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Nephroprotective Effect of Aqueous Extract of Parsley against Nephrotoxicity Induced by Carbon Tetrachloride in the Male Rats

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ABSTRACT

Background: *CCl_4 causes disorders in different body organs especially kidneys by generating free radicals. High exposure to CCl_4 can cause kidney damage. It produces renal diseases in human. Metabolism of CCl_4 involves in the production of free radicals through its activation by drug metabolizing enzymes located in the endoplasmic reticulum. Parsley has antioxidant, anti-inflammatory, anticancer activities, and probable immune boosting properties make it relevant in the traditional treatment of urinary tract infection, nephritis, and cystitis.*

Objectives: *The present study aimed to investigate the protective effects of oral administration of aqueous extract of parsley against the histopathological and biochemical alterations induced in the kidney by CCl_4 of male rats.*

Materials and Methods: *24 male rats were used for this study and divided into 4 groups. The first group was control group injected intraperitoneally with olive oil (1ml/kg of body weight) three time/week, the 2nd was injected intraperitoneally with CCl_4 (1ml/kg of body weight) three time/week, the 3rd was injected intraperitoneally with CCl_4 (1ml/kg of body weight) three time/week concurrently with receiving aqueous Parsley leaves extract (5g/kg body wt/day) by gastric intubation's, the 4th was injected intraperitoneally with CCl_4 (1ml/kg of body weight) three time/week concurrently with receiving aqueous Parsley leaves extract (20g/kg body wt/day) by gastric intubation's, respectively for 4 weeks. The Kidneys were dissected out and specimens were taken and processed for light microscopic examinations. Blood samples were obtained for assessment of serum urea, creatinine, and uric acid.*

Results: *The results showed a significant increase in serum urea, creatinine, uric acid, K+, and Cl- concentrations and a significant decrease in serum Na+ concentration in rats treated with CCl4 compared with controls. Histopathological investigation of rats treated with CCl4 showed degeneration of epithelial lining and disruption of brush borders of the proximal convoluted tubules and presence of epithelial debris inside their lumens. The renal corpuscle appeared with degeneration of the glomerulus and disrupted Bowman's capsule. Extensive perivascular infiltration of inflammatory cells and massive interstitial hemorrhage were seen. Co-administration of Parsley with CCl4 significantly dose dependent improvement in the structural changes in the kidney and the serum urea, creatinine, uric acid, K+, and Cl- concentrations were significantly declined and serum Na+ concentration was significantly increased compared with CCl4 treated group.*

Conclusion: *It can be concluded that, carbon tetrachloride has adverse effects on the kidney, it caused a serious pathophysiological changes in the kidney. Parsley aqueous extract was able to protect the kidney against these effects. So, the persons expose to CCl_4 should be advised to take parsley aqueous extract.*

Keywords: *CCl4, Parsley, Nephrotoxicity, Nephroprotective, Renal pathophysiological changes, Histopathology*

INTRODUCTION

CCl_4 does not occur naturally, it is a clear liquid with sweet smell that can be detected at low levels [1, 2]. Volatile organic compounds such as CCl_4 are a class of solvents to which many people are exposed occupationally and environmentally [2]. CCl_4 was formerly used for metal degreasing and as dry cleaning, fabric-spotting, and fire extinguisher fluids, grain fumigant and reaction medium. Because of its

harmful effects, these uses are now banned and it is only used in some industrial applications [2, 3]. The primary routes of potential human exposure to CCl_4 are inhalation, ingestion, and dermal contact [2, 4].

A number of reports clearly demonstrated that CCl_4 causes disorders in kidneys, liver, lungs, testis as well as in blood by generating free radicals [2, 5, 6]. In addition, report on various documented case studies established that CCl_4 produces renal diseases in human [7, 8]. A number of endogenous and exogenous nephropathy risk factors generate oxygen free radicals *in vivo*. Therefore the role of oxygen derived free radicals and lipid peroxidation has attracted considerable attention [2, 9, 10]. Metabolism of CCl_4 involves in the production of free radicals through its activation by drug metabolizing enzymes located in the endoplasmic reticulum [2, 11]. High exposure to CCl_4 can cause kidney damage [2, 4]. CCl4 induces oxidative stress in many settings [12]; therefore, it might be expected to contribute to nephrotoxicity [2]. CCl_4 is one of such widely used environmental toxicant to experimentally induce animal models of acute nephrotoxicity and hepatic damages [2].

Medicinal plants and herbs play an important role in the prevention and treatment of kidney diseases. Parsley, a bright green biennial shrub is widely used traditionally as a food additive and herbal remedies for many ailments [13]. Parsley (*Petroselinum crispum*) is a member of Apiaceous family that has been employed in the food, pharmaceutical, perfume, and cosmetic industries [14]. Parsley leaves are used as culinary, garnishing and medicinal herb in the Mediterranean region of Southern Europe. Parsley extract was reported to produce a diuretic effect and good antioxidant activity [15, 16]. It has been reported that parsley has anti inflammatory and probable immune boosting properties make it relevant in the traditional treatment of urinary tract infection, nephritis, cystitis and prevention of renal stones formation. Parsley increases diuresis by inhibiting the Na+/K+ - ATPase pump in the kidney, thereby enhancing sodium and water excretion while increasing potassium resorption [16, 17]. It is one of the most used medicinal plants to treat renal diseases [18], diabetes, cardiac diseases, and arterial hypertension [19]. laxative activities [20], antioxidant [21], antihepototoxic, anti-hyperlipidemic, anti-hyperg lycemic [22], anticoagulant [23], antianemic, menorrhagic, and anti-microbial [24-26]. In popular medicine, parsley is used to treat various illnesses such as Alzheimer's disease, thrombosis and strokes. Parsley is widely employed against cardiovascular diseases [27, 28]. Also, parsley leaves were used for treatment of constipation, jaundice, colic, flatulence edema, rheumatism. It was used to treat lumbago, as a blood pressure regulator, impotence and nose bleed [29], eczema, knee, ache, impotence and bleeding [26, 29], nose bleeding, hematoma, skin blemishes due to it's bleaching properties, halitosis, ear ache, otitis, and as an emenagogue favouring menstruation and alleviating it's pains. It is also widely used as a galactofuge by lactating mothers to stop excessive milk production. Large amount can have uterotonic effect and therefore it's use is contraindicated in pregnancy and ingestion of more than 10 drops a day of the oil may cause abortion [30, 31].

Parsley is a power house of nutrition, rich in tocopherol and vitamin A [22, 28, 32]. It contains starch, vitamins B, C, β-carotene and zinc. It is a good source of iron, calcium, phosphorous and antioxidants like luteolin, vitamin C, vitamin A and zinc and these may likely account for it's hepato protective effect [33, 34]. Phytochemically, the leaves and seeds of *P. crispum* has been shown to contain high levels of essential oil known as apiole, while the tender buds contain psoralen and related compounds that can induce photosensitivity and these include xanthotoxin, ficusin, bergapten, majudin, heraclin and antimicrobial furocoumarins namely 8-methoxypsoralen, 5-methoxypsoralen, oxypuecedanin, isopimpinellin, 6'-acetylopin and a new monoterpene glycoside [29, 35, 36]. Besides having significant nutritional value, parsley also exhibits antioxidant and neutralizing properties [28, 37]. The constituents of parsley which include ascorbic acid, carotenoids, flavo-noids, coumarins, apiole, various terpenoic compounds, phenyl propanoids, phthalides, furano coumarins, and tocopherol, have been chemically investigated. Phytochemical screening of parsley has revealed the presence of several classes of flavonoids [38]. Flavonols (kaempferol and quercetin) and flavones (apigenin and luteolin), which occur as glycosidic form in nature, are major flavonoids found in parsley (*Petroselinum crispum*) and other apiaceous vegetables [39]. Kaempferol and quercetin, which belong to flavonol group, possess a wide range of biochemical and pharmacological effects and have been recommended as chemopreventive agents or nutritional supplements [40, 41]. The predominant

mechanism of their biological actions is thought to result from antioxidant activity, enzyme inhibition, and the capacity to scavenge free radicals [40, 42]. Therefore, it is speculated that the health promoting effect of parsley (*Petroselinum crispum*) may be due to its flavonol constituents and the content of flavonoid compounds in parsley is about 100 mg/100 g fresh weight [43].

Parsley is rich with an antioxidant arsenal that includes luteolin, flavonoid that searches out and eradicates free radicals in the body that cause oxidative stress in cells [28, 44]. Components of fresh parsley leaf scavenge superoxide anion in vitro [45], and methanol extracts of parsley scavenge hydroxyl radical [38]. Supplementation of diets with fresh leaf can increase antioxidant capacity of rat plasma [46] and decrease the oxidative stress in humans [21]. It has been reported that parsley alcoholic extract has a protective effect against toxicity induced by sodium valproate in male rats [47]. Parsley leaves are rich in Apigenin and its glucosidal flavonoids that were found to possess anti-inflammatory especially for renal inflammation; antioxidant and anticancer activities [48, 49]. Aqueous extract of parsley reduced the number of calcium oxalate deposits and therefore parsley can be used for kidney and bladder stones [50].

Antioxidants such as melatonin, ascorbate, α-tocopherol, silibinin, lazaroid, propinonyl carnitine and superoxide dismutase/catalase, have been shown to ameliorate CCl4-induced renal toxicity [2, 51]. However, according to our knowledge, the reported literatures on the protective effect of parsley against CCl_4 nephrotoxicity are still limited. Therefore, it has become a task to prevent nephropathy damage induced by CCl_4 by eliminating free radicals and prevent lipid peroxidation with a natural antioxidant rich plant like parsley. So, the aim of this study was to investigate the protective effects of oral administration of aqueous extract of parsley against the histopathological and biochemical alterations induced by CCl4 in kidney of male F-344/NHsd Fischer rats.

Materials and Methods

Materials

Chemicals

Carbon tetrachloride (CCl4) was purchased from Sigma Aldrich, St Louis, MO. It was applied as 1ml/kg body weight of CCl4 suspended in olive oil (1:9 v/v) i.p three a week for 4 weeks as a modified method to the method of Marsillach et al., [52] and El-Baz et al., [53]. Other chemicals were xylene, formalin10%, ethanol (absolute alcohol), chloroform, purified water, and paraffin wax. All these chemicals were obtained from the Chemistry department of the Faculty of Science – Zawia.

Plant Materials

The plain leaf parsley type daily collected from a vegetable market in Zawia (Libya), carefully washed under tap water and left to dry at room temperature. Then they were weighted and completely blended in distilled water (1:1w/v). Animals were received Parsley juice orally (Haidari *et al.*, [54] by gastric intubation's at two doses of 5g/Kg parsley or 20g/ kg/day for six weeks

Animals

24 male F-344/NHsd Fischer rats (14-15 weeks, 200±25gm) were used in this study. Rats were purchased from Animal Welfare House of Libyan National Medical Research Centre, Zawia, Libya. They were housed in plastic cages, 6 rats per cage, and left one week for acclimation, before commencing the experiment. The rats were kept in a room under standard conditions of ventilation, temperature (25 ± 4°C), humidity (65 ± 5 %) with light/dark cycle. The animals were provided with tap water *ad libitum* and feed with the standard commercial chow. The animal procedures were performed by Guidelines for Ethical Conduct in the Care and Use of Animals.

Methods

Experimental Design

After one week of acclimation, the animals were randomized and divided into four groups (6 rats for each) as follow:

- Group I (control group): This group included 6 animals that was provided with tape water and fed with a normal diet and injected intraperitoneal with 1 ml/kg body weight of olive oil for 4 weeks.

- Group II (CCl_4 group): The animals were injected intraperitoneal with 1 ml/kg body weight of CCl_4 three a week for 4 weeks.

- Group III (CCl_4/ (5g/kg) Parsley leaves extract co-administration): The animals were injected intraperitoneal with 1 ml/kg body weight of CCl4 three a week concurrently with receiving aqueous Parsley leaves extract (5g/kg body wt/day) by gastric intubation's for 4 weeks.

- Group IV (CCl_4/ (20g/kg) Parsley leaves extract co-administration): The animals were injected

intraperitoneal with 1 ml/kg body weight of CCl_4 three a week concurrently with receiving aqueous Parsley leaves extract (20g/kg body wt/day) by gastric intubation's for 4 weeks.

Blood Sampling

At the end of the experiment and 24 hours after the last dose, all animals were anaesthetized with ether and blood samples were collected by heart puncture. The samples were collected in a clean dry tube and centrifuged at 3000 rpm for 15 minutes then, the serum was separated and kept in a deep freezer at -20◦C until biochemical measurements were carried out.

Biochemical Analysis

Serum urea measurement was based upon the cleavage of urea with urease [55]. Serum uric acid was determined according to Fossatti *et al.*, [56]. Serum creatinine was measured without protein precipitation [57]. Sodium and potassium ions levels were estimated spectro photometrically using commercial kits by the modified method described by Maruna and Trinder [58].

Histological Preparation

At the end of the experimentation and 24 hours after the last dose, all animals were anaesthetized with ether. The animals were immediately dissected, and small pieces of the kidney were quickly removed and fixed in formalin fluid. After fixation specimens were dehydrated in an ascending series of alcohol, then were kept in terpineol for three days to ensure complete dehydration and clearing purposes. Cleared specimens were rinsed in three changes of xylol before embedding in paraffin wax (m.p.56-58°C). Three sections of 5 microns thick were taken from each kidney sample, each being at a distance of at least 500 microns from the proceeding one and mounted on clean slides without an adhesive medium. The sections were stained with Hematoxylin and Eosin and examined under a binocular light microscope [59].

Statistical Analysis

Results were expressed as mean ± SD, Data were analyzed by one way ANOVA. The difference between means ± SD was tested at $P < 0.05$ using Duncan's multiple range test. In all statistical tests, the probability level of $P < 0.05$ was considered significant.

RESULTS

The results showed a significant (*P<0.01*) increase in serum urea, creatinine, uric acid, K+, Cl- and concentrations and a significant decrease Na+ concentration in rats treated with CCl4 compared with control group. Co-administration of Parsley with CCl_4, a dose dependent, significantly (P<0.01) decreased serum urea, creatinine, uric acid, K^+, and Cl^- concentrations and increased serum Na^+ concentration compared to CCl_4 group (Table.1& Figure.1-6).

Table1. *Effect of treatment of male rats with CCl_4 and aqueous extract of parsley on serum urea, creatinine, uric acid, Na^+, K^+, and Cl^- concentrations*

Parameters	Groups			
	Control	CCl_4	CCl_4 + 5g/kg Parsley (CCl_4+5P)	CCl_4 + 20g/kg Parsley (CCl_4+20P)
	Mean + SD	Mean + SD	Mean + SD	Mean + SD
Urea (mg/dl)	37.67 ± 1.03	50.67 ± 5.47**	42.83 ± 0.75**##	38.33 ± 1.37##
Creatinine (mg/dl)	0.37 ± 0.01	0.71 ± 0.09**	0.45 ± 0.02*##	0.40 ± 0.01##
Uric Acid (mg/dl)	1.85 ± 0.10	3.35 ± 0.26**	2.56 ± 0.14**##	1.96 ± 0.13##
Na^+ (mmol/L)	140.27 ± 1.20	128.47 ± 6.78**	140.00 ± 0.91##	142.17 ± 0.42##
K^+ (mmol/L)	4.80 ± 0.40	8.01 ± 0.79**	6.11 ± 0.45**##	5.41 ± 0.22##
Cl^- (mmol/L)	101.72 ± 2.26	106.30 ± 0.92**	106.23 ± 0.46**	105.13 ± 1.01**

*All data are mean of 6 individuals. *: Significant differences as compared with control group (P < 0.05); **: Significant differences as compared with control group (P < 0.01); ##: Significant differences as compared with control group (P < 0.01).*

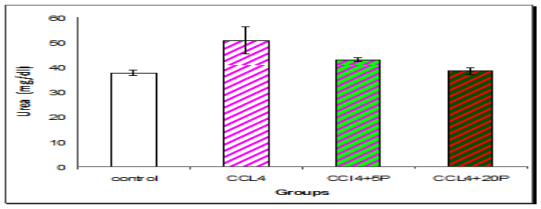

Figure1. *Effect of treatment of male rats with CCl4 and aqueous extract of parsley on serum urea concentration*

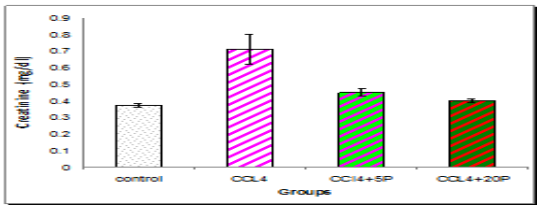

Figure2. *Effect of treatment of male rats with CCl4 and aqueous extract of parsley on serum creatinine concentration*

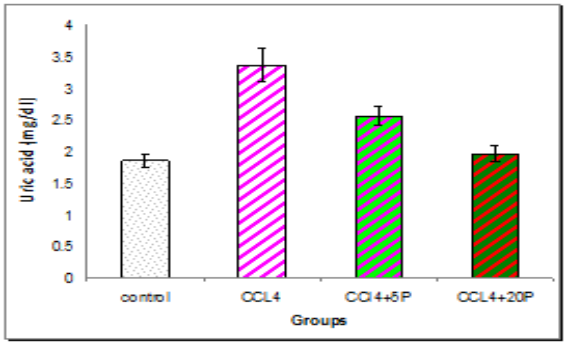

Figure3. *Effect of treatment of male rats with CCl4 and aqueous extract of parsley on serum uric acid concentration*

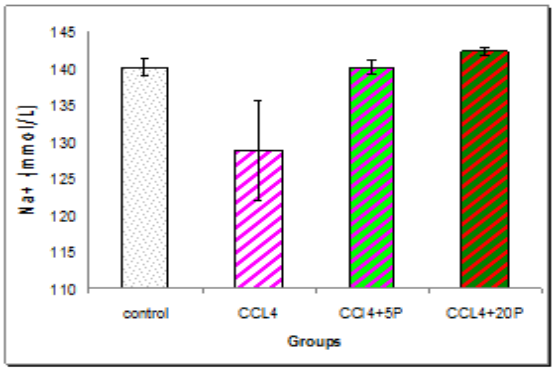

Figure4. *Effect of treatment of male rats with CCl4 and aqueous extract of parsley on serum Na+ ions concentration*

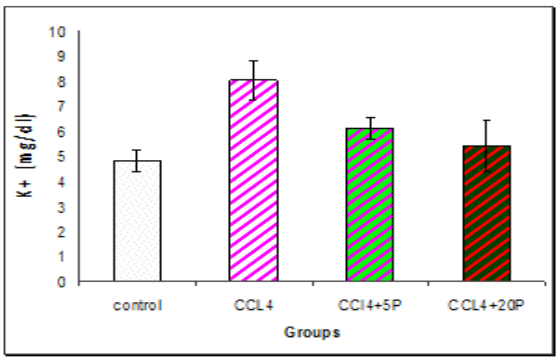

Figure5. *Effect of treatment of male rats with CCl4 and aqueous extract of parsley on serum K+ ions concentration*

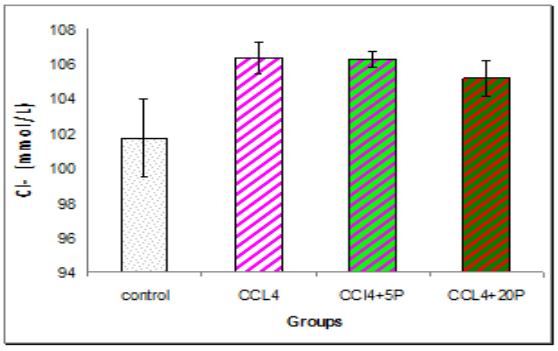

Figure6. *Effect of treatment of male rats with CCl4 and aqueous extract of parsley on serum Cl- ions concentration*

Histologically, by light microscopic examination, the kidney appeared with normal structures in normal control animals. The glomerulus appeared normal with intact Bowman's capsule and Bowman's space. The proximal convoluted tubules appeared with intact limiting basement membrane and their lumens showed intact brush borders. The distal convoluted tubules showed wide lumens with low cuboidal lining cells. An afferent arteriole appeared near the glomerulus (Fig. 7).

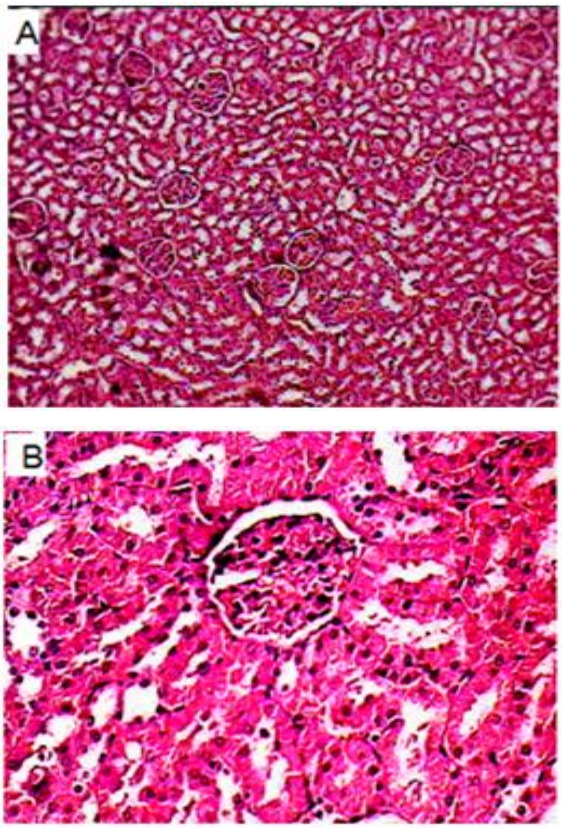

Figure7. *Light micrograph of sections in the kidney of control rats (administered olive oil) showed the glomerulus appeared normal with intact Bowman's capsule and Bowman's space. The proximal convoluted tubules appeared with intact limiting basement membrane and their lumens showed intact brush borders. The distal convoluted tubules showed wide lumens with low cuboidal lining cells. An afferent arteriole appeared near the glomerulus. (Haematoxylin &Eosin; A: ×100& B × 400).*

In CCl4 treated rats, the proximal convoluted tubules showed degenerated epithelial lining with disruption of their brush borders and presence of epithelial debris inside their lumens. The renal corpuscle appeared with degeneration of the glomerulus and disrupted Bowman's capsule. The afferent arteriole appeared near the glomerulus with thickening in its wall and degeneration of endothelial lining. Extensive perivascular infiltration of inflammatory cells

and massive interstitial hemorrhage were seen (Fig. 8).

In animals co-administered with CCl4 and 5g/kg body weight of Parsley aqueous extract, most of the proximal convoluted tubules appeared with normal epithelial lining with their brush borders, but some tubules appeared regenerating with disrupted brush borders. The glomerulus appeared more or less normal with intact Bowman's capsule. Massive interstitial hemorrhage was seen (Fig. 9).

In animals co-administered with CCl4 and 20g/kg body weight of Parsley aqueous extract, most of the proximal convoluted tubules appeared with normal epithelial lining and regained their brush borders. Some tubules still have diminished brush borders. The glomerulus appeared normal with intact Bowman's capsule (Fig. 10).

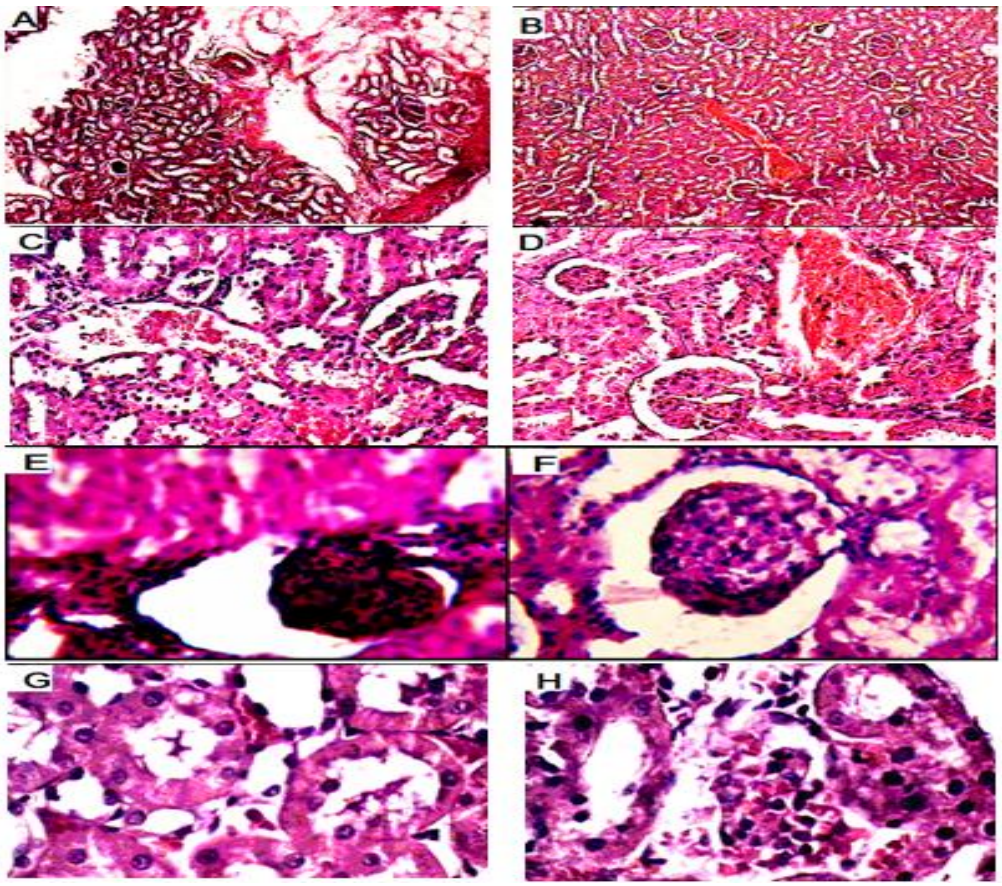

Figure8. *Light micrograph of sections in the kidney of CCl_4 treated rats. The proximal convoluted tubules showed degenerated epithelial lining with disruption of their brush borders and presence of epithelial debris inside their lumens. The renal corpuscle appeared with degeneration of the glomerulus and disrupted Bowman's capsule. Extensive perivascular infiltration of inflammatory cells and massive interstitial hemorrhage were seen. (Haematoxylin & Eosin; A&B × 100; C&D × 400; E-H ×1000)*

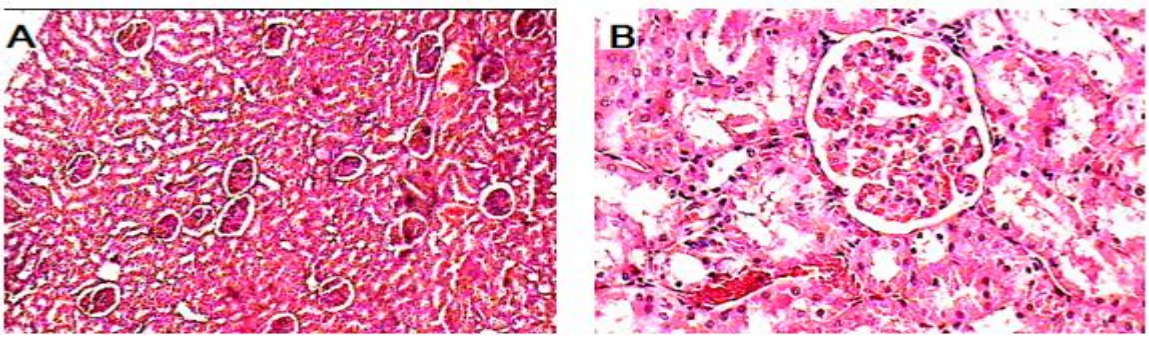

Figure9. *Light micrograph of a section in the kidney of CCl_4 treated rats co-administered 5g/kg body weight Parsley aqueous extract. Most of the proximal convoluted tubules appeared with normal epithelial lining with their brush borders, but some tubules appeared regenerating with disrupted brush borders. The glomerulus appeared more or less normal with intact Bowman's capsule. massive interstitial hemorrhage were seen (Haematoxylin &Eosin; A×100& B×400).*

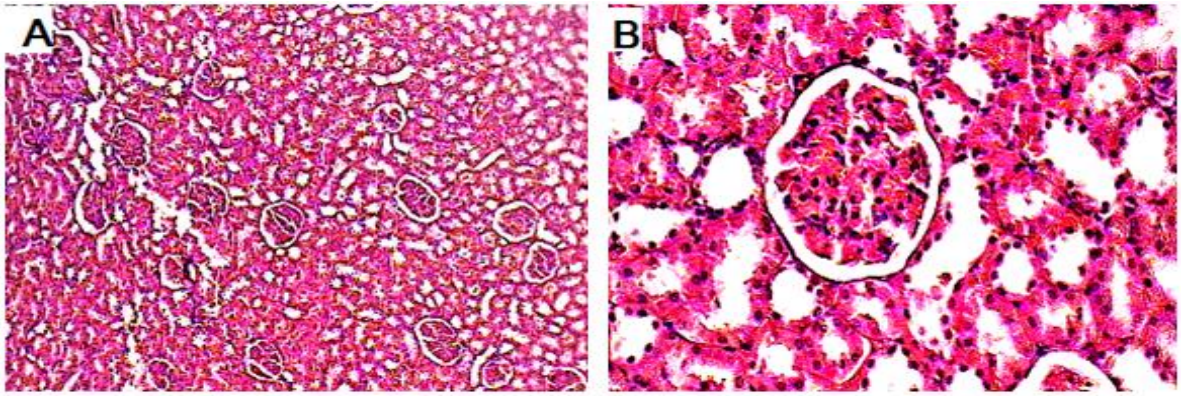

Figure9. *Light micrograph of a section in the kidney of CCl4 treated rats co-administered 5g/kg body weight Parsley aqueous extract. Most of the proximal convoluted tubules appeared with normal epithelial lining and regained their brush borders. Some tubules still have diminished brush borders. The glomerulus appeared normal with intact Bowman's capsule (Haematoxylin &Eosin; A×100& B×400).*

DISCUSSION

The results of the present study showed a significant increase in the level of urea, creatinine, and uric acid in the group treated with 1 ml/kg of body weight of carbon tetrachloride. These results were consistent with the results of Makni *et al.*, [60] who reported that intraperitoneally injection of rats with 1ml/kg body weight of CCl_4 caused a significant increase in plasma creatinine, urea and uric acid levels. Kidney histological sections showed glomerular hypertrophy and tubular dilatation in CCl_4-treated rats. Adewole *et al.*, [2] demonstrated that chronic administration of CCl_4 caused marked impairment in renal function. Serum creatinine and BUN concentrations were significantly higher in CCl_4- treated rats which are consistent with lower creatinine and BUN clearance. CCl_4 is metabolized by cytochrome P450 2E1 to trichloromethyl radical ($CCl_3^{\cdot}$). $CCl_3^{\cdot}$ and its highly reactive derivative, the trichloromethylperoxyl radical ($Cl_3COO^{\cdot}$), are assumed to initiate free radical-mediated lipid peroxidation leading to accumulation of lipid peroxidation products that causes renal injuries.

These radicals are capable of initiating a chain of lipid peroxidation reactions by abstracting hydrogen from polyunsaturated fatty acids. Peroxidation of lipids, particularly those containing polyunsaturated fatty acids, can dramatically change the properties of biological membranes, resulting in severe cell damage and play a significant role in pathogenesis of diseases [2, 61]. This phenomenon results in the generation of ROS, like superoxide anion $O^{\cdot}$, H_2O_2 and hydroxyl radical OH. Evidence suggests that various enzymatic and non-enzymatic systems have been developed by mammalian cells to cope with ROS and other free radicals [2, 62]. However, when a condition of oxidative stress establishes, the defense capacities against ROS becomes insufficient [2, 63]. Oxidative stress can promote the formation of a variety of vasoactive mediators that can affect renal function directly by initiating renal vasoconstriction or decreasing the glomerular capillary ultra filtration coefficient; and thus reducing glomerular filtration rate [2, 64]. In vitro and in vivo studies indicate that CCl_4 enhances lipid peroxidation, reduces renal microsomal NADPH cytochrome P450, and renal reduced/oxidized glutathione ratio (GSH/GSSG) in kidney cortex as well as renal microsomes and mitochondria [65].

In the present study, treatment of rats with CCl4 caused a serious histopathological changes in the kidney. The proximal convoluted tubules showed degenerated epithelial lining with disruption of their brush borders and presence of epithelial debris inside their lumens. The renal corpuscle appeared with degeneration of the glomerulus and disrupted Bowman's capsule. The afferent arteriole appeared near the glomerulus with thickening in its wall and degeneration of endothelial lining. Extensive perivascular infiltration of inflammatory cells and massive interstitial hemorrhage were seen. These results similar to those described in the study of Adewole *et al.*, [2] found that the histopathological alterations induced by CCl_4 in rats were glomerular hypercellularity, moderate to severe necrosis and tubule-interstitial alterations. Authors suggested that the capacity for tubular absorption may have been altered, thus bringing about functional overload of nephrons with subsequent renal dysfunctions. Also, Makni *et al.*, [60] reported that the Kidney of rats injected intraperitoneally with 1ml/kg

body weight of CCl_4 showed glomerular hypertrophy and tubular dilatation.

In the current study, co-administration of Parsley with CCl4, a dose dependent, significantly ($P<0.01$) decreased serum urea, creatinine, uric acid, K+, and Cl- concentrations and increased serum Na+ concentration and most of the proximal convoluted tubules appeared with normal epithelial lining and regained their brush borders. Some tubules still have diminished brush borders. The glomerulus appeared normal with intact Bowman's capsule, these results similar to those of the previous studies [66-69]. Shalaby and Hammoda, [66] repoted that treatment of rats with 80 mg/kg body weight (i.p) gentamicin for 10 days caused a significant increase in serum urea, creatinine, sodium and potassium, marked tubular necrosis in the kidney. But, rats pre-treated orally with parsley extract (100 and 200 mg/kg) for 6 weeks and intoxicated with gentamicin (80 mg/kg) during last 10 days of the experiment showed that a significant decreases in serum urea, creatinine, sodium and potassium, and increased activity of antioxidant enzymes. Also, the extracts increased urine volume and urinary excretion of Na+ and K+ electrolytes, denoting a diuretic effect. But, the small dose (100mg/kg) of parsley leaves extract caused a mild necrosis in renal tubules with protein casts in their lumen and the large dose (200 mg/kg) of parsley extract, caused only mild congestion of intertubular blood vessels. The mechanisms of nephroprotective effect may be due to inhibition of tissue lipid peroxidation and enhancement of antioxidant activity. The nephroprotective effect of parsley was attributed to the antioxidant activity due to its high content of flavonoids [66, 70]. Authors concluded that oral pre-treatments with parsley in gentamicin-nephrotoxic rats caused nephroprotective, diuretic and antioxidant effects as they reversed serum biochemical parameters and mitigated histopathological alterations in kidney induced by gentamicin in rats. These results affirm the traditional use of parsley for the prevention of kidney diseases. Elkhamisy, [67] evaluated that the nephroprotective, diuretic, and antioxidant effects of parsley leaves extract on gentamicin induced nephrotoxic rats. The results showed that gentamicin induced nephrotoxicity manifested by alterations in serum and urine biochemical parameters, increased tissue lipid peroxidation, decreased antioxidant activity and presence of renal tubular necrosis upon histopathology. Oral pretreatments with parsley leaves extract caused a nephroprotective effect evident by significant decreases in serum levels of urea, and creatinine in nephrotoxic rats. The extracts decreased serum sodium and potassium levels, and increased activity of antioxidant enzymes. Parsley leaves extract also increased urine volume and urinary excretion of Na+ and K+ electrolytes, denoting a diuretic activity and mitigated renal tubular necrosis induced by gentamicin.

The nephroprotective mechanisms of parsley leaves extract could be attributed to inhibition of lipid peroxidation and enhancement of antioxidant enzymes activity. Afzal *et al.*, [71] found that a polyherbal formulation containing parsley produced nephroprotective and diuretic effects in rats. Also, Mahmoud *et al.*, [69] investigated that the nephroprotective and antioxidant effects of parsley plant as watery extract of fresh leaves (decoction), fresh leaves, seeds and seeds oil against gentamicin-induced nephrotoxicity in male albino rats. The results showed that serum serum urea, uric acid, and creatinine were reduced significantly in rats groups received parsley products as compared to gentamicin treated group. These data suggests that parsley herb is effective in reduction of blood urea, creatinine and uric acid levels in rats with nephrotoxicity induced by gentamicin because parsley might has a diuretics effect. In addition, Khalil *et al.*, [68] confirmed that the protective effect of parsley (*Petroselinum crispum*) leaves oils against toxicity induced by carbon tetrachloride (CCl_4) in experimental rats. Results indicated that the main components in parsley oil were a-Pinene (26.6%) and Myristicin (20.3%). Kidney function tests for serum urea nitrogen, creatinine, and uric acid were found to be increased rats treated with CCl_4. Administration of 0.5 ml of parsley oil attenuated the adverse effects and biochemical alterations caused by CCl_4. Parsley oil have strong radical scavenging activity and antioxidant activity. Therefore, the results of this study show that parsley oil led to the protective effect against CCl_4 toxicity. In this study pretreatment with parsley leaves oil showed an increase in the activity of antioxidant enzymes compared to CCl_4 treated animals indicating the potentiality of parsley leaves oil to act as an antioxidant by preventing the peroxidative damage caused by CCl_4.

CONCLUSION

It can be concluded that, carbon tetrachloride has adverse effects on the kidney, it caused a serious pathophysiological changes in the kidney. Parsley aqueous extract was able to protect the kidney against these effects. So, the persons expose to

CCl_4 should be advised to take parsley aqueous extract. Our study recommended that the consumption of parsley plant parts may be useful for patients who suffer from renal diseases.

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