Assessment the therapeutic effects of aqueous extracts of *Cilantro* and *Garlic* in mercuric chloride poisoning in rats

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Summary

The objective of the present study was to evaluate the therapeutic effects of cilantro and garlic aqueous extracts in toxicopathological changes due to acute and chronic mercuric chloride poisoning in rat. The acute toxic signs appeared at short time after treatment with mercuric chloride. Severity of intoxication and mortality rate were proportional to the dose of mercuric chloride given. Chronic clinical signs were checked continuously which characterized by anorexia, body weight loss, pale –yellow mucous membranes, rough skin, ruffled hair, dyspnea and hemoglobin urea the severity of signs was dose dependant. Results showed severe toxicopathological changes in kidneys, liver and brain with precancerous lesions in liver and fore stomach, with residual accumulation of the compound in these organs reaching the highest rate in kidneys in both acute and chronic toxicity. Treatment with plant extracts resulted in decreasing the severity of the pathological changes in the mentioned organs and increase in the immune response of the body especially in the animals treated with combination of *cilantro* and *garlic* extracts together. This was reflected in decreased levels of compound accumulation in tissue. It could be concluded that mercuric chloride poisoning in rats causes toxicopathological changes in the kidneys, liver and brain with precancerous lesions in liver and highest residual accumulation in kidneys .Treatment with cilantro and garlic extracts could reduce the severity of these lesions and residual accumulation with elevation in the immune response.

Keywords: Mercuric Chloride, Cilantro, *Garlic*, Poisoning.

تقييم التاثير العلاجي للخلاصة المائية للكزبرة والثوم على التسمم بكلوريد الزئبق في الجرذان

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الخلاصة

هدفت الدراسة الى تقييم التاثير العلاجي للخلاصة المائية للكزبرة والثوم على التغيرات المرضية السمية الحادة والمزمنة لكلوريد الزئبق في الجرذان . ظهرت العلامات السريرية الحادة بعد وقت قصير من تجريع المادة المذكورة واصبحت اكثر شدة مما ادى الى معدل الهلاكات تتناسب مع تركيز الجرعة المعطاة . تم ملاحظة العلامات السريرية المزمنة بصورة مستمرة ويمكن ايجازها, فقدان شهية مع انخفاض في وزن الجسم وشحوب واصفرار الاغشية المخاطية و خشونة الجلد وتجعد الشعر و ضيق التنفس مع البيلة الهيموغلوبينية. تتناسب شدة هذه الاعراض طرديا مع زيادة الجرعة .اوضحت النتائج وجود تغيرات مرضية سمية واضحة في انسجة الكلى ,الكبد والدماغ مع حصول افات مهيأة للتسرطن في الكبد و المعدة جراء التعرض لكلوريد الزئبق مع حصول الترسب الاكثر تركيزا في كل من التسمم الحاد والمزمن .اما بعد العلاج بالخلاصة المائية لكل من الكزبرة والثوم فقد قلت شدة التغيرات المرضية بالاضافة الى زيادة الاستجابة المناعية وهذا ما انعكس ايضا على حصول قلة في متبقيات المركب في الانسجة خصوصا عند اعطاء خلاصة الكزبرة والثوم معا .نستنتج مما سبق ان التسمم بكلوريد الزئبق في الجرذان يؤدي الى حصول تغيرات مرضية شديدة في الكلى , والكبد والدماغ مع حصول افات مهيأة للتسرطن في الكبد و ان لخلاصتي الكزبرة والثوم معا دور علاجي مهم عند حدوث التسمم حيث ادى الى قلة شدة التغيرات المرضية ونسبة متبقيات المركب في الكلى ,الكبد والدماغ مع زيادة في الاستجابة المناعية .

الكلمات المفتاحية :- كلوريد الزئبق ,الكزبرة ,الثوم ,التسمم.

Introduction

Mercury is a highly toxic heavy metal (1and 2). It causes a variety of adverse health effects including: neurological, renal, respiratory, immune dermatological, reproductive, and developmental sequelae (3 and 4). Mercury was absorbed through inhalation, ingestion and dermal contact, its absorption is related to the type of mercury compound and the duration of exposure (5) . In general, mercurial are attracted to sulfhydryl radicals in body and are bound to proteins, membranes and enzymes, altering their normal functioning (6 and 7). Mercury poisoning has been reported in human following exposure to metallic mercury and its organic and inorganic derivatives (8). Inorganic mercury accumulates primarily in the kidneys, liver, spleen, bone marrow, intestine, skin and respiratory mucosa (9). Infants and small children are particularly vulnerable because of the risk of severe injury to the developing brain (10).

Mercuric chloride is a very toxic form of mercury which induces severe alterations in the tissues of both animals and men (11). It primarily affects gastro –intestinal tract (GIT) and cause severe damage (12). Cilantro (Chinese parsley)is a kitchen herb and one of the best detoxifiers for CNS , it is especially useful for removing mercury and other toxic metals rapidly from the brain when appropriate amounts are consumed daily, as brain detoxification in one of the most difficult to achieve ,the mobilized mercury appears to be either excreted via the stool , urine or translocate into more peripheral tissues .This was a revolutionary discovery and made cilantro the first known substance that mobilizes mercury from the CNS (13) . Garlic is a powerful herb which has been used for centuries as an immune stimulant and as antiseptic agent. Garlic's powerful action comes from its sulphur containing constituents, including: allicin, aillin and daily disulphide. These compounds are quite capable for binding to and eliminating mercury as a normal part of their physiological action. Therefore the aim of the study was to investigate the pathological alterations of mercuric chloride in rats and the synergistic protective effects of cilantro and garlic.

Materials and Methods

Plant was obtained from local markets in Baghdad. A voucher specimen of the plants was deposited to be identified and authenticated at the national herbarium of Iraq botany directorate in Abu – Ghraib with certificate number (3743)in (6 /12 /2010)for Garlic and Cilantro, the plant was Cilantro Family :*umbelliferae*, Genus: *Coriander* , Species: *Coriander Sativum L* while Garlic Family: *Liliaceae* Genus: *Garlic* Species*: Allium Sativum L.*

Two hundred forty albino rat aged 6-7 weeks and weight 45-60 Gms were used. Rats obtained from (Animal house colony of Embryo Research and Infertility treatment Institute/AL-Nahrain University), housed in a plastic cages 50×30×10 cm and placed in the room for 10 days for adaptation. Room temperature was maintained at 21 ± 3 ˚C, the air of the room was changed continuously by using ventilation vacuum and with the litter of the cages was changed every week. The animals hosted in animal house at College of Veterinary Medicine, Baghdad University, and were fed on pellet add labium.

In Acute toxicity, sixty male rats divided randomly in to (12) groups each group contain (5) animals treatment were done by oral gavage by stomach tube for one month as following: 13.5 mg/kg BW (mercuric chloride) once + distilled water (24 hr later) (+ive) control 13.5 mg/kg BW (mercuric chloride) once + 100mg/kg .BW garlic extract (24 hr later), 13.5 mg/kg BW (mercurric chloride) once + 50mg/kg BW cilantro extract (24 hr later), 13.5 mg/kg BW (mercuric chloride) once + 50 mg/kg BW garlic extract and 25 mg/ kg.BW (24 hr later) cilantro extract together, 0.1ml distilled water only (- ive control) , 6.75 mg/kg BW. (Mercuric chloride) once + distilled water (24 hr later) +ive control, 6.75 mg/kg BW mg/kg BW mercury chloride mercuric chloride) once + 100mg/kg .BW garlic extract (24 hr later)**,** 6.75

mg/kg BW (mercuric chloride) once + 50mg/kg BW cilantro extract (24 hr later), 6.75 mg/kg BW (mercuric chloride) once + 50 mg/kg BW garlic extract and 25 mg/ kg BW (24 hr later) cilantro extract together, 100 mg /kg BW garlic extract 4 times, 50mg/kg BW cilantro extract 4 times /week and 50 mg/kg BW garlic extract and 25 mg/ kg.BW cilantro extract together 4 times /week.

The chronic toxicity study involves (195) male rats which were divided in to 13 groups each group contain (15) animals treatment were done by oral gavage by stomach tube treated as following: 0.006 mg /kg BW (mercuric chloride) for 12 weeks + distilled water (+ive) control, 0.006 mg /kg .BW (mercuric chloride)for 12 weeks followed by 100mg/kg .BW garlic extract for one month, 0.006 mg /kg .BW (mercuric chloride) for 12 weeks followed by 50mg/kg BW cilantro extract for one month, 0.006 mg /kg .BW (mercuric chloride) for 12 weeks followed by 50 mg/kg BW garlic extract and 25 mg/ kg.BW cilantro extract together for one month, 0.1ml distilled water for 13 weeks (- ive) control, 0.012 mg /kg BW (mercuric chloride) for 12 weeks and then treated distilled water (+ive) control, 0.012 mg /kg .BW (mercuric chloride) for 12 weeks followed by 100mg/kg BW garlic extract for one month, 0.012 mg /kg .BW (mercuric chloride) for 12 weeks followed by 50mg/kg BW cilantro extract for one month, 0.012 mg /kg .BW (mercuric chloride) for 12 weeks followed by 50 mg/kg BW garlic extract and 25 mg/ kg BW cilantro extract together for one month, 0.018 mg /kg BW (mercuric chloride) for 12 weeks and then with distilled water(+ive), 0.018 mg /kg BW (mercuric chloride) for 12 weeks followed by 100mg/kg BW garlic extract for one month, 0.018 mg /kg .BW (mercuric chloride) for 12 weeks followed by 50mg/kg BW cilantro extract for one month, 0.018 mg /kg .BW (mercuric chloride) for 12 weeks followed by 50 mg/kg BW garlic extract and 25 mg/ kg.BW cilantro extract together for one month .After 13 weeks three animals from each groups were scarified monthly.

Determination of LD50 of mercury chloride by probit method the procedure explained by, Caserate (14). Determination of LD50 of Aqueous extracts of garlic and cilantro LD50 for garlic and cilantro extract were determined according to, Dixon (15).

At the end of the each period of experiment four rats from each group were sacrificed by injection of high dose of ketamine hydrochloride and post mortem were done for all animals .The macroscopic appearance were recorded to detect any abnormal gross changes in internal organs. Specimens were taken kidneys, liver and brain; the tissues were kept in 10 % formaldehyde solution, for fixation, and then processed routinely by using the histokinette. Tissue sections were embedded in paraffin blocks, and sectioned by microtome with hematoxylin and eosin then examined under light microscope, Luna (16).

Determination of residual of trace amount for mercury, the procedure of Mudacavi (17) was followed

Tissue preparation rats were weighted anesthetized with ether and killed by decapitation about 24 hr after the last dose of saline or mercury. The brain, kidney and liver were quickly removed and weighted and then half of the tissue placed in containers and frezen (- 20c) , sample of these tissue were collected for determine of mercury.

Tissue digestion was made according to the method (18) Prior to mercury analysis, sampled were allowed to thaw and then received 10 ml of HNO3 (65%) and 1 ml of hydrogen peroxide (30%) sampled were maintained at room temprature for 24 h and heated at 45 c on a sand bath for 6 hr. sample were filtered and the volume was adjusted to 25 ml with 0.5 % HNO3 (w/v). To a series of solution containing 5- 30 µg of mercury (II) chloride add 1.0ml of a 0.05 $_{M}$ EDTA solution 5 mL of pH 4.5 buffer .2 ml of 0.05 %1.10 –phenonanthraline solution and 1 ml of 0.05 % gelatine solution . Mix the contents well and add 5ml of 0.05 % eosine solution. Again mix the solutions and dilute to 25 ml measure the absorbance of the samples in 10- mm cells at 552 nm against a reagent blank.

Factorial experiment applied in completely randomized design (CRD) was used to study the effect of treatment and month in different trails. Least significant difference (L.S.D)

was used to compare the significant difference between means .Data were analyzed using statistical analysis system (SAS) (2001) program.

Results and Discussion

The calculated LD50 for mercuric chloride was 66mg /kg.BW. Garlic 2000 mg/kg.BW and cilantro 5000 mg/kg BW The present value of LD50 (66mg/kg BW) did not agreed with results of other studies (19) in rats. That may be due to environmental adaptation e.g . Long exposure for insecticides, many toxic agents change the animal's resistance to low doses of toxic metal.

In control group there are no clear macroscopic findings. The main lesion of kidneys were congestion of the cortex which appeared more severe with the higher dose (13.5 mg / kg.BW) of mercuric chloride .The organ appeared dark red with nephromegaly (Figure 1). Also the Liver was severe congestion, dark red in color hepatomegaly with rounded edges, accentuation of lobular markings with friable parenchyma with all doses of the compound (Figure 2). Macroscopically lesion of the brain treated rats orally with a single dose (13.5 mg/kg.BW) of mercuric chloride appeared swollen, edematous, with severe congestion of meningeal blood capillaries (figure3).

Fig 1: Macroscopic appearance of kidney of rat treated orally with (13.5 mg/kg.BW) of mercuric chloride shows congestion (dark red) with nephromegaly.

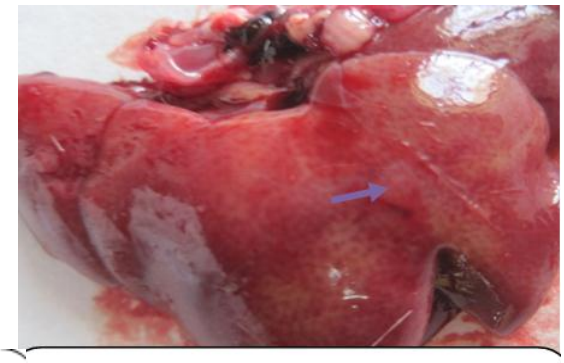


Fig2: Macroscopic appearance of liver of rat treated orally with (13.5 mg/kg.bw) mercuric chloride shows, hepatomegaly with rounded edges and accentuation of lobular markings.

(➡).

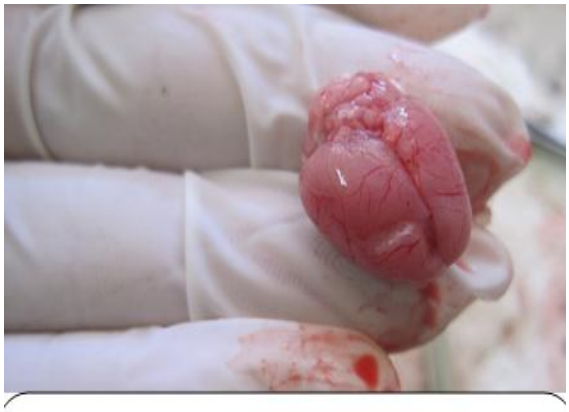


Fig 3: Macroscopic appearance of brain of rat treated orally with single dose (13.5 mg/kg.bw) mercuric chloride shows swollen, edematous brain, with severe congestion of meningeal blood capillaries

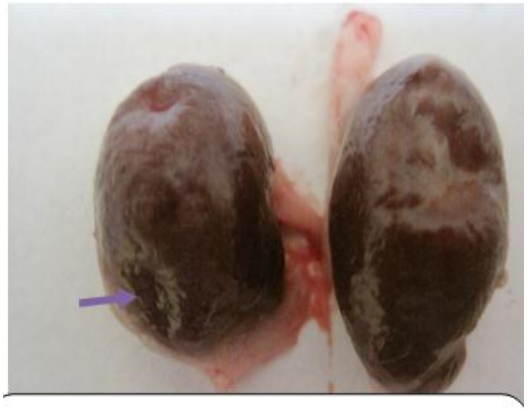


Fig4: Macroscopic appearance of kidney of rat treated orally with 0.018mg/kg.bw. of Mercuric chloride for 12 weeks the kidneys appeared small in size with contracted granular surface. ➡

Macroscopic appearance of organs in acute toxicity was characterized by severe congestion of kidneys and brain and that reflect the severe vascular effect from mercuric chloride. In addition to accentuation of lobular markings of the liver which attributed to the deference in color between the centers and the peripheries of the lobules indicating acute toxic hepatitis (20) these results agreed with (19) in experimental methyl mercuric chloride toxicosis in rats .kidneys in chronic toxicity more characterized by marked fibrosis with granular contracted surface, as the microscopic findings revealed that this disorder represents the results of chronic glomerulonephritis and severe tubular necrosis leading to atrophy of the organ .

The microscopic findings in acute toxicity study of kidneys, mercuric chloride (13.5 mg/kg BW orally) there is frank diffuse coagulative type necrosis of cortical renal tubules appeared as solid and dense masses (Figure 5). In many areas there is severe destruction of epithelial lining leaving the basement membrane only in addition, there are multiple areas of severe hemorrhages in the interstitial tissue and in the necrosed renal tubules. Depositions of hemosiderin pigment were also seen. Deposition of hyaline droplets within the proximal and distal convoluted renal tubules. These structures appeared as deep eosinophilic rounded or oval in shape (Figure 6). In mercuric chloride (6.75mg/kg BW orally) tissue sections showed cloudy swelling of epithelial cells lining the cortical renal tubules with atrophy of glomerular tuft (Figure 7) with interstitial edema and congestion of blood vessels.

In liver, Mercuric chloride (13.5 mg/kg BW orally) the microscopic findings showed extensive areas of necrosis and apoptosis (Figure 8). Other sections showed edema with severe destruction of hepatic parenchyma and oozing of blood to the necrotic area (Figure9) with infiltration of few neutrophils. Mercuric chloride (6.75mg/kg .BW orally) hepatic tissue sections of this period are characterized by vacuolation of hepatocytes. The hepatic cells have large nuclei containing many nucleoli (Figure 10).

In brain, mercuric chloride (13.5 mg/kg BW orally**)** the tissue sections of cerebellum showed severe dilation and congestion of cerebral and meningeal blood vessels (Figure11). Congestion of blood capillaries with perivascular and perineuronal edema .The cerebellum showed pyknosis of nuclei of granular layer cells of the folia, in addition to necrosis of large numbers of those cells .Furthermore there is edema between granular and molecular layer. Degeneration of many purkinji cells and complete dissolution of others (Figure12). Mercuric chloride (6.75mg/kg BW orally) the section of cerebrum showed shrinkage of neurons containing pyknotic nuclei (Figure13). Slight congestion of the choroidal blood vessels was also seen.

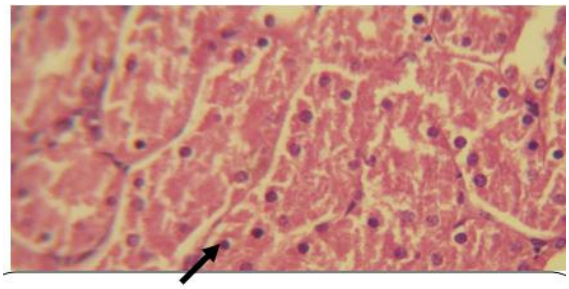
Chronic toxicity study of kidneys at first dose (0.06 mg/kg BW orally) the main histopathological lesions in all periods of experiment are the deposition of amyloid like substance in glomeruli with slight interstitial fibrosis leading to cystic dilation of renal tubules contains hyaline cast(Figure14). Second dose (0.012mg/kg BW orally) showed medullary cystic dilation of renal tubules with slight interstitial fibrosis at one weeks interval, while after 12weeks the main microscopic picture was the atrophy of glomerular tuft with proliferation of partial layer of Bowman's capsule and focal interstitial cells infiltrations (Figure15).

Chronic toxicity of liver show slight proliferation of fibrous tissue at one weeks then the fibrous connective increase in amount in late stages (three weeks) leading to atrophy of hepatocyte (Figure 16). Furthermore there is hyperplasia of bile ducts leading to formation of papillary projections. While in brain **s**light congestion, perivascular and perneuronal edema in all periods of experiment.

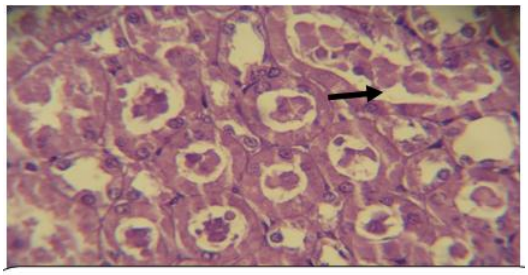
Treatment of acute toxicity of Kidneys regeneration of epithelial cells lining both proximal and distal convoluted tubules in section treated with cilantro and garlic extract for one month with perivascular cuffings (Figure17). Also in liver hepatic tissue sections showed infiltration of large numbers of mononuclear cells in the dilated and congested blood vessels in treated

group with garlic extract at two weeks periods (Figure18). While in brain tissue sections treated with both extract showed perivascular lymphocytic cuffings of cerebral and meningeal blood vessels (Figure19).

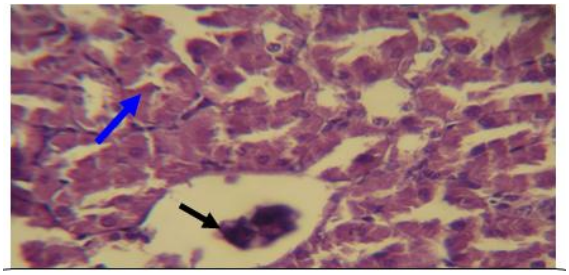
Treatment of chronic toxicity of kidneys with cilantro and/or garlic extracts: the kidneys showed perivascular lymphocytic cuffings of intertubular blood vessels in all doses seen at the one month period and continued to the end of experiment .Focal interstitial aggregation of mononuclear cells were clear in section treated with cilantro extract at a dose (0.012) for one month. In liver treated group with garlic and cilantro extraction showed dilation of sinusoids which infiltration with lymphocytes in all treated groups with formation of tiny aggregation within hepatic parenchyma especially in late stages of experiments. Early granulomas were seen in treated groups with both extracts within hepatic parenchyma and beside the congested blood vessels (figure20 and 21). Also in brain treated groups with both extracts showed focal gliosis at one month (Figure22). Groups treated with cilantro or garlic extract showed slight edema and congestion of cerebrum.



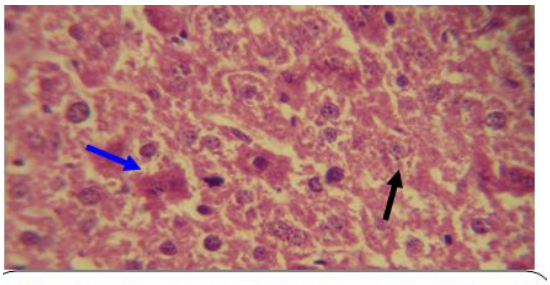
(Fig 5): Histopathological section of Kidney of rat treated orally with 13.5 mg/kg.bw /day of mercury chloride for one month showing diffuse coagulation type necrosis of cortical renal tubules ,appeared as solid and dense masses (➡) (H&E stain ×400).



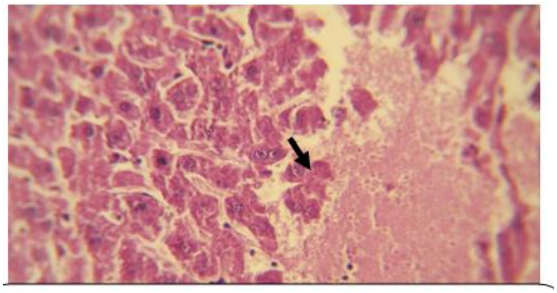
(Fig 6): Histopathological section of Kidney of rat treated with 13.5 mg/kg.bw /day of mercury chloride for one month showing large numbers of hyaline droplets within the proximal and distal convoluted renal tubules. These structures appeared as deep eosinophilic rounded or oval in shape (➡) (H& E ×400).



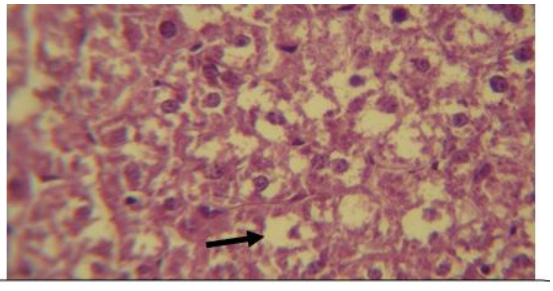
(Fig 7) :Histopathological section of liver of rat treated orally with 6.75 mg/kg.bw /day of mercury chloride for one month showing atrophy of glomerular tuft (➡) with cloudy swelling of cortical renal tubules (➡) (H&E stain ×400).



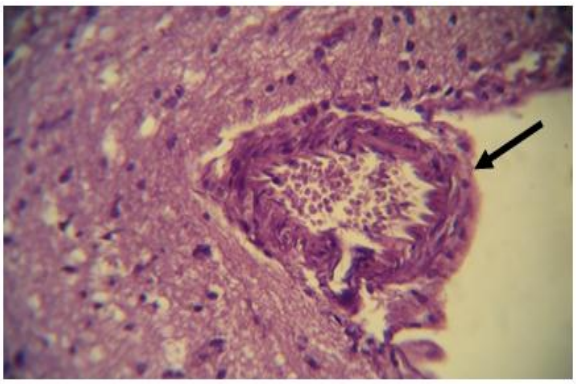
(Fig 8) :Histopathological section of liver of rat treated orally with 13.5 mg/kg.bw /day of mercury chloride for one month showing extensive areas of necrosis (➡) and apoptosis (➡) (H&E stain ×400).



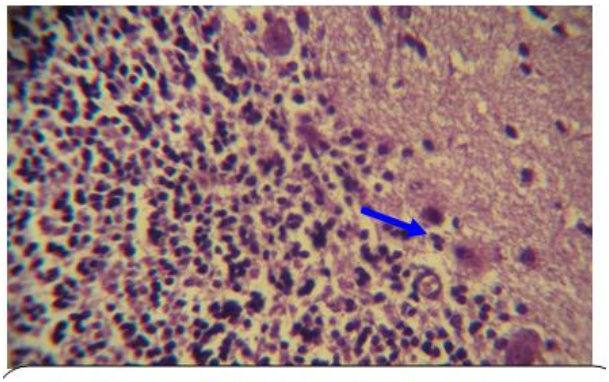
(Fig 9):Histopathological section of liver of rat treated with 13.5 mg/kg.bw /day of mercury chloride for one month showing severe destruction of hepatic parenchyma with oozing of blood to the necrotic area(➡) (H&E stain ×400).



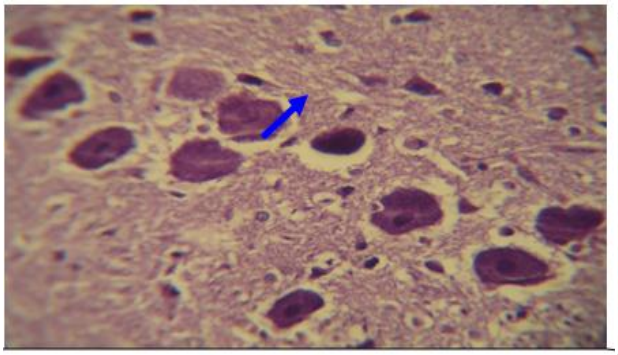
(Fig 10):Histopathological section of liver of rat treated orally with 6.75 mg/kg.bw /day of mercury chloride for one month showing vacuolation of hepatocytes (➡) the hepatic cells have large nuclei containing many nucleoli (H&E stain ×400).



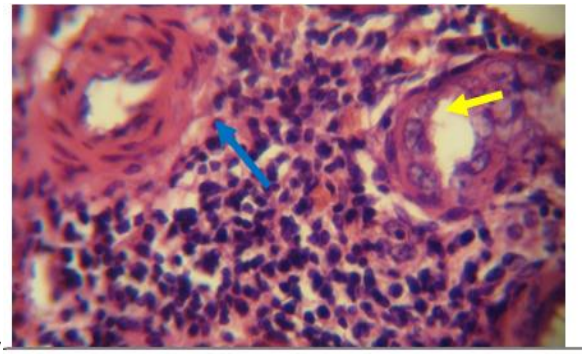
(Fig7):Histopathological section of cerebrum of rat treated orally with 13.5 mg/kg.bw /day of mercury chloride for one month showing severe dilation and congestion meningeal blood vessels (➡) (H&E stain ×400).



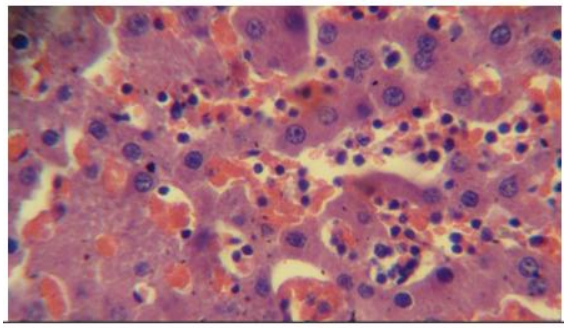
(Fig8): Histopathological section of cerebellum of rat treated orally with 13.5 mg/kg.bw /day of mercury chloride for one month showing edema between granular and molecular layer with degeneration of many purkinji cells with complete dissolution of the others (➡) (H&E stain ×400).



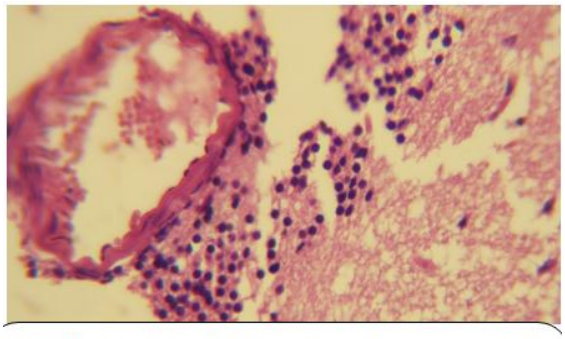
(Fig11):Histopathological section of cerebrum of rat treated with 6.75 mg/kg.bw /day of mercury chloride for one month showing shrinkage of neurons containing pyknotic nuclei with loss of Nissl substance (➡)(H&E stain ×400).



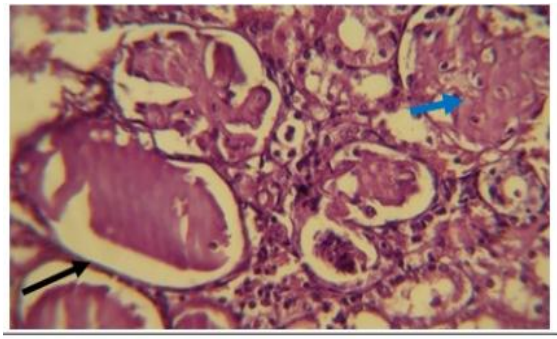
(Fig12): Histopathological section of kidney of rat treated orally with 50 mg/kg BW garlic extract and 25mg/kg.bw cilantro extract for one month period showing regeneration of epithelial cells lining the proximal (➡), with perivascular cuffing (➡) (H&E stain ×400).



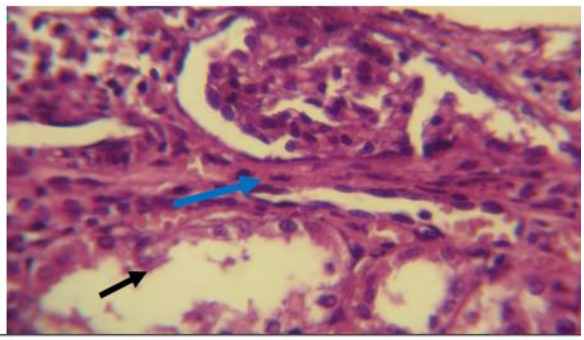
(Fig 13): Histopathological section of liver of rat treated orally with100 mg/kg.bw garlic extract for one month periods showing infiltration of large numbers of mononuclear cells in the dilated and congested blood sinusoids (H&E stain ×400).



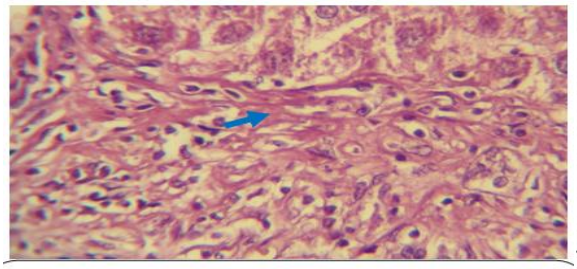
(Fig14) :Histopathological section of cerebrum of rat treated orally with 50 mg/kg BW garlic extract and 25mg/kg.bw cilantro extract for one month period showing perivascular lymphocytic cuffings of cerebral blood vessels (➡) (H&E stain ×400).



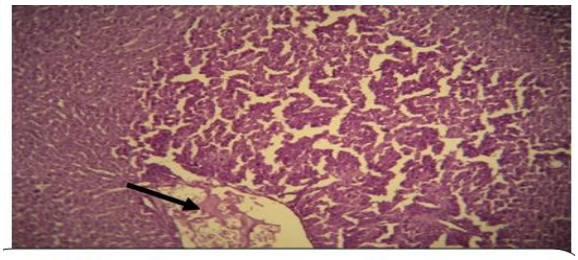
(Fig15) Histopathological section of kidney of rat treated orally with 0.018 mg/kg BW of mercury chloride for one month showing deposition of amyloid like substance in the glomeruli (➡) with hyaline cast in cystic dilated renal tubules (➡) (H&E×400).



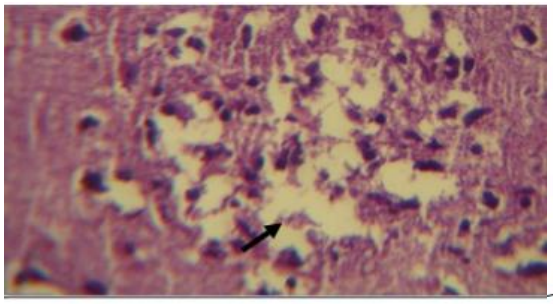
(Fig16) Histopathological section of kidney of rat treated orally with 0.018 mg/kg .Bw of mercury chloride for one month showing interstitial fibrosis leading to cystic dilation of renal tubules (➡) with thickening of Bowman's capsule (➡) (H&E stain ×400).



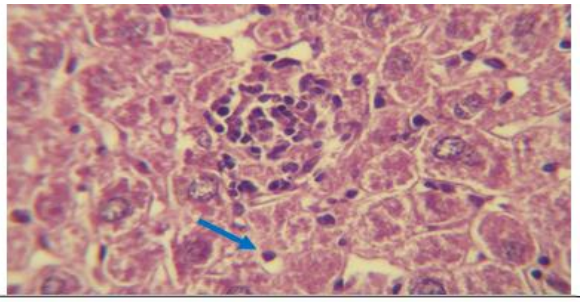
(Fig17)Histopathological section of liver of rat treated orally with 0.06 mg/kg BW of mercury chloride for three months showing moderate proliferation of fibrous connective tissue (➡)(H&E stain×400).



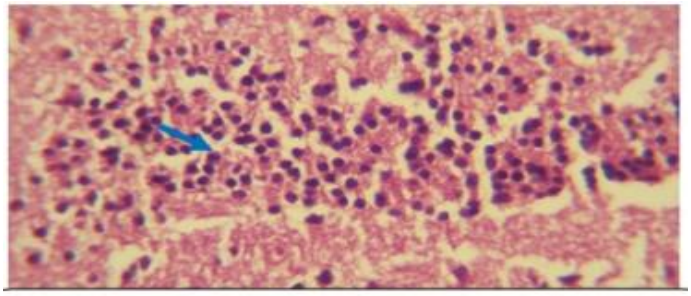
(Fig18)Histopathological section of liver of rat treated orally with 0.018 mg/kg BW of mercury chloride for three months showing formation of hyperplastic nodule lacking the central veins causing pressure atrophy to the adjacent hepatic parenchyma (➡) (H&E stain ×400).



(Fig19): Histopathological section of brain of rat treated orally with 0.018 mg/kg BW of mercury chloride for three months showing multiple areas of encephalomalacia (➡) (H&E stain ×400).



(Figu20) :Histopathological section of liver of rat treated orally with 50 mg/kg BW garlic and 25 mg/kg BW cilantro extracts for 30 days showing formation of early granuloma (➡) (H&E stain ×400).



(Fig21): Histopathological section of cerebrum of rat treated with50 mg/kg BW garlic and 25mg /kg BW cilantro extracts for one month showing focal gliosis (➡) (H&E stain ×400).

The microscopic findings of acute toxicity organs showed degenerative changes and severe coagulative necrosis of epitheial lining the renal tubules .the mechanism whereby mercury causes renal degeneration is controversial ,but many investingators believe it to be proximal excretion with some reabsorption in the distal tubuli ,it may be suggested that Hg^{+3}-sulfhydryl compounds , in which mercury deposition was ultra structurally detected. The kidneys are very sensitive to mercuric exposition (21) and that mercuric chloride – exposed rats had very serious renal disturbances that began only 15 days later (22).

Necrophosis of renal tubules leading to hyaline droplets and casts in the renal tubules Lumina .the significant change in the liver due to high dose of mercuric chloride was the centri –lobular coagulative necrosis which began centrally and progress peripherally in the lobule and it appears a constant feature of all examined section and that agreed with (19) but not agreed with (23) who stated that mercury does not induce severe necrosis to hepatic cells . We thought that these changes probably occurred due to accumulation of mercuric chloride in the mitochondria and lysosomes causing progressive hepatocyte organelle damage and cellular degeneration and necrosis, or it may result from hypoxia in the perivenular region with increase in hepatic oxygen demand without an appropriate in hepatic blood flows. the necrotic area forming agape leading to blood oozing to the necrotic area as seen in the microscopic sections .increase in apoptosis was due to depletion of all thiol reserves , which predisposes cell to reactive oxygen species (ROS) damage and at the same time , activities death – signaling pathways (24) at dose (6.75 mg/kg.BW) the therapeutic parenchyma showed diffuse minimal vacuolations and that due to toxic effects of mercuric chloride which are responsible for accumulation of fat in hepatocytes ,the edema of the brain could be related to the damage of the permeability of blood brain barrier (BBB) by mercuric chloride . (25) referred that BBB has a very important function in maintaining the fluid environment of nervous system , while other organs in the body transport molecules by simple method of diffusion , the BBB selects only contains and (essential amino acids , glucose , calcium , sodium , potassium) to be transported carriers in the plasma membrane , studies carried by (26 and 27) on rats indicated that mercury has neurotoxic effects reflected in its ability to penetrate and damage BBB system. Also ,electron – microscopic hitochemical analysis revealed that, intracellulary, mercury was bound to the membranes organelles such as mitochondria , endoplasmic reticulum, Golgi complex and nuclear envelopes(28) .

Degeneration and necrosis of purkinji cells, shrinkage of neurons was due to direct toxic effect of the compound or the role of mercuric chloride as anoxic poison (29) organs of chronic toxicity showed deposition of amyloid like substance deposition in the glomeruli of the kidneys. Amyloid of kidneys was reported in deferent chronic diseases or toxic substance(30) .this may be due to that the toxic causes over stimulation of immune cells leads to impair their function or influence the ability of monocytes to secrete enzymes that destruction the plasma AA (Amyloid- associated).The higher dose (0.018mg/kg.BW) showed interstitial fibrosis thinking of bowman's capsules and that because kidneys are very sensitive to Hg exposition (21) leading to glomerular damage (31). Liver sections showed fibrosis of hepatic parenchyma. Recent studies have identified macrophages as critical regulators of fibrosis. like myofibroblasts these cells are derived from bone marrow immigrants , and the pathogenesis of fibrosis is tightly regulated by distinct macrophage populations that extert unique functional activities throughout the initiation, maintenance ,and resolution phase of fibrosis (32) .Other important changes occurred in liver was the formation of hyperplastic nodules .this change was due to the fact that liver could maintains its regenerative ability and continous production of new hepatocytes or it might be mercuric chloride has a mutagenic activity reflected in its highly significant effects on cell cycle kinetics and frequency of chromosomal aberrations (33).the hypeplastic nodules were associated with some of the predisposing factors of primary hepatocellular carcinoma (34) . Focal encephalomylacia which noted in higher dose was due to necrosis of neurons and glial

cells and the pressure resulting from the edematous fluid (35). Concluded that chronic exposure to CNS ultimately causing permanent damage. Efforts have been done on use of dietary constituents as therapeutic agents capable of controlling or minimizing the effects of many toxic compounds. Cilantro and garlic was one of these agents (36 and 37). The present study showed that cilantro and garlic have been responsible for reducing or nullifying the injurious effects of $Hgcl_2$ in rats both in histopathological examination or in reducing the residual concentration of the compound in these vital organs especially when used together in addition to activation of immune response reflected by focal mononuclear cells infiltrations ,perivascular cuffings , formation of early granuloma in liver and focal gliosis in brain and that may be attributed to the active compounds like antioxidant alkaloids and flavoniods (38 and 39) explained that the flavinoids has a direct effect on glial cells by inducing activation of astrocytes and microglia and release of tumor necrosis factor alpha(TNF-a).Furthermore flavonoids are generally thought to be having antioxidant and free radical scavenging effects (40), in addition to neuroprotective actions (41).

Table (1): Residual of mercuric chloride in rats organs which treated orally with extracts of cilantro (50mg/kg.BW) and garlic (100 mg /kg.BW) for (4 weeks).

period / groups	4 weeks		
	Brain	**Liver**	**Kidney**
Control (–ve)	0.0002±0.0001	0.0001±0.0005	0.0002±0.0001
Control(+ve)1	60.66±0.27[a]	30.14±3.84[a]	92.12±1.45[a]
Cilantro 1	29.38±0.26[e]	17.56±0.17[e]	20.43±0.17[e]
Garlic 1	37.12±1.52[d]	20.11±8.81[c]	50.30±0.14[c]
Garlic and Cilantro 1	15.41±5.77[g]	9.13±2.08[g]	15.18±1.85[g]
Control(+ve)2	43.31±8.81[b]	25.22±1.45[b]	60.37±0.26[b]
Cilantro 2	18.10±5.77[f]	12.70±0.11[f]	17.23±0.13[f]
Garlic 2	38.32±1.45[c]	19.43±8.81[d]	42.19±8.81[d]
Garlic and cilantro 2	10.18±3.28[h]	7.68±8.81[h]	12.40±0.15[h]

Different small letters means significant (p<0.01) results between groups

Table (2): Residual of mercuric chloride in rats organs which treated orally with compounds with doses (0.006, 0.012, 0.018 mg/kg BW) for (12 weeks).
Different small letters means significant (p<0.01) results between groups

period / Group	4 weeks			8weeks			12weeks		
	Brain	Liver	Kidney	Brain	Liver	Kidney	Brain	Liver	Kidney
Control	0.0002 ±0.0001	0.0001±0.0005	0.0002±0.0001	0.0002±0.0001	0.0001±0.0005	0.0002±0.0001	0.0002±0.0001	0.0001±0.0005	0.0002 ±0.0001
0.006	60.26± 0.21c	30.31±5.77c	80.53±8.81b	70.33±8.81b	44.11±5.11a	93.74±3.05b	100.2±0.13a	55.17±6.36b	110.0± 9.28b
0.012	65.44± 3.05b	37.17±6.36b	83.12±1.52a	75.76±2.60a	45.75±2.51a	99.15±2.64a	101.8±0.37a	57.17±1.45a	112.30 ±0.17a
0.018	70.23± 0.13a	39.40±5.77a	122.47±0.11c						

Different small letters means significant (p<0.01) results between group

Table (3):Residual of mercuric chloride in rats organs which treated orally with extracts of cilantro (50mg/kg BW) and garlic (100 mg /kg BW) for (4 weeks) .

Gropes	4 weeks		
	Brain	Liver	Kidney
control	0.0002±0.0001	0.0001±0.0005	0.0002±0.0001
Cilantro 1	22.2±8.81 ^{d}	20.14±1.20^{b}	23.46±8.81c
Garlic 1	32.50±8.81^{c}	29.16±8.81^{a}	26.78±5.77^{c}
Garlic and cilantro 1	11.60±1.52^{f}	18.33±8.81^{b}	12.40±5.77^{d}
Control (+ve)1	58.53±0.33^{a}	35.53±0.21^{b}	62.70±5.77^{b}
Cilantro 2	40.20±5.77^{b}	30.73±0.12^{a}	42.78±6.99^{b}
Garlic 2	15.120±1.15^{a}	29.21±5.77^{a}	62.18±5^{a}
Garlic and cilantro 2	17.10±5.77^{e}	19.66±8.81^{b}	20.13±19.75^{c}
Control (+ve)2	57.76±57.62^{c}	35.72±.45^{b}	65.33±5.77^{a}

Different small letters means significant (p<0.01) results between groups

Although there was widespread distribution of mercury in the organs and tissues of the treated rats, the residual concentrations of mercury were not uniform, possibly owing to variations in the rates of accumulation and/or clearance of the residues from the individual tissues. The toxic effects of mercuric chloride are mainly in the kidneys as a result of acute and chronic toxicity explained. These results agreed with (19) they suggested that mercuric chloride –exposed rats had very serious renal disturbances that began only 15 day later this was stated that kidneys are very sensitive to mercury exposure. Cilantro can mobilize mercury and other toxic metals rapidly from the CNS, it is probably the only effective agent in mobilizing mercury stored in the intracellular space (attached to mitochondria, tubulin, liposome's …ect) and in the nuclear of the cell (reversing DNA damage of mercury). Because cilantro mobilizes more toxins, it can carry out the body, (42) Cilantro is especially useful for removing mercury from the brain as brain detoxification is one of the most difficult to achieve , the mobilized mercury appear to be either excreted via the stool , the urine or translocated into more peripheral tissue , this makes the cilantro the first known substance that mobilize mercury from CNC. The protective effects of garlic has been attributed to the two presence of organosulphur compounds like dialy sulphide (DAS) ,diallyl disulphide (DADS), ajoene, allixin, allyl mercaptans and allyl methyl sulphides (13). The (DADS) is one of the major volatile degenerative compounds of the garlic. Any studies on animals showed its protective effects against chemically induce toxicity (36).

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