

Observation of 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

Thirty six adult wistar rats of both sexes weighing between 120g-300g were randomly grouped into four, each cage containing nine rats. Group A rats was the control and was maintained on standard feed and water for 21days, group B received 0.2g of mercury Chloride, group C received 0.4g of mercury chloride while group D received 0.5g of mercury chloride all for 21days. The mercury chloride was administered orally on daily basis for 21days .the weights of the rats were recorded on weekly basis.

On the 22nd day all the groups of rats were sacrificed by cervical dislocation, blood was collected through cardiac puncture and homogenized for biochemical analysis, it was 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 weight of the experimental rats decreased significantly compared to the control group. the weight of the control group decreased too when compared to the initial mean weight. The organ (kidney) weight was taken, the mean of the kidney weight in groups B and C increased compared to the control group while group D decreased significantly compared to the control group.

In the biochemical analysis there was statistical increase in alanine transaminase in the experimental groups compared to the control group. In aspartate transaminase there was

statistical increase in all the experimental groups compare to the control group while in alkaline phosphatase they increased significantly in all the experimental groups compare to control group. Histological study of the kidney revealed that C-D groups 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 and hypercellularity and complete collapse of the glomerulus. It can be concluded from this study that mercury chloride induced nephrotoxic effect on the kidney of adult wistar rats.

Key words: mercury chloride, kidney, histomorphology, histological examination, aspartate transaminase, alkaline phosphatase, alanine transaminase.

INTRODUCTION

Mercury chloride is the most common bivalent compound. Although extremely toxic, odourless, colourless, white crystalline solid, a laboratory reagent, once used as a treatment for syphilis, it is no longer used for medicinal purposes because of Mercury toxicity and the availability of superior treatments (Walter, 2005).

It is soluble in alcohol, acetone, and ethyl acetate, slightly soluble in benzene. It is used in industrial agriculture to kill various types of mildews, mold and rusts. As little as 0.1 gram is enough to cause damage to body tissues and 2 grams can cause extensive tissue damage wherever high concentrations of the poison are encountered (Clarkson, 2002) many disinfectants and in photography materials. It is highly toxic (Litovitz, 2000)

The mercury (II) chloride chemical formula is HgCl_2 and its molar mass is 251.72 gmol^{-1} . The molecule is formed by a mercury (II) cation Hg^{+2} and a two chloride anions Cl^{-1} , which form two ionic binds. The crystal structure is orthorhombic. It's chemical structure can be written as below, in the common representations used for organic molecules (Yess, 1993)

Mercury (II) chloride is not produce by nature or biologic system; it is only produced by chemical processes.

Mercury chloride is synthesized through several methods such as the reaction between mercury (I) chloride and chlorine pure or also through the reaction between the hydrochloric acid and mercury (I) nitrate at 300°C . Additionally, it can be produced by the mixture between mercury (II) sulfate and sodium chloride at high temperatures and on dry atmosphere (Ostlund 1996).

Mercury (II) chloride is a colorless to white crystalline solid. Its density is 5.43 g mL⁻¹. Its melting point is 276°C and its boiling point is 304°C. Mercury (II) chloride is poorly soluble in cold water but soluble in hot water, ethanol, ethyl acetate and acetone. It is slightly soluble in pyridine and benzene (Crump *et al* 1998).

Although mercury (II) chloride is extremely toxic compound, it is still used in many industries such as the metallurgical due to it can form amalgams with other metals such as aluminum. In the century XIX was used as a material in the railroad building but it was not use anymore because its toxicity. Mercury (II) chloride is mostly used as catalyst for both: organic and inorganic synthesis. It is used many year in the treatment of syphilis and other illnesses but now, it is not used as medicine because its toxicity. Moreover, mercury (II) chloride is used as reagent to reveal photos and in preservation of biological samples (Baselt, 2000)

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 (Magos and Clarkson 2006). The present study was conducted to find out the histopathological changes in kidneys after the administration of mercury chloride through oral route. Mercury is a naturally occurring element that is found air, water, and soil exposure to mercury-even small amounts –may cause serious health problems, and is a threat to the development of the child in utero and early life. Mercury may have toxic effects on the nervous, digestive and immune systems, and on lungs, kidneys, skins, and eyes. Mercury is considered by WHO as one of the top chemicals or groups of chemicals of major public health concern. People are mainly exposed to methyl mercury, an organic compound, when they eat fish and shellfish that contain the compound. Methyl mercury is very different to ethyl mercury. Ethyl mercury is used as a preservative in some vaccines and does not pose a health risk.

Mercury exist in various forms; Elemental or metallic and inorganic to which people may be exposed through their occupation and organic e.g methyl mercury, to which diet. These forms of mercury differ in their degree of toxicity and in the effects on the nervous, digestive and immune systems, and on lungs, kidneys, skin, and eyes. Mercury occurs naturally in the earth's crust. It is released into the environment from the volcanic activity, weathering of rocks and as a result of human activity. Human activity is the main cause of mercury releases,

particularly coal-fired power stations, residential coal burning for heating and cooking, industrial processes, waste incinerators and as a result of mining for mercury, gold, and other metals. Once in the environment, mercury can be transformed by bacteria into methyl mercury. Methyl mercury then bioaccumulates [bioaccumulation occurs when an organism contains higher concentration of the substance than do the surroundings] in fish and shellfish. Methyl mercury also biomagnifies. For example, large predatory fish are more likely to have high levels of mercury as a result of eating many smaller fish that have acquired mercury through ingestion of plankton. People may be exposed to mercury in any of its forms under different circumstances. However, exposure mainly occurs through consumption of fish and shellfish contaminated with methyl mercury and through worker inhalation of elemental mercury vapours during industrial processes. Cooking does not eliminate mercury.

The kidneys are bean-shaped organs that several essential regulatory roles in vertebrates. They remove excess organic molecules from the blood, and it is by this action that their best-known function is performed: the removal of waste products of metabolism (Walter, 2004). They are essential in the urinary system and also serve homeostatic functions such as the regulation of electrolytes, maintenance of acid-base balance, and regulations of blood pressure (via maintaining salt and water balance). They serve the body as a natural filter of the blood, and remove water soluble wastes, which are diverted to the bladder. In producing urine, the kidneys excrete wastes such as urea and ammonium, and they are also responsible for the reabsorption of water, glucose, and amino acids. The kidneys also produce hormones including calcitriol, erythropoietin, and the enzyme rennin, the last of which indirectly acts on the kidney in negative feedback (Thomas, 2005).

Urine production, and the control of its composition, is exclusively the function of the kidneys .

It is located at the rear of the abdominal cavity in the retroperitoneal space; the kidneys receive blood from the paired renal arteries, and drain into the paired renal veins. Each kidney excretes urine into a ureter that empties into the bladder (Walter, 2004).

Renal physiology is the study of kidney function, while nephrology is the medical specialty concerned with kidney diseases. Diseases of the kidney are diverse, but individuals with kidney disease frequently display characteristic clinical features. Common clinical conditions involving the kidney include the nephritic and nephritic syndromes, renal cysts,

acute kidney injury, chronic kidney disease, urinary tract infection, nephrolithiasis, and urinary tract obstruction. (Contran *et al.*, 2005).

Various cancers of the kidney exist; the most common adult renal cancer is renal cell carcinoma. Cancers, cysts, and some other renal conditions can be managed with removal of the kidney, or nephrectomy. When renal functions, measured by glomerular filtration rate, are persistently poor, and kidney transplantation may be treatment options. Although they are not normally, kidney stones can be painful. (Walter, 2004).

The kidneys are not just organs of excretion but they are respiratory organs which helps to maintain homeostasis. The weight of the two kidneys is about 0.4% of the total body weight and most kidney receives 1350ml-1300ml of blood per minute. kidney is a compound tubular gland covered by a connective tissue capsule. Kidneys produces urine, ureter transport the urine to the urinary bladder. Urinary bladder stores the urine until it is voided. kidneys perform several vital functions besides formation of urine. By excreting urine, kidneys play the principal role in homeostasis. The kidneys are bean shaped organ located at behind the peritoneal line in the lumbar region of the posterior abdominal cavity (Wheihel, 1996)

The kidney Produces and excretes urine, Filter waste from blood, Maintain electrolyte balance, Regulate blood pressure by producing vasoactive substance

The kidney forms the first part of the urinary system and their principal function is to maintain electrolyte homeostasis and the acid base balance. Kidney function is vital for regulating blood pressure and the kidneys are source of several important hormones such as erythropoietin regulates the production of red blood cells. Histologically, the renal parenchyma consists of four parts: glomeruli, tubules, interstitium, and blood vessels (Hursh 2000).

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. A mechanism of action whereby mercury acts as possible promoter of carcinogen. (Park, 2007). Since mercury is not a mutagenic compound directly affecting gene expression in aborder sense, mercury might be an example of an epigenetic tumor promoter or further expanding this as metagenetic factors (Syversen kaur 2012).

There are a number of mechanism by which alkylmercury compounds cause toxic action in the body .studies reveal that there are some similarities between the mechanisms of the action of mono-alkyl mercury compounds methylmercury and ethylmercury The similarities in mechanism of toxicity for MeHg and EtHg are represented and compared . The difference is manifested toxicity of MeHg and EtHg are likely the result of the difference in exposure , metabolism, and elimination from the body, rather than differences in mechanism of action between the two (Pamela, 2017).

Acute exposure, mercuric chloride is corrosive to the eyes and throat. Persistent visual disturbance has been seen from acute systemic poisoning. Profound circulatory collapse with tachycardia and hypotension can occur from acute exposure to inorganic mercurials. Peripheral neuropathy and brain can occur even from acute exposures (Goldberg, 1996).

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 academy of science and published by the national institute of health[1985].

EXPERIMENTAL DESIGN

The rats were grouped into cages of 9 rats per cage or group. Group A as the control they were not given any substance just water and pellet and their cages were cleaned regularly. Group B were given 0.02g of mercury chloride dissolved in 90ml of water while Group C was given 0.04g of mercury chloride dissolved in 90mls of water and Group D was given 0.05g of mercury chloride dissolved in 90mls of water. After two weeks of acclimatization, mercury chloride was administered on the 14 of February and lasted for 21 days. The mode of administration was oral through the use of cannula and attached calibrated syringe in experiment animals. Each rat received 2ml of each substance for each group. The substance was iced during the administration.

STATISTICAL ANALYSIS

All data were expressed as mean \pm SD of number of experiment (n=4). The statistical analysis of the result obtained in this study was evaluated and tested for significance using t – test. If p – value of the t – test is less than 0.05 ($p < 0.05$), then result is significant. If the p-value of the t – test is greater than 0.05 ($p > 0.05$), then that means that the result is not significant.

RESULTS

Table 1; Showing the mean \pm S.E.M of the weights (g) of left and right kidney.

GROUPS	LEFT KIDNEY	RIGHT KIDNEY	% RELATIVE	% RELATIVE
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	WEIGHTS(G)	WEIGHTS(G)	WEIGHTS OF LEFT KIDNEY	WEIGHTS OF RIGHT KIDNEY
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 \pm 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).

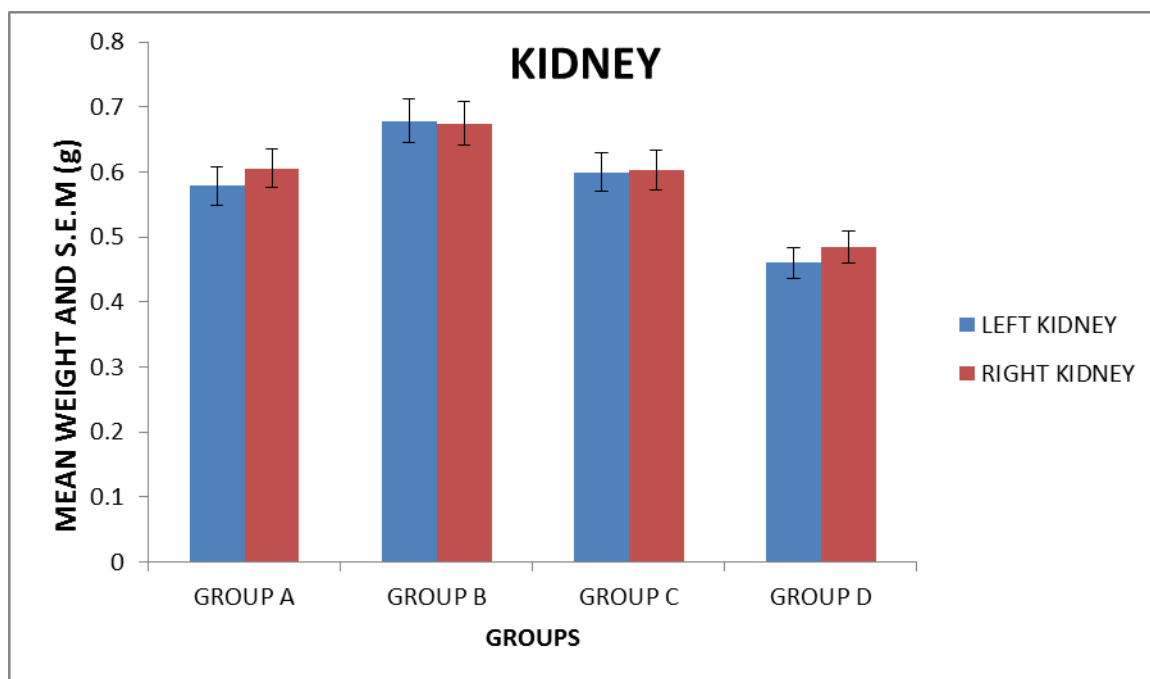


Figure 1; charts showing the graphical representation of the changes in organ weights of mercury chloride.

Weights of the organ (kidney) are expressed as mean \pm 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: showing the effect of mercury chloride on the activities of ALP, AST and ALT on the kidney

GROUPS	ALP	AST	ALT
A	30.70 \pm 2.00	63.27 \pm 4.96	25.00 \pm 1.64
B	41.99 \pm 10.75	84.65 \pm 2.82**	32.24 \pm 2.19*
C	39.97 \pm 1.01**	93.12 \pm 3.29**	38.77 \pm 2.32**
D	40.32 \pm 0.75**	115.4 \pm 2.75**	33.33 \pm 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 \pm Standard error of mean.

The t-test table of the mean \pm 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).

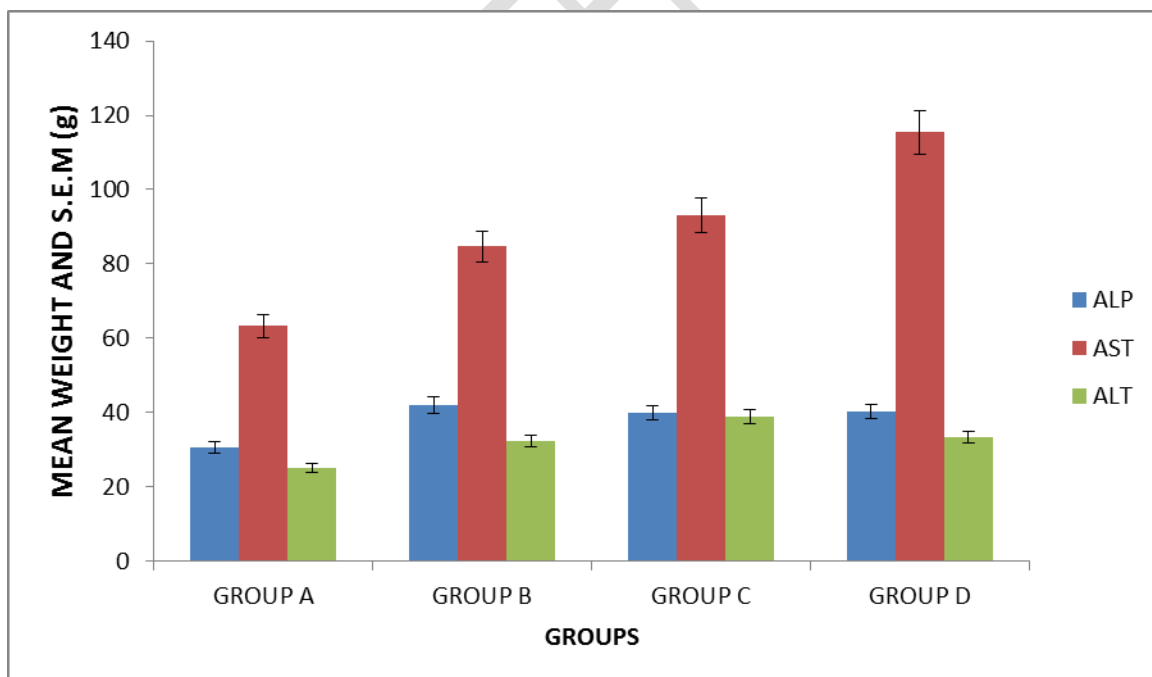


Figure 2 ; chart showing serum analysis of the kidneys of adult wistar rats after administration of HgCl₂ for 3 weeks .when comparing the parameters of experiment groups (Group B, and D) with that of control group (Group A) it could be seen that there was significant increase in ALP (Alkaline phosphatase), Aspartate transaminase increased

significantly in all the groups compared to the control group, Alanine transaminase increased more in group c , significant 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 arteriosclerosis, 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)

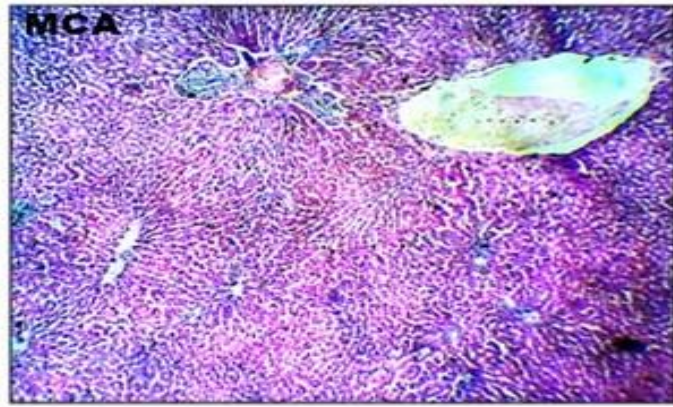


Plate 1a: (H&E X100) photomicrograph of a normal histology of kidney for control group (Group A)

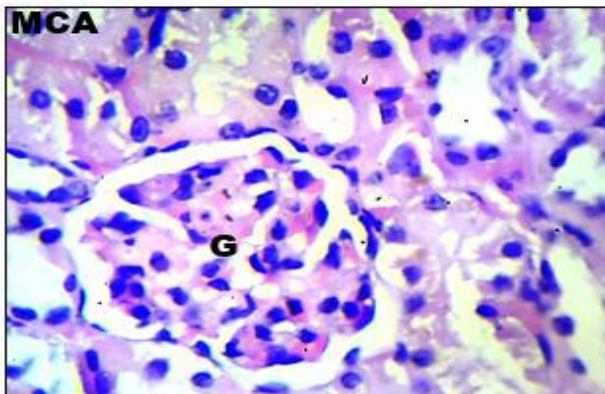


Plate 1b: (H&E X400) photomicrograph of a normal histology of kidney for control group (Group A). showing the renal glomeruli.

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).

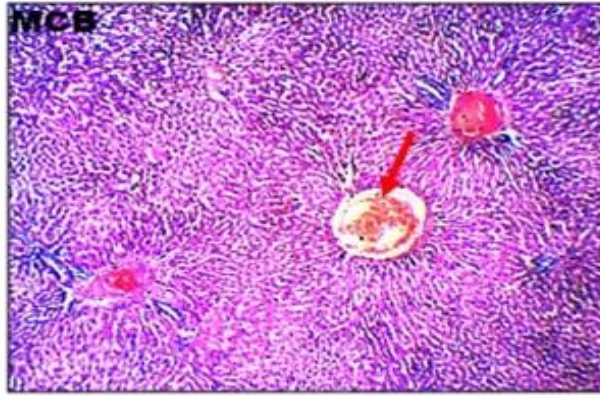


Plate 2a. Transverse section of the Kidney of the rats in group B after administration of 0.02g of Mercury chloride for 21days. (Haematoxylin and eosin X 100)

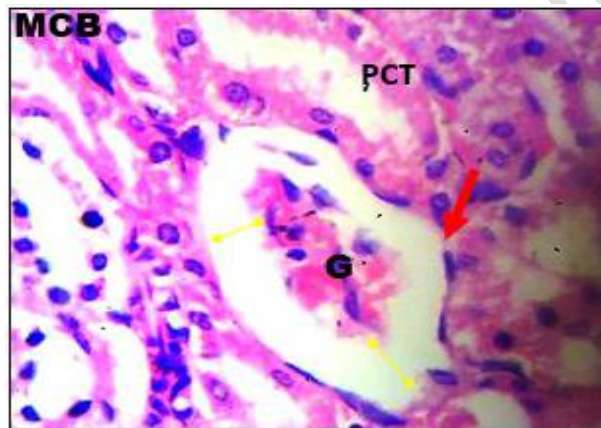


Plate 2b. Transverse section of the Kidney of the rats in group B after administration of 0.02g of Mercury chloride for 21days. (Haematoxylin and eosin X 400). Showing the proximal convoluted tubules(PCT),renal glomeruli (G), Areas with marked pathomorphological changes are indicated by red arrows. Bowman's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

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

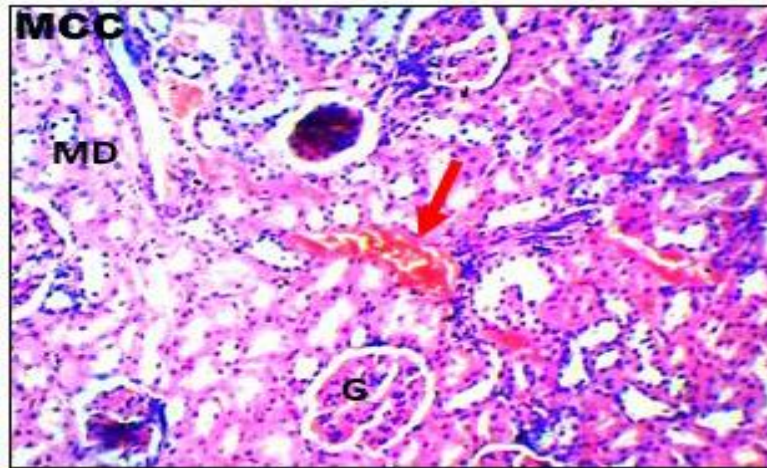


Plate 3a. Transverse section of the Kidney of the rats in group C after administration of 0.04g of Mercury chloride for 21days. (Haematoxylin and eosin X 100)

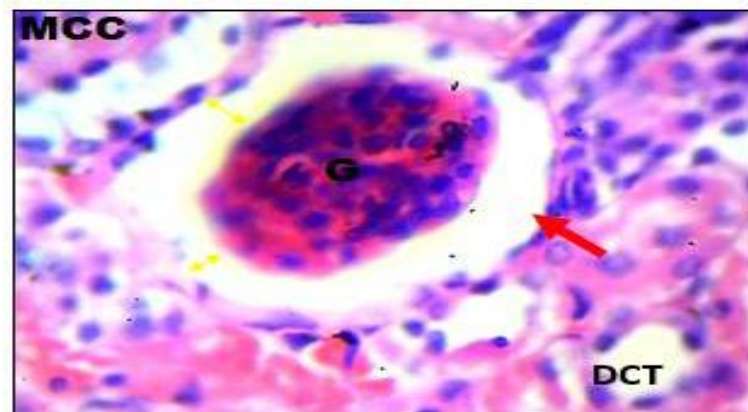


Plate 3b. Transverse section of the Kidney of the rats in group C after administration of 0.04g of Mercury chloride for 21days. (Haematoxylin and eosin X 400)

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 arteriosclerosis, 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

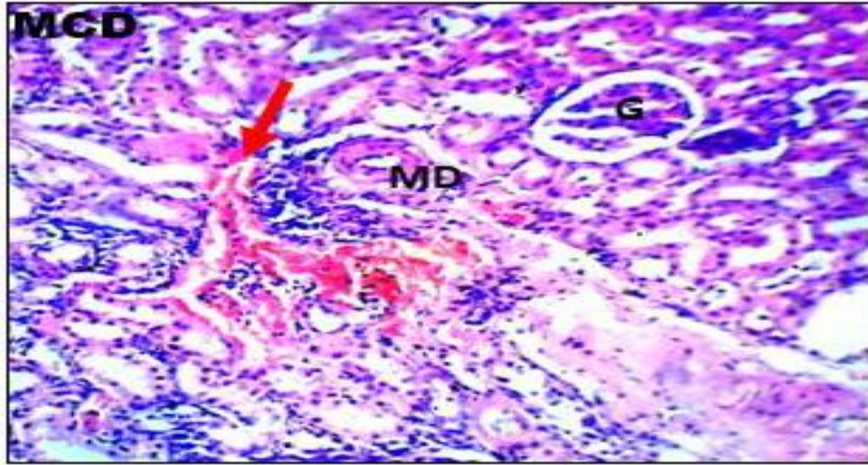


Plate 4a. Transverse section of the Kidney of the rats in group D after administration of 0.05g of Mercury chloride for 21days. (Haematoxylin and eosin X 100) .

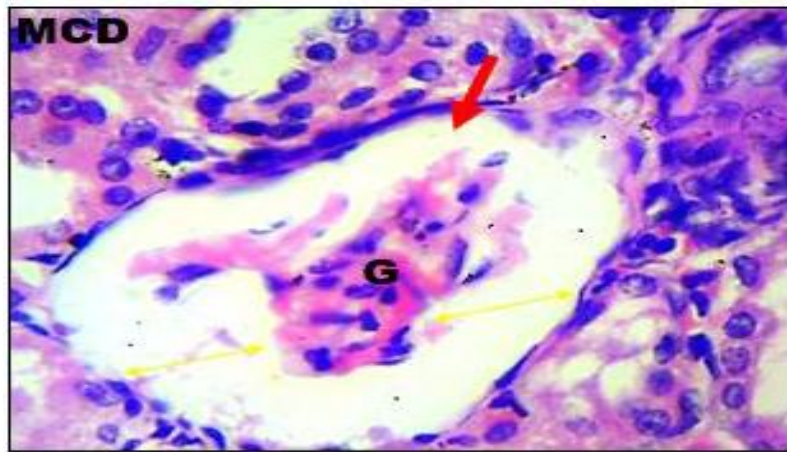


Plate 4b. Transverse section of the Kidney of the rats in group D after administration of 0.05g of Mercury chloride for 21days. (Haematoxylin and eosin X 400) .

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 the glomerulus. There is hyaline arteriosclerosis, 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 project studied 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.

The result of weight analysis showed a significant decrease on the final body weight in group A (control), group B (0.02g of mercury chloride), group C (0.04g of mercury chloride), group D (0.05g of mercury chloride). The findings in this studies showed decrease in body weights could be caused by this toxicant (Hgcl), which may decrease feed intake by modulation of appetite. In general sense, any substance which disturbs metabolism or physiology sufficiently to cause growth inhibition causes decreased feed intake. weights loss is known to be the basic aspect of Hg toxicity and has been attributed to reduced food intake by animals (Jaiswal et al 2013).

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.

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 arteriosclerosis, 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.

According to Sultan and Adel [2017] mercury can cause 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.

The present data showed that the exposure of adult wistar rats to mercuric chloride is capable of inducing alterations in some enzymatic activities, renal functions and some biochemical parameters. 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.

This investigation has presented consistent information in the results showing the histological, biochemical analysis confirming the damaging effects of mercury chloride on the kidneys of wistar rats.

Conclusion

This study concluded that mercury chloride exposure in wistar rats has adversely affected the renal tissue of adult wistar rats.

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