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# The Effect of Ginger Plant (*Zingiber officinale*) Aqueous Extract on Function and Histological Structure of Kidney in Mice Treated with Carbon Tetrachloride

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**Abstract :** The percent work was designed to determine the effect of ginger plant aqueous extract on function and histological structure of kidney in mice treated with carbon tetrachloride ($CCl_4$). Ginger plant caused a protective effect against $CCl_4$ induced kidney damage and improved the kidney weight and biochemical parameters including urea, uric acid and creatinine. The ginger plant has a protective effect against injury in the kidney of mice treated with $CCL_4$, because the ginger plant protects the tissues of kidney from toxic effect of $CCL_4$. The kidney of $CCL_4$ treated mice showed many histological alterations in the kidney included: atrophy, vascular degeneration and hemorrhage, death cell, degeneration of epithelial cells, destruction of basement membrane and reduce of interstitial connective tissue.
**Key words :** Kidney, $CCl_4$, Urea.

## 1. Introduction:

The kidney is a vital organ present in vertebrates and some other animals. It has a wide range of functions such as the excretory organ and it filtered the blood to remove waste [1, 2].The kidneys regulate the electrolytes balance including: salt, acid-base and fluid and they are also responsible for the reabsorption of amino acids, glucose and water [3, 4]. During the development, three kidney systems are formed in cranial to caudal sequence: the pronephros, (It is rudimentary and nonfunctional), mesonephros (It may function for a short time during early fetal period) and metanephros (It forms the permeant kidney) [5, 6].

The kidneys were composed of renal tubules (nephrons), which developed from intermediate mesoderm (nephromeres) [7]. Mammalian kidneys were compact organ divided into a cortex and medulla [8, 9]. The cortex and medulla contain the nephrons, which can be considered the functional units of the kidney; in human, each kidney composed of 1 to 4 million nephrons[2, 3].

The medical plants act as antiradicals and DNA cleavage protector's phytochemical are compounds found in plants that have beneficial effect on health or play an active role in amelioration of diseases. Ginger (*Zingiber officinale*) is a plant common ingredient in Asian and Indian cuisine. It is used for its medical properties for centuries among many cultures for over 5000 years[10, 11].

Ginger has been used for centuries to reduce inflammation and treat inflammatory conditions and antioxidant effect [12]. Toxic chemicals cause the kidney damages, which are induced by oxidative damages. Carbon tetrachloride ($CCL_4$), a well-known model compound for producing chemical injury [13, 14].

$CCL_4$ is regarded as highly toxic, it is known animal carcinogen and potential human carcinogen, $CCL_4$ is a clear,nonflammable, heavy liquid that evaporates readily, producing sweat odor. Its chemical stability results in an atmospheric half-life of 30 to 100 years about 1% of the total $CCL_4$ found in the environment is dissolved in surface waters and oceans. The $CCL_4$ evaporates easily, most of it released to the environment, it can remain in air for several years before it broken down and small amounts of it are found in surface water. $CCL_4$ can enter the body through lungs, brain, liver, skeletal muscles, kidney, stomach, intestine and skin and accumulates in the body fat. The aim of this study was to elevate the effect of ginger plant treatment on $CCL_4$ in mice using biochemical and histopathological parameters.

## 2. Materials and Methods

Twenty-four adult male albino mice (*Mus musculus*), weighting about (26-32 g) and aged (50-90 day), obtained from Al-Nahrain University, were housed in plastic cages at (20-24˚C) temperature, 12 hour light/ 12 hour dark cycle. The animals feeding with laboratory diet and tap water *ad libitum*. The mice were divided randomly into experimental (18) and control (6) groups. The experimental group divided into three groups, were treated with aqueous ginger extract, $CCL_4$ and $CCL_4$ with aqueous ginger extract.

Ginger (*Zinger officiale*) plant was purchased from local market and botanical identification was confirmed at the herbarium of Baghdad University, preparation of aquatic extract of ginger, the root plant was dried and ground in an electric mill (10 gram of ginger by 100 ml distilled water).

$CCL_4$ was purchased from sigma-Aldrich Co. St. USA.A single dose of $CCL_4$ (diluted in olive oil 1:1) at (0.2% ml/kg) body weight (BW). All groups were injection intraperitoneal (IP) (0.1 ml) for each dose, the control group received single dose of normal saline at one day for ten days, while three experimental groups, the first group received single dose of aqueous ginger extract (500 mg/kg) at day for ten days, the second group received single dose of $CCL_4$ (0.2%) for two days, the third group received single dose of $CCL_4$ (0.2%) for two Days and then it received single dose of aqueous root extract of ginger plant (500 mg/kg) for seven days.

Mice of all groups were given ether anesthesia and the animals were scarified. The blood samples were collected by cardiac puncture, serum was collected for determine the urea, uric acid and creatinine[15,16].

The kidney was excised from animals and weighted and then fixed in 10% formalin for 24 hours [17] and washed by tap water. Paraffin wax methods employed in histological study according to the methods of Vacca (1985); Suvarn et al. (2013)[18,19].The collected data were analyzed by SPSS. The histological sections were observed by compound light microscope (Olympus, Japan).

## 3. Results

The current study is designed to demonstrate the effect of ginger plant (*Zinger officiale*) aqueous extract on kidney in albino mice (*Mus musculus*) treated with carbon tetrachloride ($CCL_4$), the results showed as following:

*** Weight Study:**

The $CCL_4$ treatment affected on the kidney tissues. It was found that significant decrease at ($P<0.05$) in kidney weight of $CCL_4$ treated mice 0.25±0.003) gm compared with control group where the mean weight of kidney in treated group with $CCL_4$ was (0.14±0.003) gmwile control group was (0.14±0.01) gm(Table 1, Figure 1). However, a significant increase at ($P<0.05$) was observed in the kidney weight of mice treated with $CCL_4$ + ginger plant compared with $CCL_4$ treated group where the mean weight of kidney in treated group with $CCL_4$+ginger plant was (0.21±0.01) gm while treated group with $CCL_4$ was (0.14±0.003) gm are presented in (Table 1, Figure 1).

**Table (1): The statistical analysis of the mean and stander error (Mean±S.E) of kidney weight in control and treated groups.**

| | Control group (Mean±S.E.) | Treated groups (Mean ±S.E.) | | |
|---|---|---|---|---|
| | | Ginger plant | $CCL_4$ | $CCL_4$+ Ginger plant |
| Weight of kidney (gm) | 0.25±0.01 | 0.23±0.002* | 0.14±0.003* | 0.21±0.01* |

*Significant differences (P<0.05)

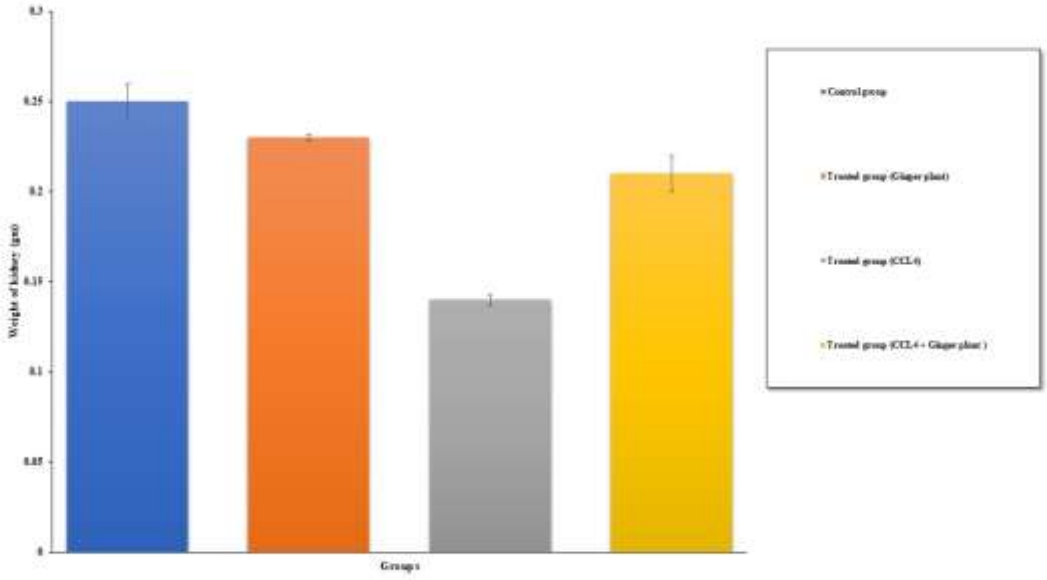

**Figure (1): The mean weight of kidney in control and treated groups.**

**Table (2): The statistical analysis of mean and stander error (Mean±S.E.) of level of urea, uric acid and creatinine in the serum blood of control and treated groups.**

| | Control group (Mean±S.E.) | Treated groups (Mean±S.E.) | | |
|---|---|---|---|---|
| | | Ginger plant | $CCL_4$ | $CCL_4$+Ginger plant |
| Urea (µmol/L) | 42.2±0.86 | 30.8±1.24* | 54.8±2.6* | 32.60±1.73*^ |
| Uric acid (µmol/L) | 2.15±0.04 | 2.02±0.04* | 3.42±0.02* | 2.25±0.02*^ |
| Creatinine (µmol/L) | 0.42±0.01 | 0.34±0.01* | 0.45±0.01* | 0.36±0.02*^ |

*^ Significant differences (P<0.05)

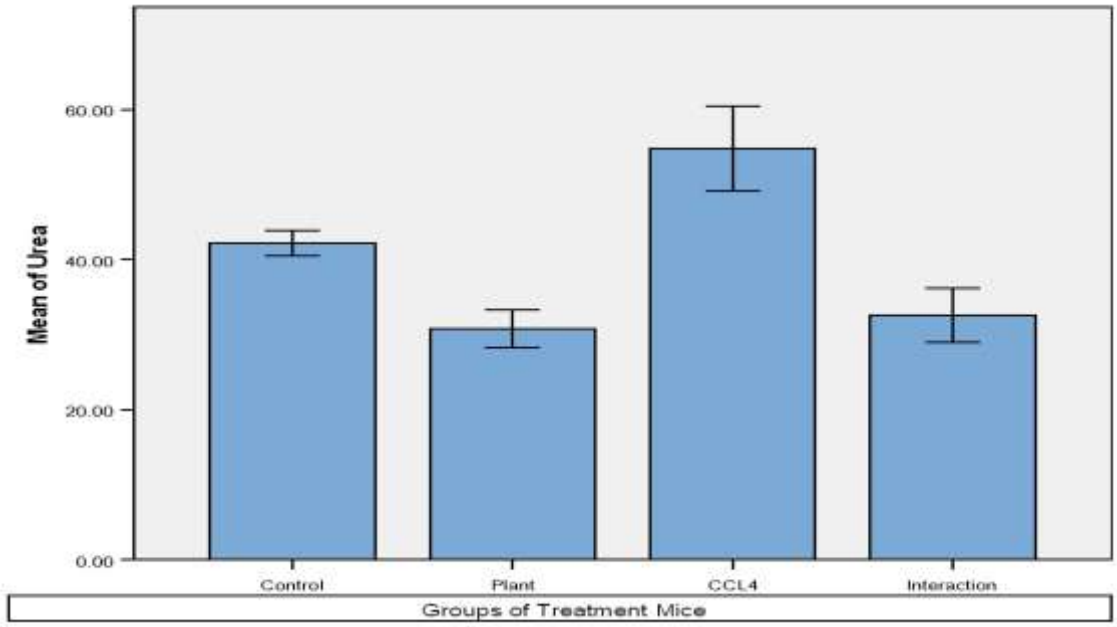


**Figure (2): The concentration of urea in the serum blood of control and treated mice.**

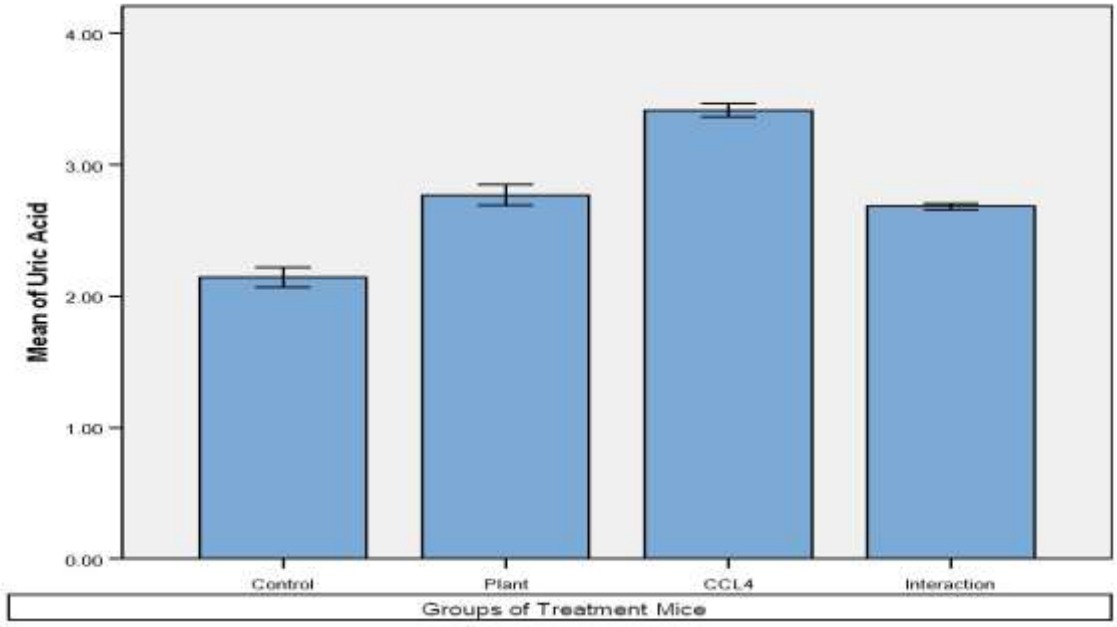


**Figure (3): The concentration of uric acid in the serum blood of control and treated mice.**

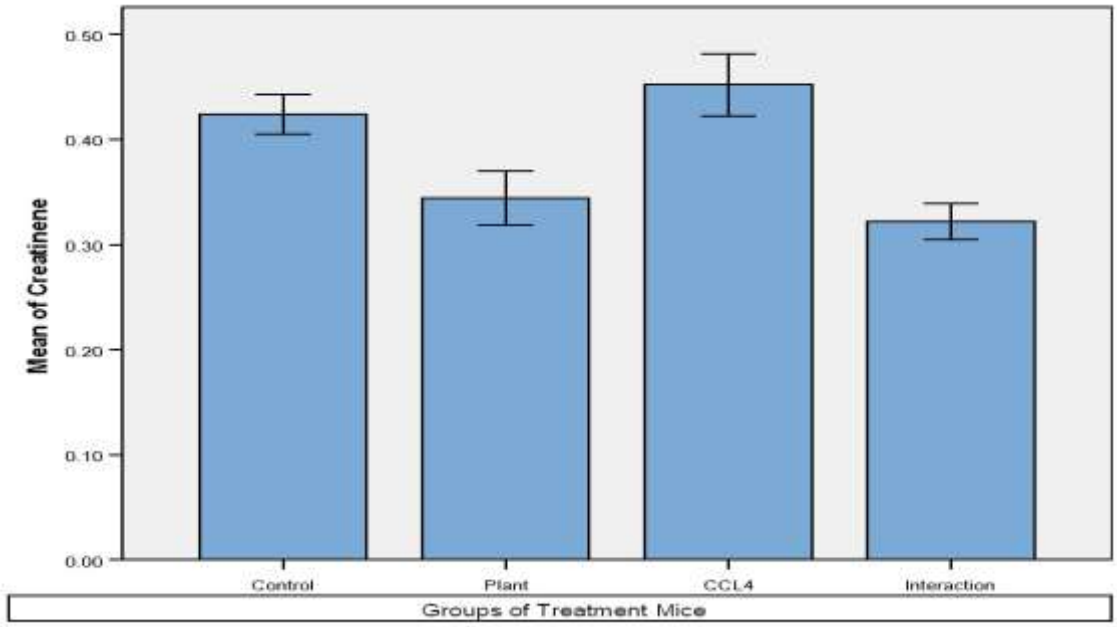


**Figure (4): The concentration of creatinine in the serum blood of control and treated mice.**

*** Biochemical Study**

In current study, the levels of urea, uric acid and creatinine were estimated in serum blood as the kidney function biomarkers. Urea and uric acid levels showed significant increase at ($P<0.05$) in the treated group with $CCL_4$compared with control group, where the mean levels of urea and uric acid in treated groups with $CCL_4$ were(54.8±2.6, 3.42±0.02 µmol/L) while control group was (42.2±0.89, 2.15±0.04 µmol/L) (Table 2, Figure 2, 3).Non-significantdifferences were noticed ($P<0.05$) in creatinine level of treated group with $CCL_4$compared with control group are presented in (Table 2, Figure 4).

Significant decrease at ($P<0.05$) was noted in urea, uric acid and creatinine levels in the $CCL_4$ +ginger plant treated mice compared with $CCL_4$ treated group, where the mean levels of urea, uric acid and creatinine in the treated group $CCL_4$ +ginger plant were (32.60±1.73, 2.35±0.02, 0.36±0.02 µmol/L) while $CCL_4$ treated group were (54.8±2.6, 3.42±0.02, 0.45±0.01 µmol/L) (Figure 2, 3, 4).

***Histological Study**

The microscopic observation of kidney sections of control group that the kidneys were located, one on either side of the vertebral column, the outer surface of the kidney was a layer of connective tissue called the renal capsule, this capsule covers the kidney.

The kidney composed of two regions: the outer layer, was cortex and the inner layer, was medulla (Figure 5). The cortex and medulla contain functional units (nephrons), which are represented by renal corpuscles (Bowman's capsules). The initial portions of a nephrons were the renal corpuscles (Bowman's capsules), which were located in the cortex of kidney. Bowman's capsule consists of double wall, the outer wall was parietal layer (capsular epithelium) and inner wall was visceral layer (glomerular layer). The epithelial tissue of Bowman's capsule was composed of simple squamous.

The thin cavity between parietal layer and visceral layer was the capsular space (Bowman's capsule) (Figure 6). The afferent arterioles form the glomerulus, which are formed a network of capillaries enclosed in Bowman's capsules (Figure 6).

The followed by renal corpuscle (Bowman's capsule) was renal tubule, that passes from the cortex to the deep part of medulla and called proximal convoluted tubule. The epithelial tissue lining of this tubule was composed of simple cuboidal epithelial tissue and its bearing a brush border in the free surface. The cytoplasm of the cells was light colored and the nuclei of cells were dark colored in central location (Figure 6).

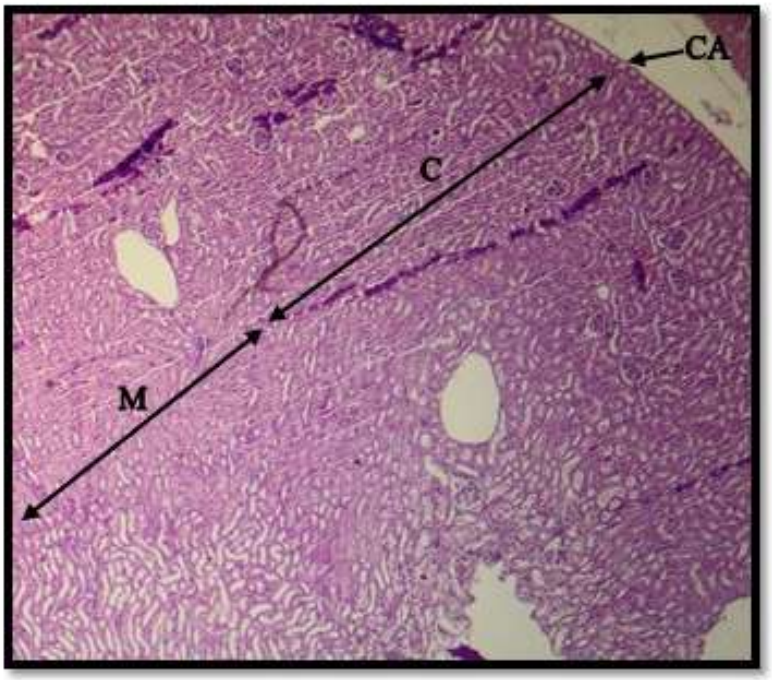


**Figure (5): Cross section through mice kidney of control group showing: Capsule (CA), Cortex (C), Medulla (M), (H&E, 4x).**

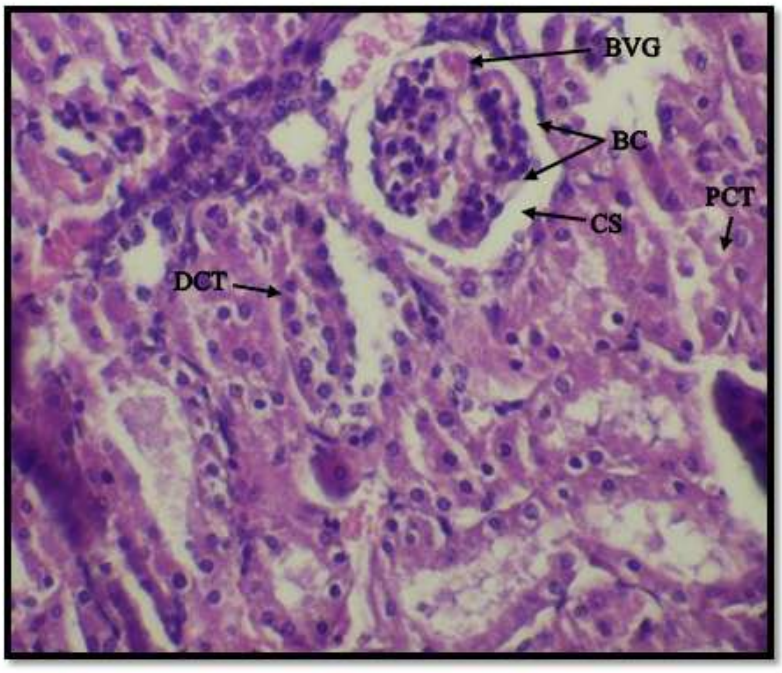


**Figure (6): Cross section through mice cortex kidney of control group showing Bowman's capsule (BC), Blood vessel glomeruli (BVG), Capsule space (CS), proximal convoluted tubule (PCT), distal convoluted tubule (DCT). (H&E, 40x).**

The distal convoluted tubule was formed from the thick segment of the ascending limb. The lining epithelial tissue of distal tubules was simple cuboidal. The cells contain nuclei in the central and dark-colored.

The lumen of distal convoluted tubule was larger than in proximal convoluted tubule. There was no brush border. The cells were more numerous than in proximal convoluted tubule (Figure 6).

The distal tubules were continuous with collecting tubules (connecting tubules). The cells of collecting tubules were simple cuboidal epithelial tissue and have nuclei and clear cytoplasm (Figure 7). The kidney of ginger plant only treated mice did not reveal any histopathological alternations (infiltration, congestion, enlargement of epithelial cells, atrophy and necrosis) (Figure 8).

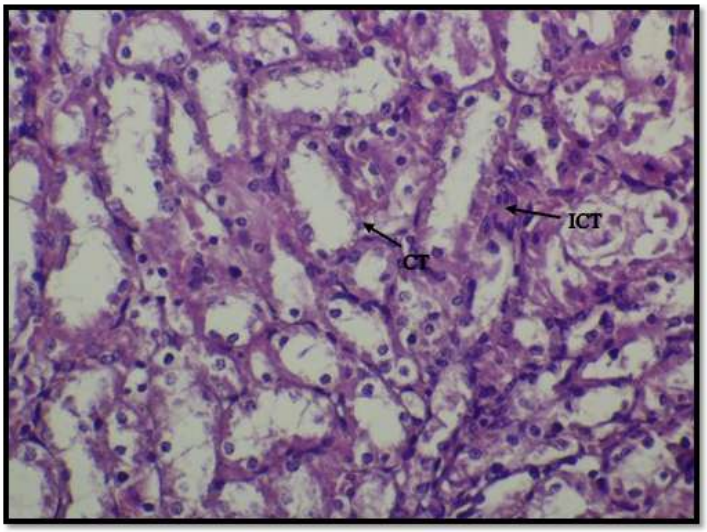


**Figure (7): Cross section through mice medulla kidney of control group showing collecting tubules (CT), interstitial connective tissue (ICT), (H&E, 40x).**

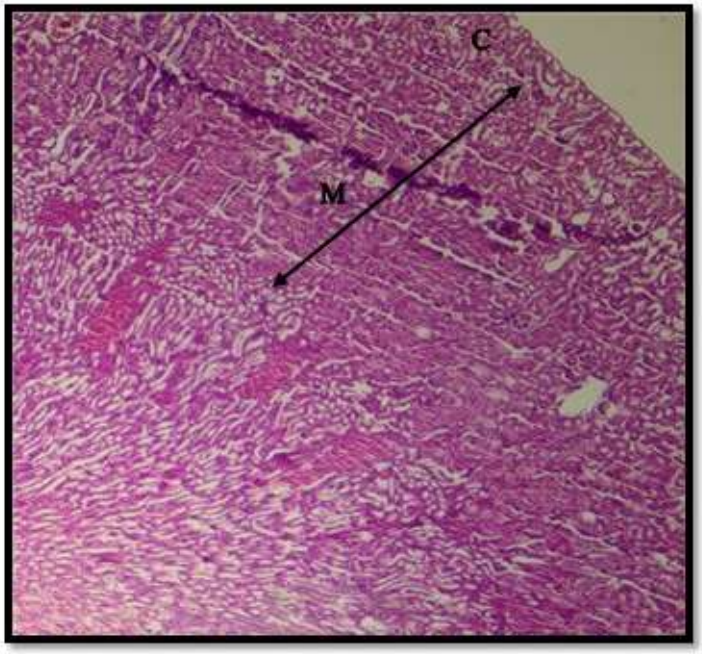


**Figure (8): Cross section through mice kidney of treated group with ginger plant showing normal cortex (C) and medulla (M) region (H&E 4x).**

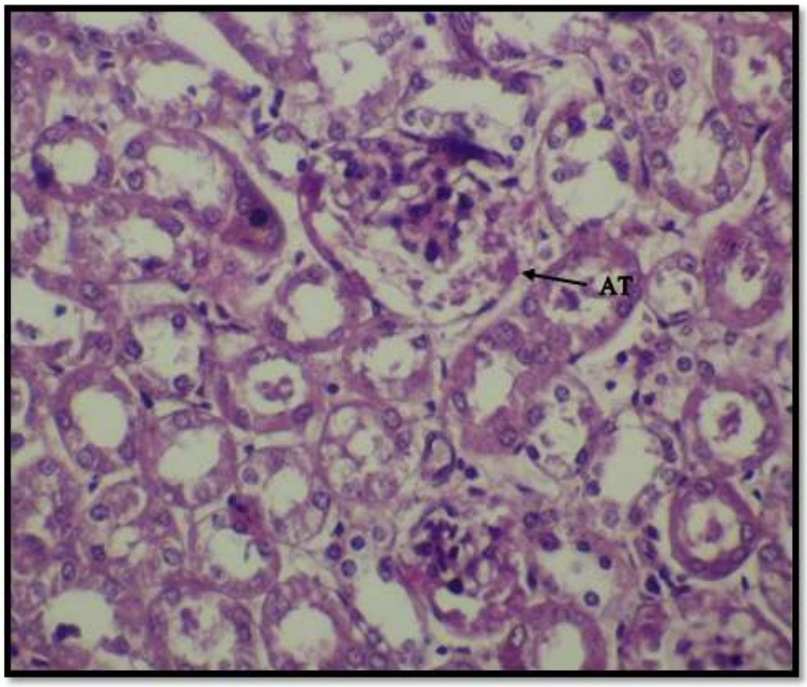


**Figure (9): Cross section through mice kidney of $CCL_4$treated group showing atrophy of glomerulus (AT) (H&E, 40x).**

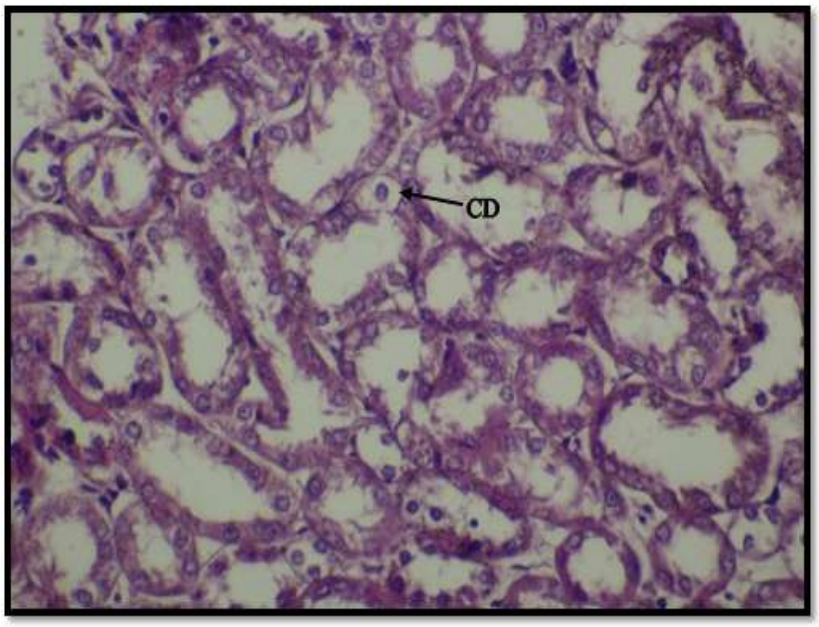


**Figure (10): Cross section through mice kidney of $CCL_4$ treated group showing cell death (CD) of convoluted tubules (H&E, 40x).**

The kidney of $CCl_4$ treated mice showed many histological alternations included atrophy of glomerulus and vascular degeneration and decrease in the diameter of glomerulus (Figure 9). Degeneration of epithelial cells and cell death of renal tubules (Figure 10).Thickening of epithelial cells layer of renal tubules (Figure

11).Destruction of basement membrane in convoluted tubule (Figure 11).Partial detachment of epithelial cells in convoluted tubule (Figure 11).Increase interstitial space with reduce interstitial connective tissue of kidney (Figure 11).

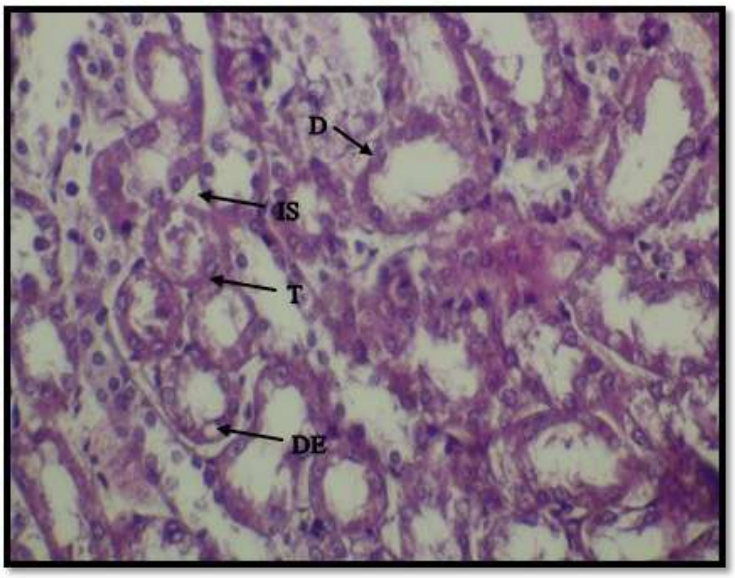


**Figure (11) : Cross section through mice kidney of $CCL_4$ treated group showing cell layer thickening (T) of convoluted tubules, destruction of basement membrane (D), detachment (DE) of epithelial cells in convoluted tubule and increase interstitial space (IS)(H&E, 40x).**

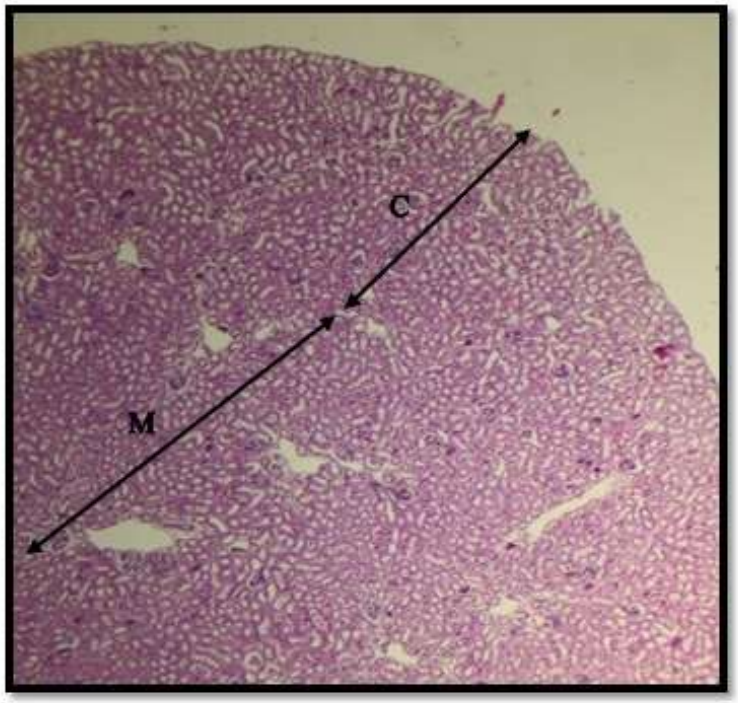


**Figure (12): Cross section through mice kidney of $CCL_4$+ginger planttreated group showing cortex (C) and Medulla (M) (AT) (H&E, 4x).**

Congested blood vessels and some of blood vessels were dilation in the kidney medulla of the treated group with $CCL_4$ and hemorrhage (Figure 12). The vascular degeneration in the treated group with $CCL_4$ compared with control, treated with ginger plant and $CCL_4$+ginger plant (Figure 12). The kidney of $CCL_4$+ginger plant treated mice showed clear recovery characterized, and the kidney sections showed intact glomerulesand normal convoluted tubules structure (Figure 12) when compared with control group (Figure 5, 6, 7).

## Discussion

In the present study, we investigated the effect of ginger aqueous extract ginger plant was reported to be present the increase in glomeruli damage. The preventive effect of ginger plant induced oxidative stress in the mice is due to its antioxidant properties. The ginger plant contains high concentrations of vitamins A, B, C and E and is very rich in magnesium and other trace elements, these vitamins act as antioxidants and preventing toxicant induced tissue injury [12]. $CCL_4$ is used as solvent for oils and fats, and widely used as a cleaning fluid (dry cleaning) and used in fire extinguishers and used as pesticide kill insects in grain, but this was stopped in 1986. $CCL_4$ is poisoning by the skin absorption, ingestion and inhalation. Inhalation of $CCL_4$ vaporous can depress central nervous system activity and cause degeneration of the kidneys [13, 14].

After analyzing different sections from control and treated groups animals by using compound light microscope, the result declared difference in the mice kidney associated with administration of $CCL_4$ in a dose (0.2% ml/kg) on albino mice (*Mus musculus*).

In the kidney of mice treated with $CCL_4$ induced severe changes, glomeruli look small, atrophied and loosely arranged in Bowman's capsule [20].

In the present work showed detachment of epithelial cells from basement membrane of convoluted tubules in mice kidney treated with $CCL_4$, this result agrees with Althnaian *et al.* (2013)[21] who pointed detachment of cells associated with impairment in meiosis [21,22].

In this study, it was found that destruction of basement membrane of the convoluted tubules this result agrees with Cordeiro and Kaliwal (2013)[20] who showed the similar change in basement membrane of convoluted tubules (Cordeiro and Kaliwal, 2013)[20].

Also, degeneration of proximal and distal tubules and interstitial connective tissue in mice kidney treated with CCL4, this result agree with Brattin et al. (1985)[13], who documented that the $CCL_4$ cause histopathological effect, represented by degeneration and destruction of interstitial connective tissue other studies suggest the cause of degeneration due to $CCL_4$ accumulation in lysosomes then rapture the cells and death or due to inhibition of protein synthesis [21].

Also, cell death in mice kidney treated with $CCL_4$, other studies documented that the cell death due to the DNA repair synthesis was detected, the $CCL_4$ is bio transformed to trichloromethyl free radicals ($CCL_3$) is considered the initial step in a chain of events, that lead to bind to lipids, contributing to the breakdown of cell structure and protein synthesis ad disrupting cell energy process lead to cell apoptosis (necrosis) [22, 23].

The histological sections showed congested blood vessels, which caused by inflammation that causes changes in blood flow and gets relaxation and dilation in the blood vessels that lead to blood accumulates in dilated capillaries and venules[24, 25].

The present study showed hemorrhage in mice kidney treated with $CCL_4$ this result agree with Ogeturk*et al*. (2005)[26] who revealed hemorrhage occur in the rat kidney.

Ginger root inhibit the induction of genes encoding cytokines and chemokines that synthesized and secreted at sites of inflammation, ginger reported to inhibit amyloid AB peptide induced cytokine and chemokine expression in cultured THP-1 monocytes [27]. The ginger inhibits platelet aggregation [28].

The kidney has a higher affinity to $CCL_4$ than liver due to the predominance of cytochrome p-450 in the renal cortex [20, 29]. The kidney is sensitive to $CCL_4$ leading to build up of water in the body and waste products in the blood causing kidney failure [29].The biochemical finding was confirmed by histological observation. The

changes include cellular necrosis, blood vessel damage and infiltration of inflammatory cells, atrophy and other histological changes which were consistent with the other authors [13, 20, 21].

Consistent with previous studies, the administration of $CCL_4$ induced a renal failure indicated by elevation of serum urea, creatinine and uric acid [30, 31].In the present study serum blood biomarkers, urea and uric acid levels were increased ($P<0.05$) and no significant change in serum creatinine in mice group with $CCL_4$ compare to mice control group.

This result agree with other studies Khan *et al*. (2009)[29] and Mbarki *et al*. (2016)[33,] and it disagree with Ogeturk *et al*. (2005)[26] in the level of serum urea, while this result agree with Althnaian *et al*. (2012)[32], and it disagree with Mbarki *et al*. (2016)[33]in the level of serum uric acid, the level of creatinine in this study agree with Khan and Al-Zohairy (2011) and Althnaian *et al*. (2012)[32, 34], while it disagree with Khan *et al*. (2009)[29] and Mbraki *et al*. (2016)[33], the increased serum blood level of renal markers have been attributed to the kidney injury [20].

The present study declared a significant decrease in weight of kidney mice treated with $CCL_4$ compared with control group, this decrease may be due to decrease mass of tissues lead to decrease weight of kidney.

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