood was collected through cardiac puncture and the kidneys were carefully removed and weighed immediately with a sensitive balance and then fixed in 10% formol saline. The tissues were processed and sectioned and stained with haematoxylin and eosin stain for histological studies. The results showed that the mean kidney weight in groups B and C increased insignificantly ($P>0.05$), compared to the control group while group D decreased significantly compared to the control group.

In the biochemical analysis there was significant increase ($P<0.05$).in alanine transaminase,, aspartate transaminase and alkaline phosphatase activities in mercury- treated group B, C and D compared to the control group A. Histological study of the kidney revealed that C and D treated groups showed marked degenerative changes, fibrosis and hemorrhage showing varying degrees of renal injury marked by focal sclerosis of the glomerulus, widening of the Bowman's space and hyper cellularity and complete coll apse of the glomerulus.

The study concluded that exposure to mercury chloride induced nephrotoxic effect on the kidney of adult wistar rats.

Keywords: *mercury chloride, kidney, histomorphology, histological changes aspartate transaminase, alkaline phospatase, alanine transaminase.*

INTRODUCTION

There is an increasing concern over the contamination induced by some trace elements which invariably leads to a high risk to public health as a result of their poor metabolization and elimination processes , thereby accumulating in organisms[1]. Similarly, it has been observed that increasing industrialization is invariably associated with an increased release of toxicants including heavy metal into the environment, with resultant effects on the public health [2].

Previous studies have documented that Methyl mercury (MeHg) intoxication has been a concern and a public health problem for many decades [3]

Mercury has been implicated among the trace elements in view of the nature of their accumulation capacity the food chain pattern. Previous studies have indicated that mercury is a naturally occurring element in crust of the earth, in water, biota and atmosphere. Moreover, environmental mercury has not only natural sources, such as volcanic emissions, it has been reported that mercury also has anthropogenic sources like emissions to the atmosphere from coal combustion, biomass, fossil fuel burning and combustion[4]

Mercury has been known to be a naturally occurring element, occurring in multiple forms and in different in oxidation states. It is many applications and uses as a wide variety of products and processes. Additionally, mercury intoxication and contamination can occur from occupational and environmental exposure[5]. It has been shown that mercury may exist in the organic or inorganic forms, while organic species (e.g., methylmercury CH_3Hg^+) being the most toxic to mammals including man. Considering that about 75-95% of the mercury in fish is CH_3Hg^+, it is known that intoxication by chronic exposures commonly occurs through contaminated seafood and fish intake.[6,7] The inorganic mercury species include elemental mercury (Hg^0), mercurous ion (Hg_2^{2+}) and mercuric ion (Hg^{2+}). Studies have indicated that Hg^0 is poorly absorbed and has a low health risk when it is in liquid form at room temperature, but its vapor is rapidly absorbed through the lungs. Report has shown that divalent mercury salts are the most important toxic forms among , the inorganic compounds[8,9].

Previous investigations have shown that Organic mercury is readily distributed throughout the body but tends to concentrate and accumulate in the brain and kidneys[5]

(ATSDR,1989;Goyer, 1991). Similarly, it has been observed that mercury binds to microsomal and mitochondrial enzymes resulting in cell injury and death[10]. Findings from previous studies have revealed that once organic mercury is absorbed into the tissues, it is almost totally oxidized to the divalent form (inorganic mercury) in erythrocytes, liver and kidneys[11].

Research finding has revealed that mercury is not destroyed by metabolism, but rather converted to various forms and oxidation state which involves an oxidation –reduction cycles[5]. The primary route of its excretion is known to be urine and faeces[5]. Consequently, this may account for the renal damage and failure associated with mercury toxicity [12,13]

Mercury (II) chloride is extremely toxic by ingestion or inhalation. It is fatal when in contact with skin and causes severe eyes damage. It is also a suspected mutagenic agent and can also cause fertility problems. It is toxic to aquatic environment. It is not flammable, but is incompatible with strong acids, ammonia, metallic salts and carbonate[14]

. Histologically, the renal parenchyma consists of four parts: glomeruli, tubules, interstitum, and blood vessels[15] A number of observation Indicate that heavy metals are able to alter cellular metabolic pathways through induction of a prooxidative state. Nevertheless, the outcome of heavy metal – mediated effects in the development of human diseases is debated and needs further insights [16] The present study was conducted to find out the histopathological changes in kidneys after the administration of mercury chloride through oral route.

MATERIALS AND METHODS

EXPERIMENTAL ANIMAL

Thirty six healthy adult rats weighing 120 -300g of both male and female were used for this experiment. The rats were divided into four groups and housed in a conducive and serene place free of harms .Group A as the control group ,group B, group C, group D. the rules of

regulations governing animal handling were strictly observed, they were housed in plastic cages, no artificial light was used.

Contact bedding (wood shaving) was used in the bottom of the cages, in order to allow the animals to form their own microenvironment. Each cage contains 9 adult wistar rats of either sex .The animals were acclimatized for two weeks. During the two weeks of acclimatization the animals were very active, fed properly, water and feed were given .The rats were carefully and routinely screened, inspected and confirmed to be healthy throughout the period of acclimatization .There cages were cleaned regularly. The animals were kept in the animal house of Anatomy Department, Ladoke Akintola university of technology, ogbomoso , Nigeria and treated in accordance with the 'guide for the care and use of laboratory animals prepared and compiled by the national Research Council [17]

EXPERIMENTAL DESIGN

The rats were grouped into cages of 9 rats per cage or group. Group A served as the control they received distilled water during the administration period. The wistar rats in groups B, C, and D mercury-treated groups and were administered mercury chloride orally at the concentration of 0.2mg/kg, 0.4mg and 0.5 mg/kg body weights respectively for 21 consecutive days and on the 22nd day all the groups of rats were sacrificed by cervical dislocation . The administration lasted for a period of 21 days and on the 22nd day all the groups of rats were sacrificed by cervical dislocation, blood was collected through cardiac puncture and the kidneys were carefully removed and weighed immediately with sensitive balance and then fixed in 10% formol saline. The tissues were processed and sectioned and stained with haematoxylin and eosin stain for histological studies. The results showed that the mean kidney weight in groups B and C increased insignificantly compared to the control group while group D decreased significantly compared to the control group.

STATISTICAL ANALYSIS

Experimental data that were obtained from this study were expressed as Mean± SEM (standard error of mean) and data were analyzed by analysis of variance (ANOVA). Student's t-test was employed to compare differences between the groups using Graph pad Prism for windows (GraphPad Prism, Inc Chicago). A value of $P < 0.5$ was considered to be significant

RESULTS

Table 1; The Mean ± S.E.M of the weights (g) of left and right kidney.

GROUPS	LEFT KIDNEY WEIGHTS(G)	RIGHT KIDNEY WEIGHTS(G)	% RELATIVE WEIGHTS OF LEFT KIDNEY	**% RELATIVE WEIGHTS OF RIGHT KIGNEY**
A(Contol)	0.58±0.04	0.61±0.03	**0.28**	**0.29**
B (0.02g/kg)	0.68±0.04	0.67±0.03	**0.34**	**0.33**
C(0.04g/kg)	0.60±0.03	0.60±0.02	**0.35**	**0.35**
D(0.05g/kg)	0.46±0.02*	0.48±0.02**	**0.33**	**0.35**

Significance: $P < 0.05$, value greater than 0.05 were considered insignificant while values less than 0.05 were considered significant (*). Values were expressed as mean ± Standard error of mean.

Table 1 above showed relative organ weights and also compares the effects of administration of mercury chloride on the rats in experiment groups with those in the control group.

There was significant decrease ($P<0.05$) in organ weight of right and left kidney in group D as compared to the control group while group B and C show insignificant increase as compared with the control group ($P > 0.05$).

There was significant increase ($P < 0.05$) in group B, C and D as compared to group control group (group A).

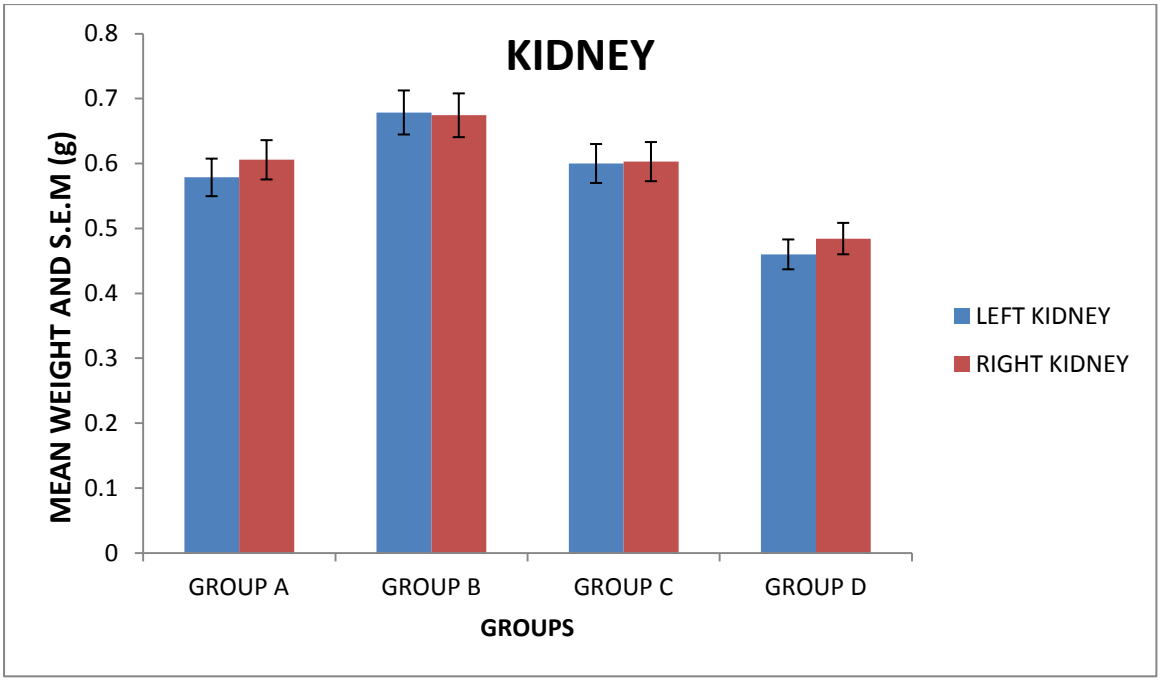

Figure 1; charts showing the graphical representation of the changes in organ weights of mercury chloride.

Weights of the organ (kidney) were expressed as mean ± S.E.M using student t-test. Values less than 0.05 were considered significant while values greater than 0.05 were considered insignificant.($p<0.05$).

BIOCHEMICAL EVALUATION

Table 2: The effect of mercury chloride on the activities of ALP, AST and ALT on the kidney

GROUPS	ALP	AST	ALT
A	30.70 ± 2.00	63.27 ± 4.96	25.00 ± 1.64
B	41.99 ± 10.75	84.65 ± 2.82**	32.24 ± 2.19*
C	39.97 ± 1.01**	93.12 ± 3.29**	38.77 ± 2.32**
D	40.32 ± 0.75**	115.4 ± 2.75**	33.33 ± 0.51**

Significance: $P < 0.05$, value greater than 0.05 were considered insignificant while values less than 0.05 were considered significant (*). Values were expressed as mean ± Standard error of mean.

The t-test table of the mean ± S.E.M of the biochemical parameters of kidney after administration of mercury chloride for 21days is shown above. when comparing the parameters of experimental groups (Group B, Group C, and Group D) with that of the control group (Group A), it could be seen that there was significant increase (P< 0.05) in ALP (Alkaline phosphatase) level in group B,C and D,

In aspartate transaminase, there was a significant increase (P< 0.05) in all the groups but significant increase in group D compare to the control group.

In alanine transaminase, there was insignificant increase (P< 0.05) in all the groups compare to control group (group A).

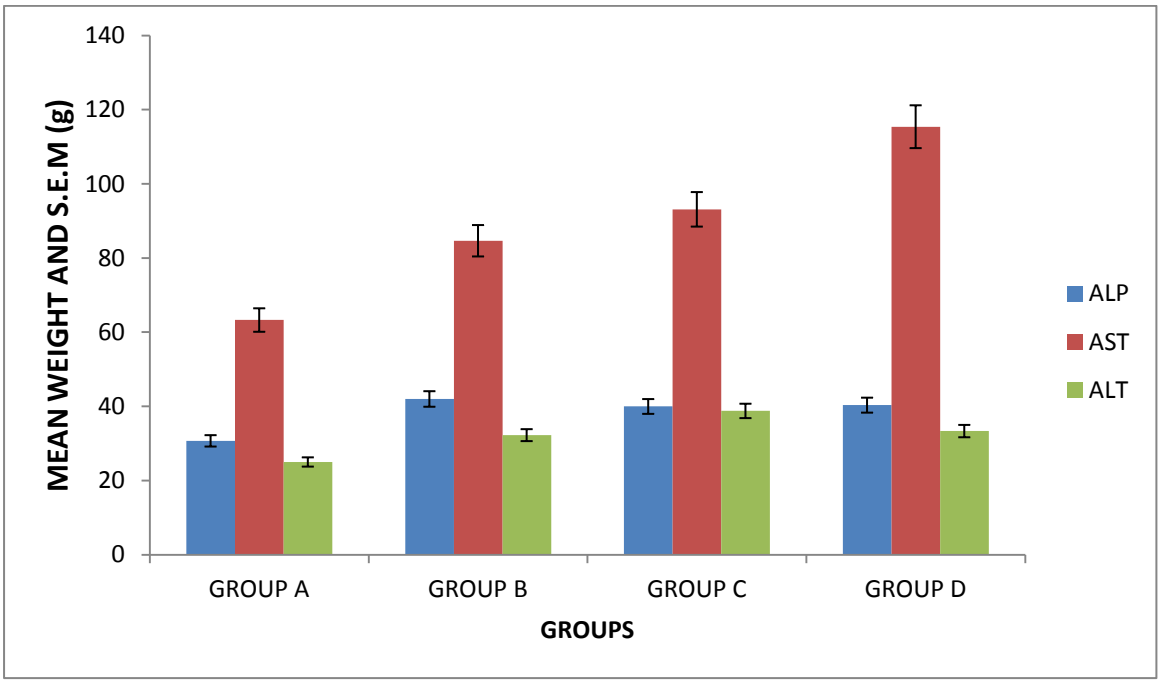

Figure 2 ; chart showing serum analysis of the kidneys of adult wistar rats after administration of Hgcl for 3 weeks .when comparing the parameters of experiment groups (Group B, and D) with that of control group (Group A) it could be seen that there was significant increase in ALP (Alkaline phosphatase), Aspartate transaminase increased significantly in all the groups compared to the control group, Alanine transaminase increased more in group c , signicant increase in group b and D compare to the control group.

HISTOLOGICAL OBSERVATIONS

This show the micrograph representation of H&E staining showing the general cytoarchitecture of the kidney in adult Wistar rats in group A (control), Group B (Treated with 0.02g/kg of mercury chloride for 21days), Group C (Treated with 0.04g/kg of mercury chloride for 21days), Group D (Treated with 0.05g/kg of mercury chloride for 21days). Magnification: X100, X400 respectively.

Photomicrographs of the renal cortex showing panoramic views of Kidney general micromorphological presentations in Adult Wistar rats across the study groups. Hematoxylin and Eosin stain (X100). The Renal Corpuscles (RC), Renal glomeruli (G), Macula densa (MD), Distal and Proximal (DCT & PCT) convoluted tubules and the bowman's capsule are demonstrated across study groups. Areas with marked pathomorphological changes are indicated by red arrows. Bowman's capsule as well as bowman's space in indicated by yellow arrow heads

The collagen (type IV) of the basement membrane outlines the glomerular capillaries. The collagen of the parietal layer (PL) of Bowman's capsule (BC) and the basal membrane (BM) of a distal tubule are observable from the photomicrographs. C-D groups showed marked degenerative changes fibrosis and hemorrhage (red arrows)showing varying degrees of renal injury evidenced by focal sclerosis of the glomerulus, widening of the Bowman's space and hyper cellularity and complete collapse of the glomerulus. There is hyaline arteriolosclerosis, interstitial fibrosis, poor staining intensity which indicative of low glycogen deposits, interstitial inflammation as well as acute tubular necrosis are all observed in relative to A group that appears normal. Features are consistent with chronic glomerulonephritis and or glomerulosclerosis

GROUP A(CONTROL RATS)

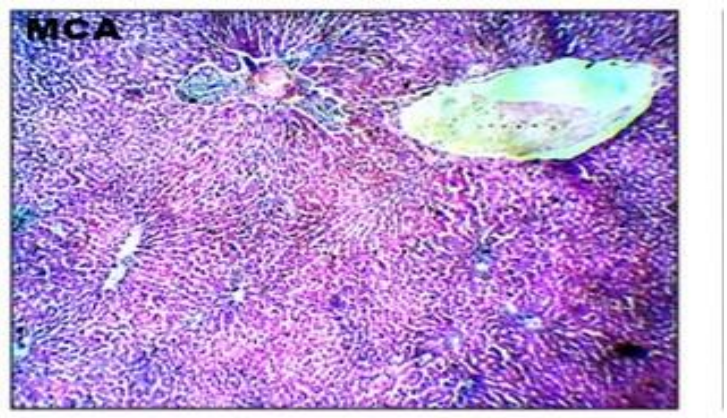

Plate 1a: (H&E X100) photomicrograph of a normal histology of kidney for control group (Group A)

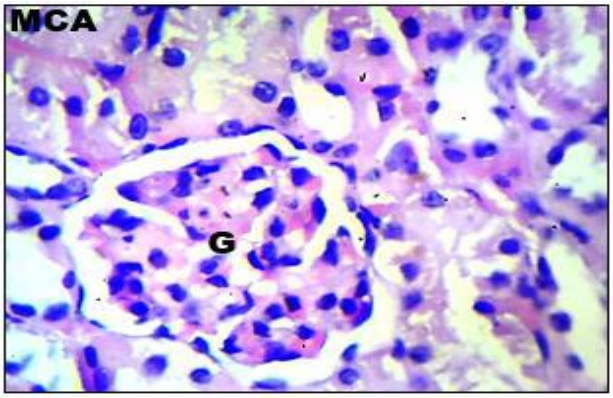

Plate 1b: (H&E X400) photo photomicrograph of a normal histology of kidney for control group (Group A).showing the renal glomerui.

The two micrograph above shows the transverse section of kidney in the control group (groups that received water and stock food diet only for four weeks). The collagen (type IV) of the basement membrane outlines the glomerular capillaries. The collagen of the parietal layer (PL) of Bowman's capsule (BC) and the basal membrane (BM) of a distal tubule are observable from the photomicrographs.

GROUP B (Rats which received 0.02g of Mercury chloride for 3weeks).

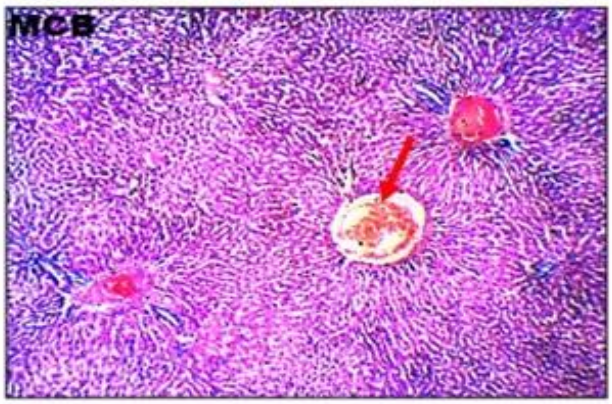

Plate 2a. Transverse section of the Kidney of the rats in group B after adiministration of 0.02g of Mercury chloride for 21days. (Haematoxylin and eosin X 100)

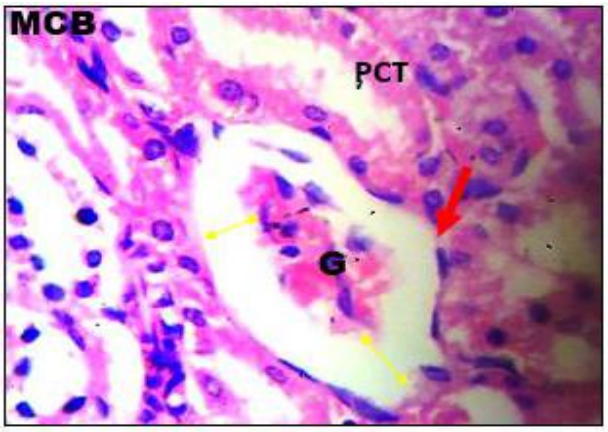

Plate 2b. Transverse section of the Kidney of the rats in group B after adiministration of 0.02g of Mercury chloride for 21days. (Haematoxylin and eosin X 400). Showing the proximal convoluted tubules(PCT),renal glomeruli (G), Areas with marked pathomorphological changes are indicated by red arrows. Bowman's capsule as well as bowman's space in indicated by yellow arrow heads.

GROUP C (Rats which received 0.04g of Mercury chloride for 3weeks).

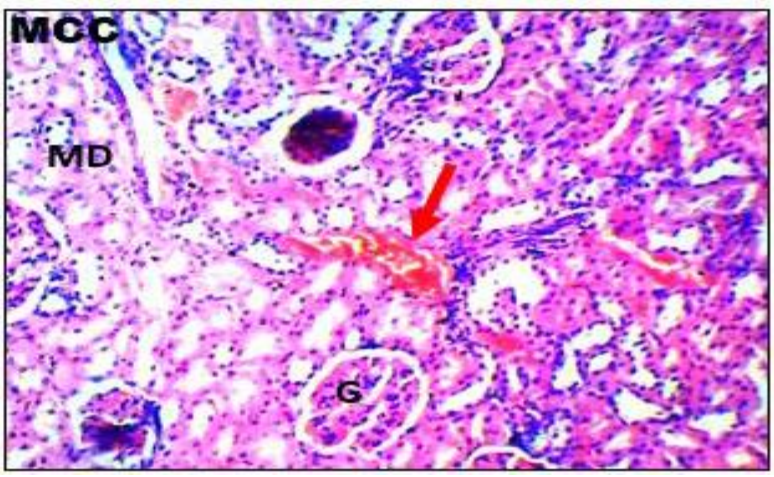

Plate 3a. Transverse section of the Kidney of the rats in group C after adiministration of 0.04g of Mercury chloride for 21days. (Haematoxylin and eosin X 100)

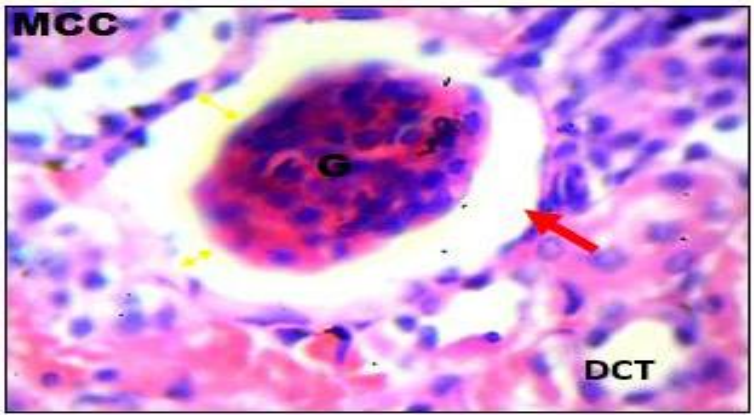

Plate 3b. Transverse section of the Kidney of the rats in group C after adiministration of 0.04g of Mercury chloride for 21days. (Haematoxylin and eosin X 400)

Showing the distal convoluted tubules(DCT),renal glomeruli (G).This group showed marked degenerative changes fibrosis and hemorrhage (red arrows)showing varying degrees of renal injury evidenced by focal sclerosis of the glomerulus, widening of the Bowman's space and hyper cellularity and complete collapse of the glomerulus. There is hyaline arteriolosclerosis, interstitial fibrosis, poor staining intensity which indicative of low glycogen deposits, interstitial inflammation as well as acute tubular necrosis are all observed in relative to A group that appears normal. Features are consistent with chronic glomerulonephritis and or glomerulosclerosis

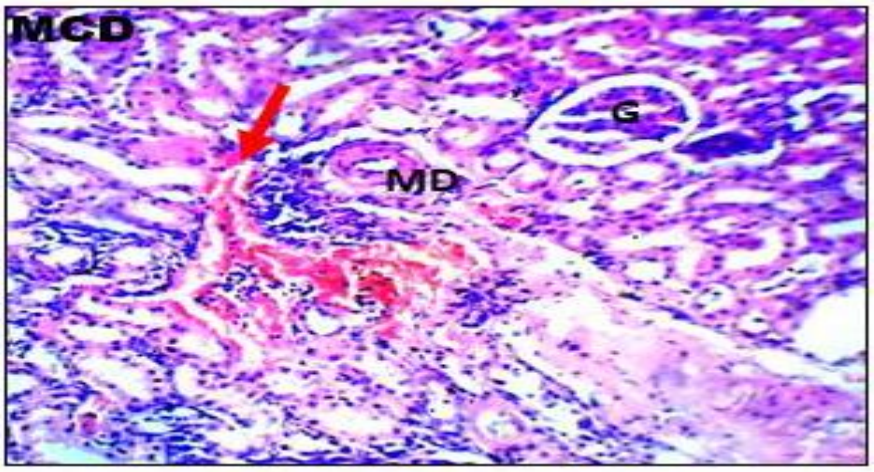

Plate 4a. Transverse section of the Kidney of the rats in group D after adiministration of 0.05g of Mercury chloride for 21days. (Haematoxylin and eosin X 100) .

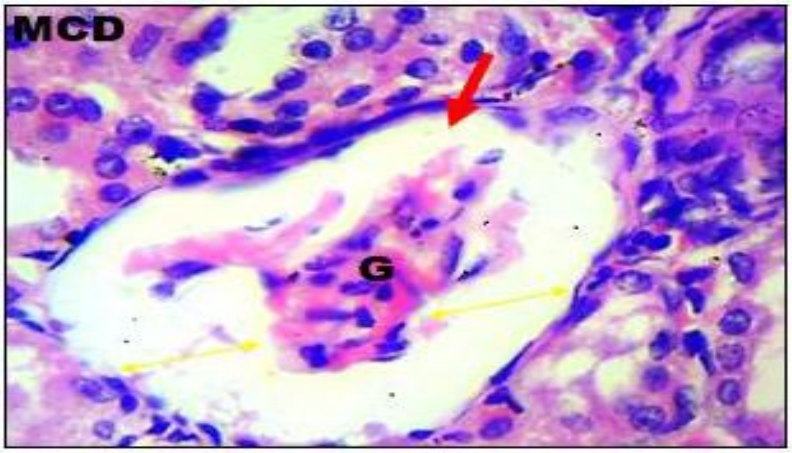

Plate 4b. Transverse section of the Kidney of the rats in group D after adiministration of 0.05g of Mercury chloride for 21days. (Haematoxylin and eosin X 400) .

D groups showed marked degenerative changes fibrosis and hemorrhage (red arrows) showing varying degrees of renal injury evidenced by focal sclerosis of the glomerulus, widening of the Bowman's space and hyper cellularity and complete collapse of theglomerulus. There is hyaline arteriolosclerosis, interstitial fibrosis, poor staining intensity which indicative of low glycogen deposits, interstitial inflammation as well as acute tubular necrosis are all observed in relative to A group that appears normal. Features are consistent with chronic glomerulonephritis and or glomerulosclerosis

DISCUSSION

This study assessed the histomorphological effects of mercury chloride on the kidneys of adult wistar rats. Mercury is a well-known toxic heavy metal to animals as well as humans, Heavy metals are hazardous substances that cause serious health risk to ecosystems and organisms due to their high toxicity conferred by nature of their environmental persistence.. Rats induced with mercury chloride in various groups was reported to have increase in the organ weight (kidney) in groups B and C, compared to control groups while group D decreased significantly($P<0.05$)..Findings from previous study have shown that renal size and weight increased significantly following mercury exposure compared with agematched controls, additionally, Light microscope morphometry revealed that the growth of the kidneys was due primarily to an increase in proximal tubule volume and caused partly by dilatation of the tubule lumen and partly by an increase in the volume of the proximal tubule cells as

reported in the study [18] .The changes in the kidney weights in the mercury treated rat may be partly due dilatation and enlargement of the tubules in association with earlier reports Microscopic examination of the left and right kidney in group A (control group) showed the normal histological structure of the renal corpuscles and renal tubules. The renal corpuscle consisted of tuft of blood capillaries surrounded by the Bowman's capsule. The renal tubules included proximal convoluted tubules lined by large pyramidal cells with a brush border of microvilli and appears with small lumen, and distal convoluted tubules lined by cuboidal cells without brush border so its appears with large and clear lumen. In group B (induced 0.2g of mercury chloride), group C (0.04g of mercury chloride), and group D induced(0.05g of mercury chloride) showed marked degenerative changes fibrosis and hemorrhage showing varying degrees of renal injury evidenced by focal sclerosis of the glomerulus, widening of the Bowman's space , hyper cellularity and complete collapse of the glomerulus. There is hyaline arteriolosclerosis, interstitial fibrosis, poor staining intensity which indicative of low glycogen deposits, interstitial inflammation as well as acute tubular necrosis are all observed in relative to A group that appears normal. Features are consistent with chronic glomerulonephritis and or glomerulosclerosis.

Previous studies showed that vacuolar changes and pyknotic nuclei of glomerular and tubular cells, and also tubular necrosis were observed in mercury vapor inhaled rat. While on the other hand, glomerular sclerosis and glomerular degeneration were similarly revealed coupled with dilated Bowman's space in the Hg treated group [19] Similarly, histopathological findings characterized by a decreased number of glomeruli due to glomerular sclerosis which may be segmental or total necrosis in association with decreased volumes of both proximal and distal tubules caused from tubular necrosis have previously been reported in rats following treatment with mercury[19]

Mercury exposure has been implicated in the histopathology of kidney damage and evidence suggests a linkage between mercury exposure and acute tubular necrosis, glomerulonephritis, chronic renal disease, renal cancer and nephrotic syndrome. Various reports have shown mercury exposure can lead to various kidney injuries including: subacute-onset nephrotic syndrome, tubular dysfunction, secondary focal segmental glomerulosclerosis, syncreticatic nephrotic syndrome, nephritic syndrome, nephrotic-range proteinuria, glomerular disease, and membranous glomerulonephritis[20].

The present data showed that the exposure of adult wistar rats to mercuric chloride is capable of inducing alterations in some enzymatic activities.. Whereas results of the differences in between the exposed adult wistar rats and unexposed group suggest that exposure to mercury could cause renal dysfunction. Also, increase in serum AST, ALT and ALP can be used as potential enzyme biomarkers for mercury-induced hepatotoxicosis which ultimately affects the general health by altering the functional and structural integrity of kidney. The present findings clearly demonstrate that mercuric chloride is capable of inducing dose dependent

histopathological changes in kidney of the exposed adult wistar rats. Interestingly, histopathological findings were consistent with biochemical reports in the present study as shown in the results. This investigation has presented consistent information in the results showing the histological changes following mercury exposure in wistar rats. This study concluded that mercury chloride exposure in wistar rats has adversely affected the kidneys of adult wistar rats.

Ethical Approval

Animal Ethic committee approval has been taken to carry out this study.

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