

**AMELIORATIVE POTENTIALS OF ADMINISTRATION OF VITAMIN C AND GARLIC  
FOLLOWING MERCURIC CHLORIDE INDUCTION IN THE KIDNEY MORPHOLOGY  
OF ALBINO WISTAR RATS**

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**ABSTRACT**

This study was aimed at assessing the perils of mercury (II) chloride poisoning in the morphology of the kidney of Albino Wistar rats and the ameliorating tendencies of administration of garlic and Vitamin C following mercury (II) chloride poisoning. 35 adult Albino rats of Wistar strain weighing 190-235g were used for this investigation. The animals were divided into 7 groups of 5 animals each. Group 1 rats received 5ml of distilled water, group 2 received 50.6mg/kg of HgCl<sub>2</sub>, group 3 received 50.6mg/kg of HgCl<sub>2</sub> + 90mg/kg of Vit C, group 4 received 50.6mg/kg of HgCl<sub>2</sub> + 5ml of garlic solution, group 5 received 5ml of garlic solution, group 6 received 50.6mg/kg HgCl<sub>2</sub> + 10 ml of garlic solution + 90mg/kg of Vit C and group 7 received 50.6mg/kg HgCl<sub>2</sub> + 5ml of garlic solution + 90mg/kg of Vit C for 7 days. After the last dose schedule, the animals were sacrificed and the kidneys removed for histopathological examination. Results from this study reveals shrunken glomeruli, degeneration of Bowman capsule and increased periglomerular space in animals administered with 50.6mg/kg of HgCl<sub>2</sub>. Similarly, mild degeneration of Bowman capsule and normal glomeruli was evident in animals administered with 50.6mg/kg of HgCl<sub>2</sub> + 90mg/kg of Vit C. Sections of rats administered with 50.6mg/kg of HgCl<sub>2</sub> + 5ml of garlic solution showed mild shrunken glomeruli, mild increases in the periglomerular space and cytoplasmic vacuolation. Sections of rats administered with 5ml of garlic solution showed normal Bowman capsule, glomeruli and vacuolation. Sections of rats administered 50.6mg/kg of HgCl<sub>2</sub> + 10ml of garlic solution + 90mg/kg of Vit C showed improved glomeruli architecture and improved Bowman capsule while sections of rats administered with 50.6mg/kg of HgCl<sub>2</sub> + 5ml of garlic solution + 90mg/kg of Vit C showed mild improvement in glomeruli architecture and reduced enlargement of periglomerular space. It can therefore be concluded that mercury (II) chloride is poisonous to the kidney and that consumption of vitamin C and garlic may pose ameliorating impact on the morphology of the kidney.

**KEY WORDS:** histopathological, periglomerular, Wistar, vacuolation.

**INTRODUCTION**

Mercury (II) chloride or mercuric chloride is the chemical compound of mercury and chlorine with the formula HgCl<sub>2</sub>. This white crystalline solid is a laboratory reagent and a molecular compound. 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. Mercuric chloride exists not as a salt composed of discrete ions, but rather is composed of linear triatomic molecules, hence its tendency to sublime. In the crystal, each mercury atom is bonded to two close chloride ligands with Hg—Cl distance of 2.38 Å; six more chlorides are more distant at 3.38 Å (Wells, 1984). Mercuric chloride is obtained by the action of chlorine on mercury or mercury(I) chloride, by the addition of hydrochloric acid to a hot,

concentrated solution of mercury(I) compounds such as the nitrate:  $\text{HgNO}_3 + 2 \text{HCl} \rightarrow \text{HgCl}_2 + \text{H}_2\text{O} + \text{NO}_2$ ,

Heating a mixture of solid mercury (II) sulfate and sodium chloride also affords volatile HgCl<sub>2</sub>, which sublimes and condenses in the form of small rhombic crystals. Its solubility increases from 6% at 20 °C (68 °F) to 36% in 100 °C (212 °F). In the presence of chloride ions, it dissolves to give the tetrahedral coordination complex [HgCl<sub>4</sub>]<sup>2-</sup>. The main application of mercuric chloride is as a catalyst for the conversion of acetylene to vinyl chloride, the precursor to polyvinylchloride:  $\text{C}_2\text{H}_2 + \text{HCl} \rightarrow \text{CH}_2=\text{CHCl}$

For this application, the mercuric chloride is supported on carbon in concentrations of about 5 weight percent.

This technology has been eclipsed by the thermal cracking of 1,2-dichloroethane. Other significant applications of mercuric chloride include its use as a depolarizer in batteries and as a reagent in organic synthesis and analytical chemistry (Mathias *et al.*, 2006). It is being used in plant tissue culture for surface sterilisation of explants such as leaf or stem nodes.

For the preservation of anthropological and biological specimens during the late 19th and early 20th centuries, objects were dipped in or were painted with a "mercuric solution". This was done to prevent the specimens' destruction by moths, mites and mould. Objects in drawers were protected by scattering crystalline mercuric chloride over them (Goldberg, 1996). It finds minor use in tanning, and wood was preserved by kyanizing (soaking in mercuric chloride) (Freeman *et al.*, 2003). Mercuric chloride was one of the three chemicals used for railroad tie wood treatment between 1830 and 1856 in Europe and the United States. Limited railroad ties were treated in the United States until there were concerns over lumber shortages in the 1890s. The process was generally abandoned because mercuric chloride was water-soluble and not effective for the long term, as well as poisonous. Furthermore, alternative treatment processes, such as copper sulfate, zinc chloride, and ultimately creosote; were found to be less toxic. Limited kyanizing was used for some railroad ties in the 1890s and early 1900s. Elemental mercury was known to the ancient Greeks, Romans, Chinese, and Hindus. Mercuric chloride was used to disinfect wounds by Arab physicians in the Middle Ages (Maillard *et al.*, 2007). It continued to be used by Arab doctors into the twentieth century, until modern medicine deemed it unsafe for use. Syphilis was frequently treated with mercuric chloride before the advent of antibiotics. It was inhaled, ingested, injected, and applied topically. Both mercuric-chloride treatment for syphilis and poisoning during the course of treatment was so common that the latter's symptoms were often confused with those of syphilis. This use of "salts of white mercury" is referred to in the English-language folk song "The Unfortunate Rake" (Pimple, 2002). Yaws was treated with mercuric chloride (labeled as Corrosive Sublimate) before the advent of antibiotics. It was applied topically to alleviate ulcerative symptoms.

Garlic is a bulbous plant. It grows up to 1.2 m (4 ft) in height. It produces hermaphrodite flowers. It is pollinated by bees and other insects (Meredith *et al.*, 2014). Garlic is widely used around the world for its pungent flavor as a seasoning or condiment. A great deal of low quality clinical research has been conducted to determine the effect of garlic on preventing cardiovascular diseases and on various biomarkers of cardiovascular health. Garlic contain vitamins B6 and C, and the dietary minerals, manganese and phosphorus. Garlic is also a moderate source of certain B vitamins, including thiamin and pantothenic acid, as well as the dietary minerals, calcium, iron, and zinc. The composition of a garlic bulb is 59% water, 33%

carbohydrates, 6% protein, 2% dietary fiber and less than 1% fat (USDA, 2014).

Vitamin C, also known as ascorbic acid is a vitamin found in food and used as a dietary supplement. As a supplement it is used to treat and prevent scurvy (ASHSP, 2016). Evidence does not support use in the general population for the prevention of the common cold (WHO, 2008). It may be taken by mouth or by injection (ASHSP, 2016). Vitamin C is an essential nutrient for certain animals including humans. Vitamin C describes several vitamers that have vitamin C activity in animals, including ascorbic acid and its salts, and some oxidized forms of the molecule like dehydroascorbic acid. Ascorbate and ascorbic acid are both naturally present in the body when either of these is introduced into cells, since the forms interconvert according to pH. Vitamin C is a cofactor in at least eight enzymatic reactions, including several collagen synthesis reactions that, when dysfunctional, cause the most severe symptoms of scurvy (FSA, 2010). In animals, these reactions are especially important in wound-healing and in preventing bleeding from capillaries. Ascorbate also acts as an antioxidant, protecting against oxidative stress (Padayatty *et al.*, 2003).

## MATERIALS AND METHODS

35 adult Albino rats of Wistar strain weighing 190-235g were used for this investigation. They were housed in the animal house of the Faculty of Basic Medical Sciences, University of Uyo. They were housed under standard laboratory conditions and had free access to water. The experiment was carried out under compliance of the Ethics committee of the Faculty of Basic Medical Sciences, University of Uyo on the guiding principles of the care and use of animals.

### Procurement of Mercury (II) chloride

Mercuric (II) chloride was obtained in a white crystalline form from the Department of Chemistry, University of Uyo, Nigeria.

### Preparation of Mercury (II) chloride

100 g of mercuric (II) chloride was reconstituted in 5% toluene 80 to make up the test solution of known concentration.

### Procurement of Garlic and Vitamin C

Garlic was purchased from a local market situated along Calabar-Itu road in Itu Local Government Area of Akwa Ibom State, Nigeria. Vitamin C was obtained as pellets from Pharmablaze Pharmacy situated along Oron road in Uyo Local Government of Akwa Ibom State.

### Preparation of Garlic Solution

The outermost covering of Garlic was peeled off using a kitchen knife to expose mesocarp. The mesocarp was massaged with mortar and Pestle into fine particles. The particles were reconstituted in 500ml of distilled water for 3 days to ferment.

**Preparation of Vitamin C:** Vitamin C pellets were grinded into fine granules and reconstituted in 5% toluene 80 to make up the test solution of known concentration.

**Experimental Design:** 35 adult Albino rats of Wistar strain weighing 190-235g were used for this investigation and were randomly divided into 7 groups of 5 animals each. The animals were treated as follows

Group 1: rats were administered with 5ml of distilled water

Group 2: rats were administered with 50.6mg/kg of HgCl<sub>2</sub>

Group 3: rats were administered with 50.6mg/kg of HgCl<sub>2</sub>+90mg/kg of Vit C

Group 4: rats were administered with 50.6mg/kg of HgCl<sub>2</sub>+ 5ml of garlic solution

Group 5: rats were administered with 5ml of garlic solution

Group 6: rats were administered 50.6mg/kg HgCl<sub>2</sub> + 10 ml of garlic solution + 90mg/kg of Vit C

Group 7: rats were administered 50.6mg/kg HgCl<sub>2</sub> + 5ml of garlic solution + 90mg/kg of Vit C.

The test agents were administered for 7 days. After the last dose schedule, the animals were anaesthetised using chloroform and kidney removed for histology.

**Kidney Histology:** Kidneys were obtained and fixed in 10% buffered formalin. The first stage of processing was the removal of unbound water and aqueous fixatives from the tissue components. The tissues were dehydrated using graded series of alcohol (70%, 95% and absolute alcohol). Xylene was used for clearing to remove dehydrating solutions thus making the tissue components receptive to infiltrating medium. Two changes of xylene were used to clear the tissues one hour each thus making the tissues to have translucent appearance. Paraffin wax was used for infiltration, the tissues were impregnated with wax which forms a matrix preventing tissue structure distortion during microtomy. The impregnated tissues were placed in an oven whose temperature was regulated to 60°C which corresponds to about the melting point of paraffin wax. Paraffin wax was also used for embedding to provide external support for the tissues during microscopy.

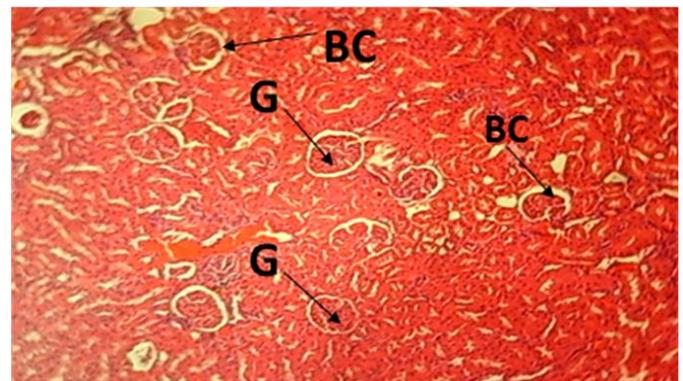
The paraffin blocks were cut into ribbons by setting the micrometer of the microtome at about 5mm or by advancing the block using the coarse feed mechanism. Haematoxylin and Eosin staining techniques was used.

## RESULTS

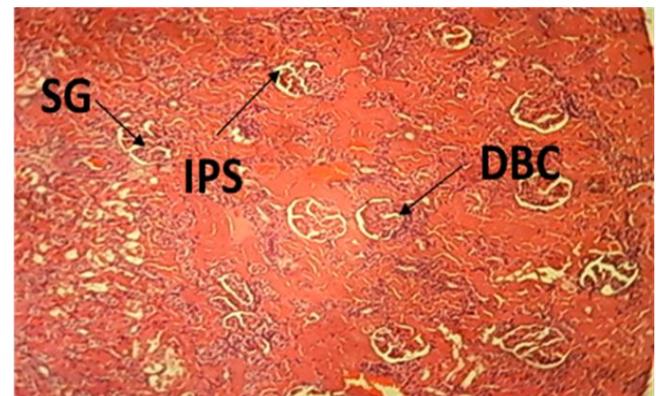
From this investigation, the H& E sections of kidney of control rats administered with 5ml of distilled water showed normal architecture of Bowman capsule and Glomeruli. Sections of kidney of rats administered with 50.6mg/kg of HgCl<sub>2</sub> showed shrunken glomeruli, degeneration of Bowman capsule and increased periglomerular space. Similarly the sections of kidney of

rats administered with 50.6mg/kg of HgCl<sub>2</sub> +90mg/kg of Vit C showed mild degeneration of Bowman capsule and normal glomeruli. Sections of rats administered with 50.6mg/kg of HgCl<sub>2</sub> +5ml of garlic solution showed mild shrunken glomeruli, mild increases in the periglomerular space and cytoplasmic vacuolation. Sections of rats administered with 5ml of garlic solution showed normal Bowman capsule, glomeruli and vacuolation.

Sections of rats administered 50.6mg/kg of HgCl<sub>2</sub> + 10ml of garlic solution + 90mg/kg of Vit C showed improved glomeruli architecture and improved Bowman capsule while sections of rats administered with 50.6mg/kg of HgCl<sub>2</sub> + 5ml of garlic solution + 90mg/kg of Vit C showed mild improvement in glomeruli architecture and reduced enlargement of periglomerular space.



**Fig. 1: Photomicrograph of kidney of control rat administered with 5ml of distilled water showing normal architecture of Bowman capsule (BC) and Glomeruli (G) at X10 magnification**



**Fig. 2: Photomicrograph of group 2 rats administered with 50.6mg/kg of HgCl<sub>2</sub> showing shrunken glomeruli (SG), degeneration of Bowman capsule (DBC), and increased periglomerular space (IPS) at X10 magnification**

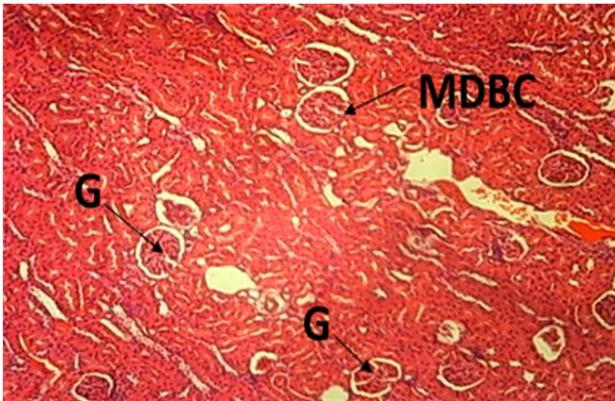


Fig. 3: Photomicrograph of group 3 rats administered with 50.6mg/kg of  $\text{HgCl}_2$  + 90mg/kg of Vit C showing mild degeneration of Bowman capsule (MDBC) and normal glomeruli (G) at X10 magnification.

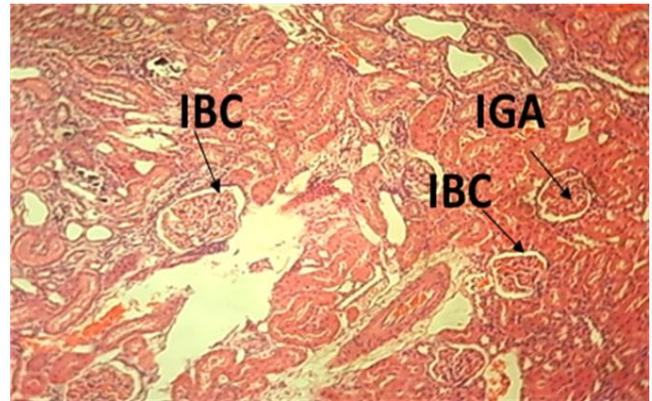


Fig. 6: Photomicrograph of group 6 rats administered with 50.6mg/kg  $\text{HgCl}_2$  + 10 ml of garlic solution + 90mg/kg of Vit C showing improved glomeruli architecture (IGA) and improved Bowman capsule (IBC) at X10 magnification

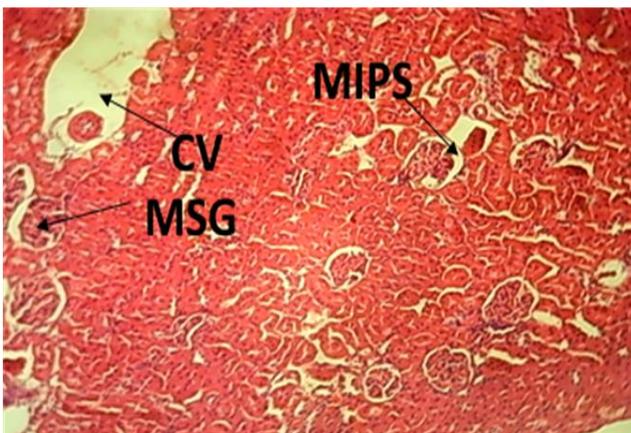


Fig. 4: Photomicrograph of group 4 rats administered with 50.6mg/kg of  $\text{HgCl}_2$  + 5ml of garlic solution showing mild shrunken glomeruli (MSG), mild increases in the periglomerular space (MIPS) and cytoplasmic vacuolation (CV) at X10 magnification

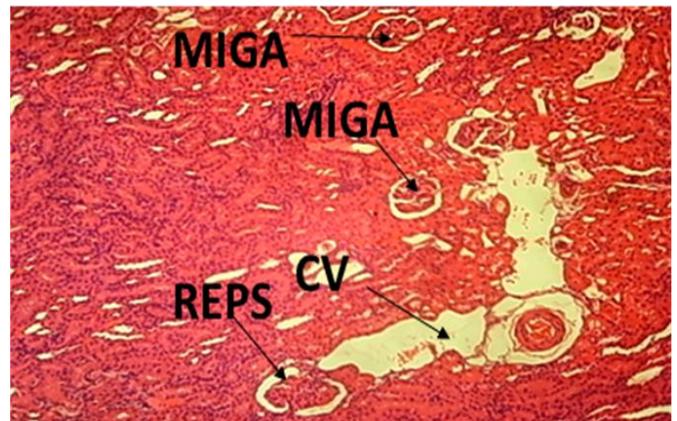


Fig. 7: Photomicrograph of group 7 rats administered 50.6mg/kg  $\text{HgCl}_2$  + 5ml of garlic solution + 90mg/kg of Vit C showing mild improvement in glomeruli architecture (MIGA), cytoplasmic vacuolation (CV) and reduced enlargement of periglomerular space (REPS) at X10 magnification.

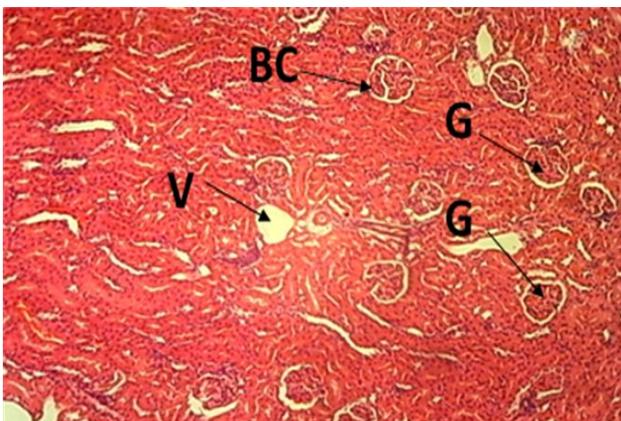


Fig. 5: Photomicrograph of group 5 rats administered with 5ml of garlic solution showing normal Bowman capsule (BC) glomeruli (G) and vacuolation (V) at X10 magnification

## DISCUSSION

Lesion due to lethal effects of some chemical compounds are very significant in delineating the health status of the common populace. Specific lesions occurring in the organs of organisms exposed to toxic substances under laboratory conditions are helpful as important biomarkers of exposure. As a result of this, histopathological examination is widely being used as a valuable tool for evaluating the impact of contaminants like mercuric chloride in animals and humans. In this study it is observed that mercuric chloride has induced histopathological changes such as shrunken glomeruli, degeneration of Bowman capsule, increased periglomerular space and cytoplasmic vacuolation in the kidney of albino Wistar rats. Similarly the study has also shown that co-administration of the mercuric chloride with either vitamin C and garlic or garlic indicated improved cytoarchitectural appearance in the glomeruli and other components of the kidney, such features noted include improved glomeruli architecture, improved

Bowman capsule, mild shrunken glomeruli and mild increases in the periglomerular space.

Investigations have identified histological alterations earlier at the level of the tubular epithelium and glomerulus in the kidney of other animals such the fish (Das and Mukherjee 2000). Cengiz, 2006 noticed degeneration in the epithelial cells of renal tubule, pycnotic nuclei in the hematopoietic tissue, dilation of glomerular capillaries, degeneration of glomerulus, intracytoplasmic vacuoles in epithelial cells of renal tubules with hypertrophied cells and narrowing of the tubular lumen in the kidney of the fish *C. carpio* exposed to deltamethrin. Ram Nayan Singh, 2012 reported shrinkage of glomerulus, dilation of tubular lumen, vacuolization, desquamation, hydropic swelling and hyaline degeneration of tubular epithelium, cyst formation and hemorrhage in the kidney of common carp, *C. carpio* after sub lethal exposure to dimethoate and stated that the duration of exposure appears to have a profound effect on the kidney.

The glomerular distortion and increased periglomerular spaces seen in the kidneys of experimental animals may be due to the spasmolytic and vasodilatory effects of the mercury chloride. These conditions are associated with prolonged reduction in the percentage of cardiac output to the kidneys, which causes reduced glomerular pressure and collapse of the glomerular capillaries. Dilation of vessels all over the body for a prolonged time can cause cardiac depression, which can eventually lead to reduced glomerular capillary pressure and increased urinary spaces. The mesangial cells of the kidneys are contractile and modify the diameter of the glomerular capillaries in response to vasoactive substances. This agrees with Ojewole and Odebiyi (1980) findings that teramethylpyrazine (TMPZ), isolated from stems of *Jatropha* specie, possesses specific spasmolytic and vasodilator activity. General reduction in body fluid and extracellular fluid caused by haemorrhage, vomiting and diarrhoea can cause glomerular hypotension, which can also result in glomerular shrinkage, distortion and increased periglomerular spaces. From this investigation it was observed that administration of mercury chloride can produce histopathological changes in the kidney of albino Wistar rats, moroso administration of antioxidants elicited a positive protective potentials on the kidney. Vitamin C and Garlic contain antioxidants. vitamin C is essential to a healthy diet as well as being a highly effective antioxidant, acting to lessen oxidative stress; a substrate for ascorbate peroxidase in plants (APX is plant specific enzyme) (Higdon, 2007) and an enzyme cofactor for the biosynthesis of many important biochemicals. Vitamin C acts as an electron donor for important enzymes (Levine *et al.*, 2000). Vitamin C is found in high concentrations in immune cells, and is consumed quickly during infections. It has been hypothesized to modulate the activities of phagocytes, the production of cytokines and lymphocytes, and the number of cell adhesion molecules in monocytes (Lindblad *et al.*, 1970).

Since Garlic contain Allicin which is an oily, slightly yellow liquid that gives garlic its unique odor. It is a thioester of sulfenic acid and is also known as allyl thiosulfinate (Nikolic *et al.*, 2004). Its biological activity can be attributed to both its antioxidant activity and its reaction with thiol-containing proteins (Rabinkov *et al.*, 1998).

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. Antioxidants such vitamin C terminate these chain reactions. Anti-oxidants are involved in protecting the body from oxidation, a natural process but some could also be attributed to the development of chronic diseases and the changes associated with ageing (Wendy *et al.*, 2003). Kidney serves as a major route of excretion of metabolites of xenobiotics, receives the largest proportion of postbranchial blood and therefore it is more likely to undergo histopathological alterations under pesticide stress (Ortiz *et al.*, 2003). Hence the kidney lesions might be expected to be good indicators of environmental pollution (Ortiz *et al.*, 2003).

## CONCLUSION

It may be concluded that Vitamin C and garlic could alleviate the alterations posed by mercury (II) chloride.

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