Role of *Petroselinum crispum* (Parsley) against gentamicin induced nephrotoxicity in albino mice

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Abstract: The present study was conducted to evaluate the beneficial effect of *Petroselinum crispum* (Parsley) against harmful effects and nephrotoxicity of gentamicin in albino mice. Healthy male albino mice were divided into 4 groups (n = 10 per group). The first group was considered as a control and received only distilled water, the second group was injected (i.m.) with gentamicin at dose level 80 mg/kg bw for a week, third group received orally aqueous extract of *P. crispum* only at dose level 40 mg/kg bw, while animals of the fourth group received aqueous extract of *P. crispum* and gentamicin. Blood samples were collected for assessment some biochemical markers of kidney functions (Urea, creatinine and total protein). The specimens of kidney were processed for histological study by light microscopy. No changes in biochemical marker was recorded in mice treated with aqueous extract of *P. crispum*. Intramuscular injection with gentamicin only caused an increase in the serum marker of kidney function comparing to control group. While, administration of *P. crispum* with gentamicin induced obvious ameliorating changes in all tested parameters and restored creatinine concentrations to nearly normal level. Examination of hematoxylin and eosin stained sections of mice treated orally with aqueous extract of *P. crispum* only showed normal architectural of kidney. While, marked glomerular and renal tubules lesions with congestion and dilation blood vessels as well as hemorrhage within interstitial tissue were noticed in kidney histological sections of gentamicin only treated group. Our results clearly demonstrate that oral administration of aqueous extract of *P. crispum* was accompanied by an improvement in the kidney tissue of mice injected with gentamicin. It is concluded that treatment with gentamicin for a week has harmful effects on the renal tissue of mice and consumption of aqueous extract of *P.crispum* should be recommended to lessened nephrotoxicity of gentamicin**.**

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Key words: Gentamicin, *Petroselinum crispum*, biochemical marker and histopathological kidney mice

1. Introduction

Gentamicin is aminoglycoside drug and it is one of the important antibiotics widely used to treat gram negative and positive bacterial infections **(Taha,1993; Salgado *et al*., 2007 and Ramhariya *et al*., 2015)**. It is still widely used and constitutes the only effective therapeutic alternative against serious microorganisms such as Pseudomonas, Proteus and Serratia that are insensitive to other antibiotics **(Balakumar *et al*., 2008; Alarifi *et al*.,2012; Corona *et al*.,2014 and Shrestha *et al*., 2014)**. Also, it is an effective drug against resistant bacterial strains to other antibiotics **(Salgado *et al*., 2007)**, but nephrotoxicity is a major side effect associated with gentamicin and has limited its therapeutic use **(Salgado *et al*., 2007 and Ramhariya *et al*., 2015).** Several other side effects have been reported by many investigators such as ototoxicity **(Rybak and Whitworth,2005)**, neurotoxicity **(Bischoff *et al*.,1977 and Paradelis *et al*., 1980)** and liver toxicity **(Aubrecht *et al*.,1997 and Nale *et al*.,2012)**. Nephrotoxicity and oxidative damage limits its long-term clinical use **(Alarifi *et al*., 2012)**. Also, gentamicin induced nephrotoxicity was reported in previous studies **(Erdem *et al*., 2000, Karahan *et al*., 2005; Babu *et al*., 2011; Chaware *et al*., 2011; Kore *et al*., 2011 and Sharma *et al*., 2011).** It has been estimated that up to 30 % of patients treated with gentamicin (aminoglycosides) for more than 7 days show some signs of nephrotoxicity **(Mattew, 1992 and Ali,2003)**. Nearly 10-25% of human patients treated with gentamicin exhibit increased blood urea nitrogen concentration subsequent to a reduction of glomerular filtration rate **(Kaloyanides, 1991 and DeBroe *et al*.,1989)**. The recommended routes of administration of gentamicin are intravenous, intramuscular, intraperitoneal or topical as it is not sufficiently absorbed by the intestinal tract **(Ali and Goetz,1997 and Qadir *et al*., 2011)**. **Ahmadvand *et al*. (2016)** concluded that gentamicin sulphate injection in rats for 12 days can lead to renal disorder. Gentamicin induced nephrotoxicity can cause renal injuries due to reactive oxygen species (ROS) generation **(Tavafi, 2013)**. Examination of renal sections of rats treated with

gentamicin at dose level 60 and 80mg/kg b.w for 10 days revealed many pathological lesions included more than half of proximal tubules showed complete or almost complete tubular necrosis with vacuolar degeneration of tubular epithelial cells and granular deposits in tubular lumens with evidence of tubular epithelial cell desquamation and lymphocytic infiltration around the proximal convoluted tubules with hyaline cast formation in proximal convoluted tubules, appearance of cells with alterations typical of apoptosis (cell shrinkage with eosinophilic cytoplasm and presence of a small and shrunken nucleus with chromatin condensation), interrupted tubular basement membrane, glomerular congestion, disruption of glomerular capillaries, atrophic half glomeruli and increase lymphocytic infiltration in cortical region **(Padmini and Kumar, 2012)**. Gentamicin nephrotoxicity is characterized functionally by an increase of serum creatinine, blood urea nitrogen, and decrease in glomerular filtration rate **(Romero *et al*., 2009)** which morphologically characterized by proximal tubule epithelial desquamation, tubular necrosis, tubular fibrosis, epithelial edema and glomerular hypertrophy **(Lakshmi *et al*.,2009**). **Baradaran and Rafieian-kopaei (2013**) reported that the rats treated with gentamicin showed renal histopathological injuries including epithelial cell vacuolization, degeneration, tubular cell flattening, hyaline cast, tubular dilatation, and debris materials in tubular lumen. The same authors reported that the gentamicin might be induced renal tubular damages via energy depletion in renal tubular cells besides inducing of oxidative stress and other mechanisms. Gentamicin renal cells damage manifested by tubular necrosis, congestion of the glomeruli with glomerular atrophy, degeneration of tubular epithelial cells with casts in the tubular lumen and infiltration of inflammatory cells in the interstitium was confirmed on histopathological examination by **Shirwaikar *et al*. (2003)**. Gentamicin induced nephrotoxicity increases the level of serum creatinine and urea, tubular necrosis and glomerular congestion but decrease glomerular filtration rate **(Nasri, 2012)**. Previous literatures indicated that gentamicin treatment results in many pathological lesions in form epithelial desquamation in proximal tubules, acute proximal tubular necrosis apoptosis in glomeruli and tubules, intracellular oedema, basement membrane interruption, glomerular narrowing of the Bowman's capsule and acute tubular necrosis degenerative changes in glomeruli with hypercellularity and also atrophy of the glomeruli, interstitial hemorrhage, filled lumens of the tubules with degenerated and desquamated cells, massive hyaline cast and severe inflammatory cell infiltrations which were prominent at many foci in both proximal and distal tubules **(Ali and Basher, 1994; Elfarra *et al*., 1994; Souza *et al*.,2008; Lakshmi *et al*., 2009; Lakshmi and Sudhakar,2010; Nale *et al*.,2012; Kang *et al*.,2013 and Sahu *et al*., 2014)**. Male albino mice treated intraperitoneally with gentamicin (80 mg/kg/day dissolved in 1 ml of distilled water) for fifteen days showed decrease in the kidneys and body weight and there was significant increase in mean serum urea, creatinine and intraluminal diameter of proximal convoluted tubules as compared to the control **(Qadir *et al*.,2011)**. **Alarifi *et al.* (2012)** reported that injected gentamicin at dose 80 mg/kg/day and 150 mg/kg/day for four consecutive weeks produced outstanding tubular, glomerular and interstitial alterations that included degeneration, necrosis, cytolysis and cortical tubular desquamation together with mesangial hypercellularity, endothelial cell proliferation and blood capillary congestion in rats. The loop of Henle showed very little or no alterations in rats treated with gentamicin **(Alarifi *et al*., 2012)**. Also the same author reported that severe tubular degenerative and necrotic changes over extensive areas were observed in the rats died at the end of the first week of treatment with gentamicin at 150 mg/kg/day. **Ullah *et al*. (2013)** observed loss of the cellular pattern with the presence of necrosis mostly in the proximal tubule in rabbit kidney treated with gentamicin. **He *et al.* (2015)** stated that the female rats injected intraperitoneal with gentamicin sulfate solution at a dose of 80 mg/kg body mass for 8 consecutive days showed marked deterioration of renal function. **El-Kashef *et al.* (2016)** reported that administration of gentamicin (100 mg/kg, i.p.) significantly increase kidney/body mass ratio, serum creatinine, lactate dehydrogenase, renal malondialdehyde (MDA), myeloperoxidase and tumor necrosis factor-alpha (TNF-α) compared to control group and it was found to induce renal injury by oxygen free radicals and lipid peroxidation. Orally administration of gentamicin at dose 80 mg/kg body weight for 7 days caused significant renal damage as evident by the rise in BUN levels, diminished glomeruli hypocellularity, moderately dilated tubules, and mild loss of brush border, severe infiltration, extensive tubular degeneration and presence of tubularcast in male mice. Also histochemistry results and immuno-histochemical reactions showed presence of collagen and reticular fibres and an increase apoptosis **(Aldahmash *et al*.,2016).**

Concerning 80% of the globe's populace is dependent on the exercise of conventional medication, which is commodiously supported on plant material **(Dahiru *et al*., 2006).** *Peteroselinum crispsum* (Parsley) is a bright green, biennial herb, which belongs to the family Apiaceae locally known as Baqdunis. It has been used medicinally for many

centuries in European, Mediterranean and Asian countries **(Vora *et al*.,2009; Awe and Banjoko,2013 and Allam *et al*.,2016)**. It is commonly used as a garnish in soups, salads, meats, vegetables, sauces, spice and herbal remedy **(Awe and Banjoko,2013 and Yanardağ *et al*.,2003).** Parsley has been employed in the food, pharmaceutical, perfume, and cosmetics industries **(Lopez *et al*.,1999)**. Traditionally, the leaf, seed and root of parsley are used in herbal medicine as enema, orally as tea to control high blood pressure, as tonic to strengthen the bladder, treatment of nose bleeding, halitosis, otitis, and menstruation pains **(Awe and Banjoko,2013)**. It has also been used in folk medicine as a diuretic, to treat eczema stomachic, jaundice, colic, rheumatism, diseases of prostate and liver **(Manderfeld *et al*., 1997; Kreydiyyeh *et al*., 2001 and Ozsoy-Sacan *et al*., 2006)**. It also used as antianemic, anticoagulant, antihyperlipidemic, antihepatotoxic, antioxidative, antimicrobial, and laxative **(Zhang *et al*.,2006; Wong and Kitts, 2006; Yanardağ *et al*.,2003; Kreydiyyeh *et al*.,2001 and Nielsen *et al*.,1999)**. parsley is used also to treat various illnesses such as Alzheimer's disease, thrombosis, strokes and it is widely employed against renal diseases and cardiovascular diseases in different countries **(Mahmood *et al*.,2014; Al-Daraji *et al*.,2012 and Jouad *et al*.,2001)**. Parsley is a medicinal plant with various proven pharmacological properties including antioxidant, hepatoprotective, neuroprotective, anti-diabeic, analgesic, spasmolytic, immunosuppressant, anti-coagulant, antiulcer, laxative, estrogenic, diuretic, hypotensive, anti-bacterial and antifungal activities **(Farzaei *et al*., 2013)**. In addition, the use of the plant is discouraged in heart and kidney disorders due to its water retention capabilities, however, its 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. In addition, it is also a common home remedy for obesity and reduction of itching in insect bites **(Pattison *et al*., 2004 & Awe and Banjoko, 2013)**. Moreever, parsley have diuretic effect that aid remove excretion of toxic material from body tissue **(Adnan *et al*.,2013)**. Parsley is a good source of mineral as iron, calcium, phosphorous, manganese, zinc and contains starch, vitamin B, vitamin C, vitamin A, and E, β-carotene, and antioxidants **(Tunali *et al*.,1999; Russo *et al*.,2003; Pattison *et al*., 2004; Ozsoy-Sacan *et al*.,2006; Vora *et al*.,2012 & Awe and Banjoko,2013)**. Ethanolic extract of parsley leaves contains tannins, flavonoids, sterols and triterpenes **(Vora *et al*.,2009)**. Besides having significant nutritional value, parsley also exhibits antioxidant and neutralizing properties **(Mahmood *et al*., 2014)**. Parsley is rich with an antioxidant arsenal as flavonoid that searches out and eradicates free radicals in the body that cause oxidative stress in cells **(Rashwan, 2012)**. **Pattison *et al*. (2004)** also stated that parsley (*P. crispum*) is considered to be the rich source of antioxidants that can help to break the chain reactions of free radical formation. Previous studies also stated that components of fresh parsley leaf and the methanol extracts of parsley scavenge free radical and protecting against membrane oxidation **(Tunali *et al*., 2000)**. **Allam *et al*. (2016)** suggest that parsley may be an important therapeutic tool to combat oxidative stress-associated diseases and prevent neuronal damage caused by oxidative stress. Parsley has a protective effect against cadmium neurotoxicity and teratogenicity in albino mice **(Maodaa *et al*., 2016)**. Overall, previous study demonstrated that low dose of parsley supplementation significantly improved pathological alterations in mice as reported by **Zhang *et al*. (2006)**. Parsley juice components were found to be significant suppressors to H2O2 and ROS levels in brain and other tissues in mice by stimulating production of glutathione synthesis and thereby boosting cellular antioxidant defense **(Zhang *et al*.,2006)**.

The kidney is a vital organ in health and disease. Many environmental contaminants and chemical variables, including drugs, alter the functions of the kidney **(Mahmood and Waters, 1994 and Begg and Barclay, 1995)**. The kidney is a common target for toxic xenobiotics due to its capacity to extract and concentrate toxic substances by highly specialized cells and also, due to its large blood flow **(Azab *et al*., 2014)**. Herbal products including parsley has been reported to possess protective effects against drugs induced nephrotoxicity in experimental animals **(Kang *et al*.,2006).** Several antioxidants that scavenge or interfere with production of ROS have been used successfully to ameliorate gentamicin nephropathy **(Nakajima *et al*.,1994)**. Several agents and strategies have been attempted to ameliorate gentamicin nephrotoxicity **(Ali *et al*.,2003; Nagai and Takano, 2004 & Cekmen *et al*., 2013)** with main focus on the use of various antioxidant agents including the extracts from medicinal plants with antioxidant properties. Therefore, the present work aimed to evaluate the effectiveness of *P. crispum* (Parsley) against the biochemical and histological alterations of gentamicin induced nephrotoxicity in mice.

2. Material and Methods

Experimental animals and treatment

Forty healthy adult male Swiss albino mice *(Mus-musculus)* 8-10 week old and weighing between 20-26 gm were used in this study. The animals were obtained from the animal breeding house of faculty of veterinary medicine, Omar Al mukhtar University, AL

Bayda - Libya. They were housed in plastic cages and kept under a controlled conditions of 20 ± 2, 12 h light/dark cycle, 50±10% humidity and fed commercial standard diet and allowed tap water *ad libitum* for seven days before starting the experiment for acclimatization. The mice were divided into 4 groups of 10 mice each and subjected to the following treatments. The first group was considered as a control (GI) and received only distilled water, the second group (GII) were injected intramuscular with gentamicin at dose level 80 mg/kg bw dissolved in 1 ml of normal physiological saline for a week (The dose of gentamicin was selected according to **Bibu *et al.* (2010)** to induce nephrotoxicity), third group (GIII) received orally by oral gavage aqueous extract of *P. crispum* only at dose level 40 mg/kg bw for 7 successive days, while animals of the fourth group received aqueous extract of *P. crispum* & gentamicin as GII and GIII.

Material used:

Fresh plant *P. crispum* (Parsley) was daily purchased from vegetable market in AL Beida Libya.

Fig. (1): *Petroselinum crispum*

At the end of the experimental period, the animals from both control and treated groups were dissected without anesthesia. A minimum of 6 animals from each group were necropsied after sacrificed by cervical dislocation on days 8 post-treatment to evaluate biochemical histopathological alterations. The present investigation comprises the following studies.

I- Clinical signs and morphological study

Animals were observed daily to note and record any changes in the behavior, depression, food intake and signs of difficulty breathing, salivation, diarrhea, muscular weakness and any signs of toxicity and mortality. Also, body weights of mice in all groups were measured at the beginning and the end of the experiment using electronic balance. Weight gains and the body weight changes (%) were calculated according to **Tütüncü *et al.* (2010)**.

II - Biochemical study

The plant was authenticated by Department of Botany, Faculty of Agriculture, Omar AL Mukhtar university, AL Bayda - Libya. All unwanted materials were removed. The plant (fresh parts of leaves and stems) was carefully washed under tap water and used to prepare fresh aqueous extract of the *P. crispum* (Parsley) as used in traditional medicine.

Preparation of the aqueous extracts of *Petroselinum crispum* (Parsley):-

Leaves of *P. crispum* (40mg) were mixed with 100 ml boiling water and steeped in boiled water in a closed vessel for 10 minute. The fresh prepared extract has been filtered using piece of gauze and and each mouse received orally 0.03ml/mouse at dose level 40mg/kg bw.

Gentamicin and dose Preparation

Gentamicin was purchased from (Sigma Co, Germany). Mice were injected intramuscular with gentamicin 0.01ml/kg for a week. A dose was determined according to **Paget and Barnes (1964)**.

Fig. (2): Gentamicin

Twenty four hours after the end of experimental period, un anesthetized mice from both control and experimental groups were sacrificed by cervical dislocation. Peripheral blood samples were collected from the neck blood vessels into free anticoagulated containers and allowed to stand for half an hour then centrifuged at 3000 rpm at 4 ºC for 15 minutes and the superanated serum was collected in eppendorf and utilized for estimation various biochemical parameters. Total protein was measured according to **Lowry *et al.*, (1951)**. Serum creatinine and urea were determined according to procedures of **Henry (1974)** and **Fawcett and Scott (1960)** respectively. Determinations of parameters were performed using an automated biochemical analyzer (Chemistry analyzer photometer by used commercial available kits from Analyticon Biotechnologies (Germany).

III -Histopathological studies

A portion of the kidneys were cut into small pieces of approximately 3-5 mm size and fixed in aqueous Bouin's fluid for -18-20 hour. After embedding in paraffin wax, thin sections of 5 µm thickness of kidney tissues were cut and stained with Harri's hematoxylin and eosin (H & E) according to **Bancroft and Gamble (2008).** The thin sections of kidney were made into permanent slides and examined under high resolution microscope with photographic facility (Nikon Eclipse E400, Japan) and histopathological changes were recognized and photographed

Statistical analysis

Data were presented as mean ± standard error (S.E.). Data were analyzed using a one way analysis of variance (ANOVA SPSS version 19) followed by post hoc test and Duncan's multiple range test. P values ≤0.05 were considered to be statistically significant. Excel programs also was used to analysis and draw the figures.

3. Results

I- Result of clinical signs and morphological study

Neither clinical signs nor abnormalities in behavior and external features were observed in mice treated with aqueous extract of *P. crispum* (Parsley) with and without gentamicin except slight hypoactivty, dull hair coat and moisture feces were observed in those animals treated with gentamicin only. No deaths were recorded during the experiment period in control and experimental treated group.

Our data of body weight was illustrated in Table (1) and Figure (3). The baseline weight of the mice at the beginning of the study was similar in all groups. No significant weight change could be documented at the end of the study. Although, the mice treated with gentamicin only showed obvious but insignificant decrease in the final body weight compared to control group. The final body weight gain in those animals increased only by 3. 6% compared to 9.24% in control group. While, the animals treated with aqueous extract of *P. crispum* only showed insignificant increase in the final body weight gain compared to control group. In comparison to control group, insignificant increase in the final body weight was observed in the mice Co-treated with *P. crispum* and gentamicin, whereas the final body weight in *P. crispum* and gentamicin treated group increased by 9.92% compared to 9.24% in control group. It was found that administration of *P. crispum* succeed to ameliorate the final body weight in the mice Co-treated with *P. crispum* and gentamicin comparing to gentamicin only treated group (Fig 3).

Table (1): The influence of aqueous extract of *P. crispum* (Parsley) with and without gentamicin on body weight gain of mice.

Time Groups	Mean of Initial body weight (gm)	Mean of final body weight (gm) after one week	The mean of change in body weight (%)
Control group	24.9±1.6[a]	27.2±1.2[a]	9.24%
Gentamicin intramuscular injected group	25±0.7[a]	25.9± 0.9[a]	3.6%
Aqueous extract of *P.crispum* only treated group	25.1±1.5[a]	28.3±1.1[a]	12.75%
Aqueous extract of *P.crispum* with gentamicin treated group	25.2±2.2[a]	27.7± 1.3[a]	9.92%

Each value represent the mean ±S.E body weight of the animals in each group.
Values, within raw and columns with common superscripts (a) are statistically insignificant, P≤ 0.05

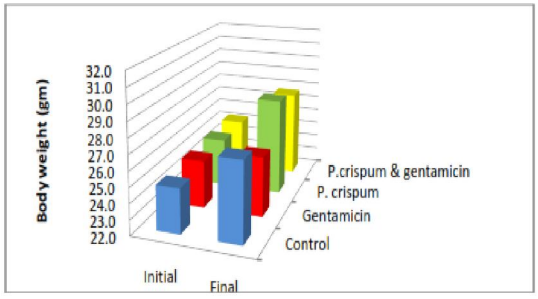

Fig. (3): The influence of aqueous extract of *P. crispum* (Parsley) with and without gentamicin on body weight gain of mice.

II - Result of biochemical study

The effects of aqueous extract of *P. crispum* (Parsley) with and without gentamicin on biochemical parameters of kidney functions are illustrated in Table (2) and Figures (4-6). Orally administration of aqueous extract of *P. crispum* (Parsley) at does level 40mg/kg b.w for a week showed menial insignificant alterations in the kidney marker parameters including serum level of total protein, urea and creatinine concentrations comparing to control group. While, intramuscular injection of gentamicin (80 mg/kg, i.m.) for 7 consecutive days to mice caused insignificant increase in all tested parameters (total protein, urea and creatinine concentrations) comparing to control

group. However, an improvement in the serum levels of total protein, urea and creatinine concentrations were recorded in mice Co-treated with aqueous extract of *P.crispum* and gentamicin comparing to gentamicin only treated group.

Table (2): The influence of aqueous extract of *P. crispum* (Parsley) with and without gentamicin on some kidney biomarker function in mice.

Groups Items	Control	Gentamicin intramuscular injected group	Aqueous extract of *P.crispum* only treated group	Aqueous extract of *P.crispum* with gentamicin
Total protein (g/dl)	5.9±0.1[a]	6.4±0.2[a]	5.9±0.3[a]	6.2±0.1[a]
Creatinine (mg/dl)	0.5±0.1[b]	0.7±0.1[b]	0.5±0.2[b]	0.5±0.3[b]
Urea (mg/dl)	48.0±10.1[c]	52.3±2.9[c]	49.0±1.7[c]	50.3±1.8[c]

Each value represent the mean ±S.E. of 5 animals in each group.
Values, within row with common superscripts (a, b, or c) are statistically insignificant, P≤ 0.05

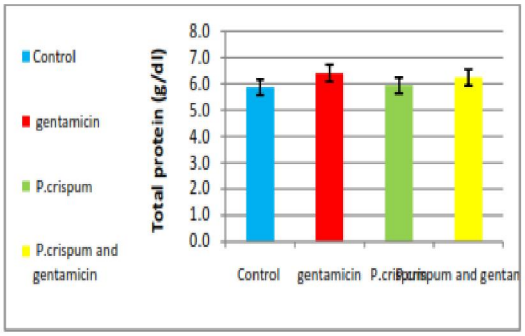

Fig. (4): Effect of aqueous extract of *P. crispum* with and without gentamicin on total protein (g/dl).

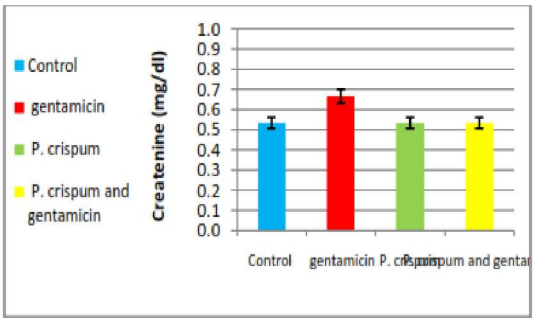

Fig. (5): Effect of aqueous extract of *P. crispum* with and without gentamicin on creatinine (mg/dl).

III - Result of histopathological studies

Postmortem examination did not reveal any abnormal gross changes in the visceral organs of control and treated mice. Kidney sections of mice from control group revealed normal architecture of renal cortex which mostly appeared occupied by renal corpuscles and surrounding proximal and distal convoluted tubules. The renal corpuscle is formed of glomerular tuft of blood capillaries surrounded by capsular space and Bowman's capsule with clear parietal and visceral epithelium. The proximal convoluted tubules are lined with large cuboidal cells with deeply stained acidophilic cytoplasm with apical brush border and rounded vesicular nuclei. The boundaries between the adjacent cells are indistinct. The distal convoluted tubules are lined by low cuboidal cells with distinct cell boundaries and less acidophilic cytoplasm. Also, normal appearance of distal convoluted tubules with macula densa was noticed. As well as normal appearance of collecting tubules, ascending and descending limbs of Henle loop were seen. Normal architecture of renal medulla was also detected (Figs. 7and 8). No visible change was observed in histological sections of kidney of mice treated orally with aqueous extract of *P. crispum* (Parsley). Normal appearance of renal corpuscles and surrounding proximal and distal convoluted tubules were detected (Fig. 9).

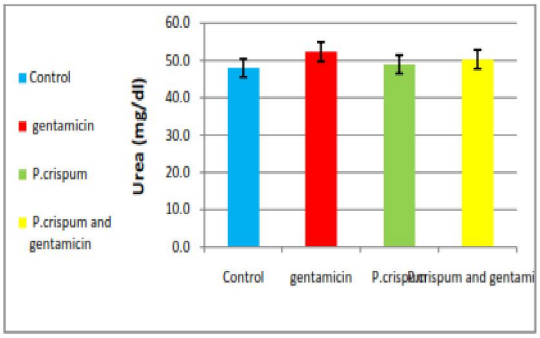

Fig. (6): Effect of aqueous extract of *P. crispum* with and without gentamicin on urea (mg/dl)

In contrast, histopathological examination of kidney sections of mice injected intramuscular with gentamicin showed marked deleterious histological changes which were more prominent in proximal convoluted tubules and renal corpuscles. Such lesions manifested in form necrotic hypertrophy vacuolization, degeneration lining tubular epithelial cells of some renal tubules lead to stenosis or completely occlusion of their lumen, with damage or

loss brush borders, necrotic and degenerative renal tubules with desquamation lining tubular epithelial cells and debris materials in tubular lumen were frequentlyseen. Also histological examination revealed presence atrophy and shrinkage of the glomeruli with widening of the capsular space. However, stenosis or occlusion capsular space of some renal corpuscles was occasionally observed. Furthermore, dilated and congested intertubular blood vessels and hemorrhage within interstitial tissue in cortex and medulla region associated with leucocytic cells infiltration were seen (Figs.10-15). The histological observations also supported the results obtained from the serum marker assay of kidney functions. The Co-treated with aqueous extract of *P. crispum* and gentamicin showed marked improvement in the histological structure of kidney in comparison to gentamicin alone treated group. Our results clearly demonstrate that repeated oral administration of aqueous extract of *P. crispum* was accompanied by an improvement in the kidney tissue of mice injected with gentamicin. Histological sections of aqueous extract of *P. crispum* and gentamicin treated group showed that the most renal tubules appeared with nearly normal feature and renal corpuscles with intact glomeruli and open capsular space was frequently observed. Whereas, the severity of the above cited histological abnormalities observed in gentamicin alone treated group were markedly decrease. However, vacuolization and swelling of the lining epithelium of some renal tubules, necrotic and degeneration renal tubules with desquamation of epithelium lining renal tubules in the lumen, hemorrhage within interstitial tissue were still found in decrease manner. Also, stenosis of capsular space of few renal corpuscles and heavy inflammatory cells infiltration around few renal corpuscles were observed (Figs.16-19).

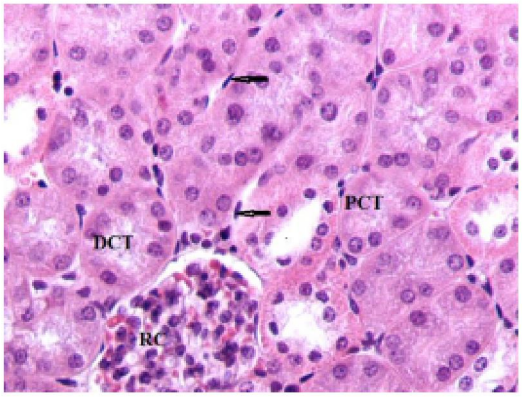

Fig. (7): A section of renal cortex of male mouse from control group showing normal architecture of distal convoluted tubules (DCT), proximal convoluted tubules (PCT), renal corpuscles (RC) and interstitial cells (Arrows) (H & E stain, X400).

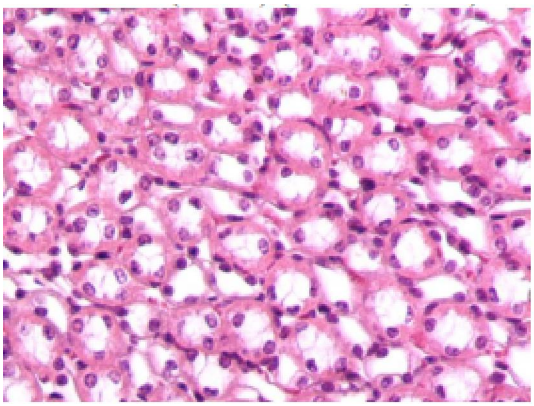

Fig. (8): A section of renal medulla of male mouse from control group showing normal architecture (H & E stain, X400).

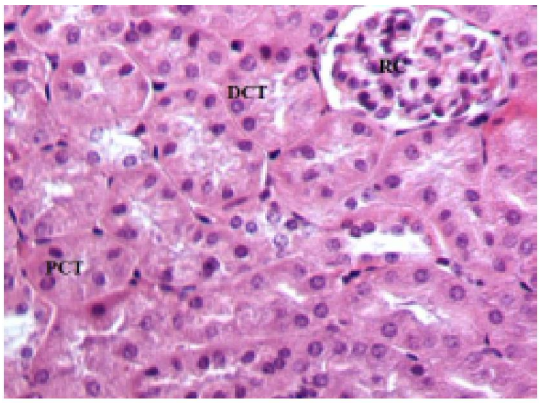

Fig. (9): A section of renal cortex of mouse treated with aqueous extract of P. crispum only showing normal architecture of distal convoluted tubules (DCT), proximal convoluted tubules (PCT), renal corpuscles (RC) and interstitial cells (Arrows) (H & E stain, X400).

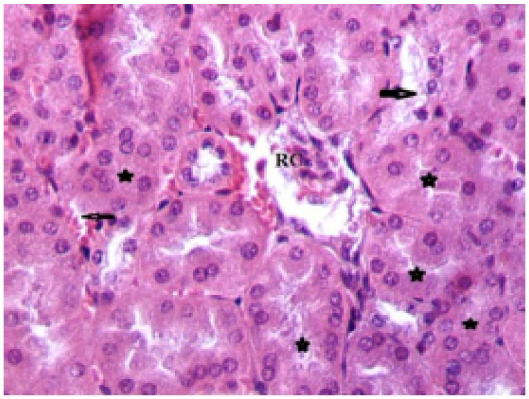

Fig. (10): A section of renal cortex of mouse injected intramuscular with gentamicin only for a week showing degeneration of renal tubules with stenosis or occlusion lumen (Stars). Note: necrotic epithelium lining renal tubule (Thick Arrow). Renal corpuscles (RC) with shrinkage glomerulus and widening urinary space, hemorrhage within interstitial tissue in cortex region (Arrows) (H & E stain, x400).

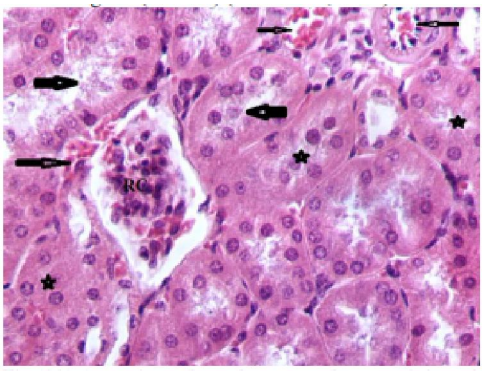

Fig. (11): A section of renal cortex of mouse injected intramuscular with gentamicin only for a week showing degeneration of renal tubules with stenosis or occlusion lumen (Stars). Necrotic and degenerative renal tubules with desquamation lining epithelial cell and debris in tubular lumen (Thick arrows). Renal corpuscles (RC) with shrinkage glomerulus and widening urinary space, dilated and congestion of intertubular blood vessels (Thin Arrows) and hemorrhage within interstitial tissue in cortex region (Arrow) (H & E stain, x400).

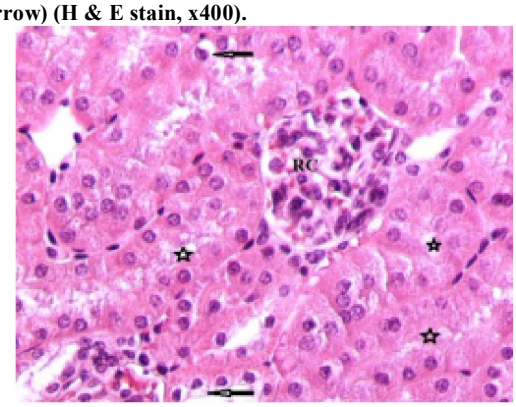

Fig. (12): A section of renal cortex of mouse injected intramuscular with gentamicin only for a week showing necrotic, hypertrophy, vacuolization degeneration lining tubular epithelial cells (Arrows) with stenosis or occlusion tubular lumen (Stars). Stenosis of urinary space of renal corpuscles (RC) (H & E stain, x400).

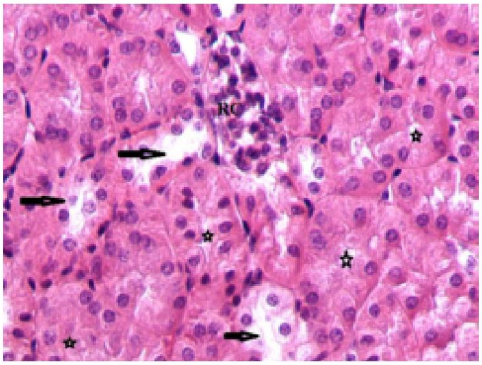

Fig. (13): A section of renal cortex of mouse injected intramuscular with gentamicin only for a week showing degeneration renal tubules with stenosis or occlusion lumen (Star), necrotic, hypertrophy vacuolated epithelial cells lining renal tubules (Thick arrows). Disappearance of urinary space of renal corpuscles (RC) (H & E stain, x400).

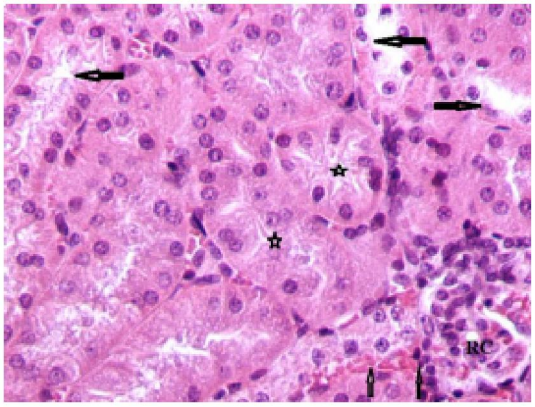

Fig. (14): A section of renal cortex of mouse injected intramuscular with gentamicin only for a week showing degeneration of renal tubules with stenosis or occlusion lumen (Stars). Necrotic and degenerative renal tubules with desquamation tubular lining epithelial and presence of debris in tubular lumen (Thick arrows). Stenosis of urinary space of renal corpuscles (RC), hemorrhage within interstitial tissue in cortex region associated with leucocytes cells infiltration (Arrows) (H & E stain, x400).

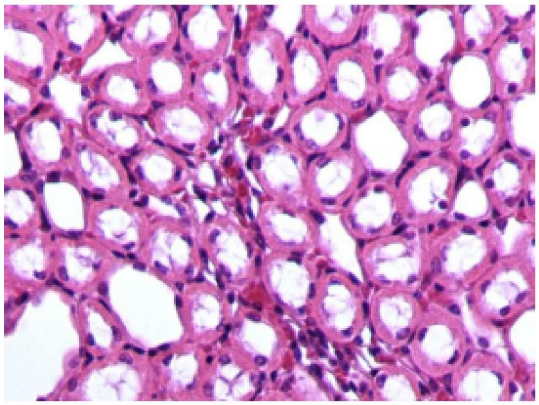

Fig. (15): A section of renal medullar of mouse injected intramuscular with gentamicin only for a week showing hemorrhage within interstitial tissue in medulla region (H & E stain, x400).

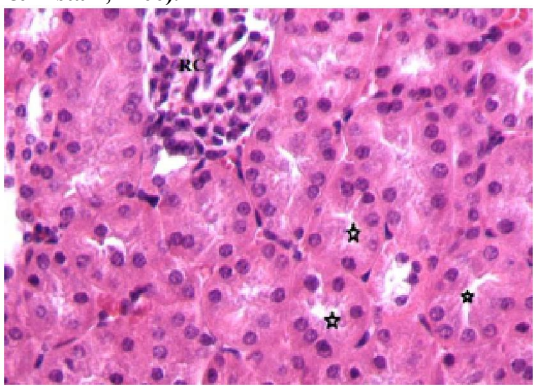

Fig. (16): A section of renal cortex of mouse Co- treated with aqueous extract of *P. crispum* & gentamicin showing most renal tubules with nearly normal feature (Stars) and renal corpuscle (RC) with stenosis of capsular space (H & E stain, x400).

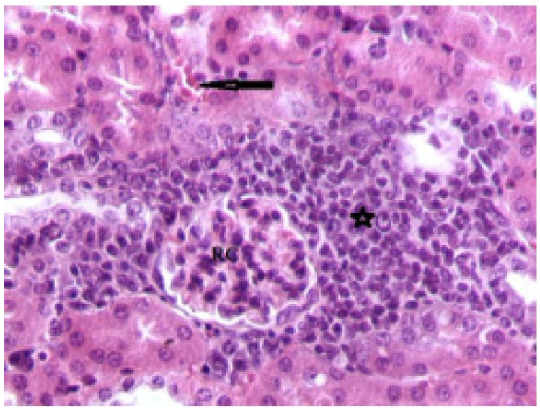

Fig. (17): A section of renal cortex of mouse Co- treated with aqueous extract of *P. crispum* & gentamicin showing hemorrhage within interstitial tissue (Arrow), stenosis of capsular space of renal corpuscle (RC), note heavy inflammatory cells infiltration around renal corpuscle (Star) (H & E stain, x400).

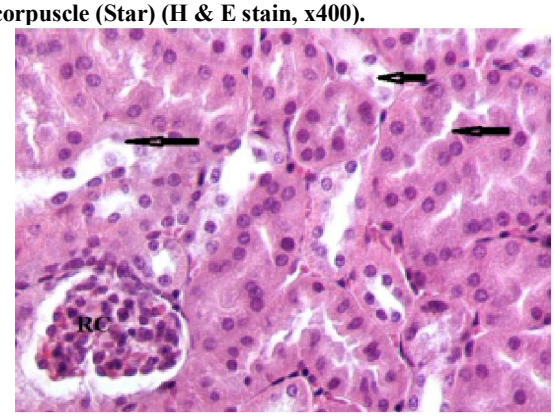

Fig. (18): A section of renal cortex of mouse Co- treated with aqueous extract of *P. crispum* & gentamicin showing renal corpuscles with intact glomeruli and open capsular space (RC), necrotic and swelling degeneration renal tubules (Arrows) (H & E stain, x400).

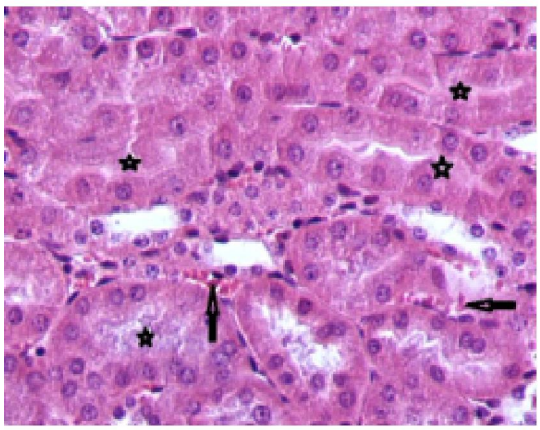

Fig. (19): A section of renal cortex of mouse Co- treated with aqueous extract of *P. crispum* & gentamicin showing swelling degeneration renal tubules (Stars), hemorrhage within interstitial tissue (Arrows) (H & E stain, x400).

4. Discussion

In the present study, neither clinical signs nor abnormalities in behavior and external features were observed in mice treated with aqueous extract of *P. crispum* (Parsley) and administration of parsley with gentamicin ameliorating the abnormalities (slight hypoactivity, dull hair coat and moisture feces) compared to gentamicin only treated group. No deaths were recorded during the experiment period in control and all treated groups. Likewise, our results are in line with the previous report by **Awe and Banjoko (2013)** who published that there were no abnormal signs of toxicity or death recorded in rats treated orally with *P. crispsum* extract daily at doses of range from 10- 1000 mg/kg body weight for 8 weeks. Our result regarding gentamicin were in agreement with previous study which had demonstrated that irritating behaviour in the male mice treatment intraperitoneally with gentamicin at dose level 80 mg/Kg/day for fifteen days **(Qdiar *et al*.,2011).** Also, our result regarding protective effects Parsley may be support and explain from previous study by **Maodaa *et al*. (2016)** who reported that parsley has protective effects against cadmium neurotoxicity in albino mice. Parsley juice supplementation (by gastric intubation at two doses of 10 and 20 g/ kg/day for 28 days) improves the abnormal behavior of cadmium intoxicated mice and reduces neuronal aberrations in the brain. Also, **Allam *et al*. (2016)** who investigated the protective role of parsley juice (*P. crispum*) at doses of 20mg/kg and 10mg/kg against cadmium teratogenicity in brain of newborns albino mouse (exposed to cadmium during pregnancy) stated that the low dose of parsley 10 g/kg/day exhibited significant effects in neutralizing and reducing the neuronal degeneration deleterious changes on the behavioral activities and oxidative stress in newborns mice. These may be due to the significant effect of parsley in the excretion of heavy metals such as cadmium from mother's bodies so the complications of cadmium toxicity reduced and disappeared in the pups of parsley ingested groups **(Darias *et al*.,2001)**.

Body weight changes serve as a sensitive indication of the general health status of animal **(Salawu *et al*.,2009)**, and used as an indicator of adverse effect of drugs and chemicals **(Mukinda and Syce, 2007).** In the present study, the mice treated with gentamicin only showed obvious but insignificant decrease in the final body weight. While, treatment with *P. crispum* only showed insignificant increase in the final body weight gain compared to control group may be related to the fact that the plant is known to be a good appetite stimulant. It was found that administration of *P. crispum* success to ameliorate the final body weight in the mice Co-treated with *P. crispum* and gentamicin comparing to

gentamicin only treated group. Our results regarding body weight in line with previous reports of other investigators who stated that administration of gentamicin alon at dose level 80 mg/Kg/day for fifteen days produced statistically significant loss of body and kidney weight **(Qdiar *et al*.,2011).** This decrease is probably due to the anorexia and partial renal failure leading to acidosis as reported earlier by **(Chen *et al*., 1984)**. Also, **Ramhariya *et al*. (2015)** demonstrated that administration of gentamicin (80 mg/kg for 10 days by intraperitoneal route) caused decrement in weight of animals. Toxicological studies have illustrated that many toxicants are usually associated with weight loss in exposed animals **(Maodaa *et al*.,2016)**. Regarding increase in the final body weight of mice treated with *P. crispum* only in a good agreement with **Awe and Banjoko (2013)** who reported that body weight gradually increased in rats treated orally with *P. crispsum* extract (Parsley) daily at doses level range of 10- 1000 mg/kg body weight for 8 weeks comparable with that of control. Our finding concerning ameliorating final body weight in the mice Co-treated with *P. crispum* and gentamicin in harmony with **Maodaa *et al*. (2016)** who reported that administration of parsley juice at two doses of 10 and 20 g/ kg/day for 28 days exhibited a similar pattern of body weight change in comparison to the control group. Also**, Maodaa *et al*. (2016)** reported that supplemented parsley juice at two doses of 10 and 20 g/ kg/day for 28 days has restored the loss of body weight in cadmium intoxicated mice.

Blood serum levels of creatinine and urea were also estimated since these two parameters are of special significance to evaluate renal function **(Frank, 1993 and Tietz, 1996).** Creatinine is considered as one of the most reliable indicators of the efficiency of renal function **(Travlos *et al*., 1996; Kore *et al*., 2011 and Babu *et al*., 2011).** In the present study, treatment with aqueous extract of *P. crispum* (40 mg/kg bw) only for a week showed menial insignificant alterations in the kidney marker including serum total protein, urea and creatinine concentrations comparing to control group. While, mice injected with gentamicin (80 mg/kg, i.m.) only showed obvious but insignificant increase in total protein, urea and creatinine concentrations comparing to control group. In contrast to this result, administration of aqueous extract of *P.crispum* with gentamicin ameliorated serum levels of total protein, urea and creatinine concentrations comparing to gentamicin only treated group. These results were in agreement with previous studies which had demonstrated that kidney function test had been affected after 5–7 days of gentamicin injection **(Abdel-Naim *et al*.,1999 and Karahan *et al*., 2005)**. Serum urea and creatinine increased in the male mice treated intraperitoneally with gentamicin at dose level 80 mg/Kg/day for fifteen days **(Qdiar *et al*., 2011). Alarifi *et al*. (2012)** reported that compared with control animals significant blood chemical changes including free radicals, serum creatinine and serum urea were recorded in rats injected daily with intramuscular injection of gentamicin at dose 80 mg/kg/day and 150 mg/kg/day for four consecutive weeks and these alterations were detected at the end of first week (the highest level of creatinine was recorded at second week, while that of urea was at first week). The findings revealed that exposure to gentamicin can induce significant histological alterations in the kidney as well as remarkable blood chemical changes that might indicate marked renal failure **(Alarifi *et al*.,2012).** Acute renal failure is characterized by disorders in some biochemical parameters in gentamicin treated rats. Gentamicin administration into rats induced impairment of renal function through release of oxygen free radical **(Heibashy and Abdel Moneim, 1999 and Heibashy *et al*.,2009).** In this context **Padmini and Kumar (2012)** reported that treatment with gentamicin at dose level 80mg/kg. b.w for 10 days produced increase in the concentration of serum urea, creatinine and uric acid in rats. The same author stated that more than half of proximal tubules showing desquamation and necrosis. These results confirmed that gentamicin produced nephrotoxicity as previously reported by **Ali *et al*. (2003), Goto (2004) and Heibashy *et al*. (2009)**. Also, serum creatinine level and blood urea nitrogen were increased in gentamicin (80 mg/kg for 10 days by intraperitoneal route) treated animals **(Ramhariya *et al*., 2015)**. Our finding also in agreement with **Moghaddam *et al*. (2010)** and **Sivachandran and Hariharan (2012)** who found that the gentamicin nephrotoxicity is functionally characterized by increase in serum creatinine, urea, and blood urea nitrogen. Gentamicin increased serum creatinine, cholesterol, blood urea nitrogen (BUN), lipid peroxidation (LPO) and suppressed superoxide dismutase (SOD) and catalase activities in rats renal tissues **(Khan *et al*.,2009 b)**. Also, the serum urea, creatinine, and uric acid were elevated in rats received intraperitoneal injection of gentamicin only (100 mg/kg bw /day) for 10 days **(Azab *et al*., 2014)**. **Ahmadvand *et al*. (2016)** demonstrated that the rats treated (i.p.**)** with gentamicin (induced nephrotoxicity) at dose 100 mg/kg for 12 days showed significant increase in the levels of serum creatinine and urea 4 fold higher than that of the control animals. The increase in levels of serum urea and creatinine may be not only due to the glomerular injuries, but also due to the decrease of glomerular filtration **(Tavafi *et al*., 2012).** Increased blood creatinine is strongly related with renal damage **(Soliman *et al*., 2007 and Kore *et al*., 2011).** A creatinine is known as an effective

indicator of renal function and any rise in creatinine levels is observed if there is marked damage to functional nephrons **(Ravichandran *et al*.,2014)** and **Khan *et al*. (2009a)** confirmed that serum creatinine level does not rise until at least half of the kidney nephrons are destroyed. Gentamicin is known to generate reactive oxygen species associated with an increase in lipid peroxidation and decrease in antioxidant enzyme activity in the kidney **(Banday *et al*., 2008).** In the present work, oral administration of *P. crispsum* (parsley) caused nephroprotective effects as it reversed the biochemical alterations induced by gentamicin in mice. The nephroprotective effect of parsley was attributed to its *in vitro* antioxidant activity (free radical scavenger activity) due to its high content of flavonoids **(Fejes *et al*., 1998).** Medicinal plants containing active chemical constituents with high antioxidant property play an important role in the prevention of various degenerative diseases **(Lukmanul *et al*.,2008)**. Flavonoids are phytophenolic compounds with strong antioxidant effects that function against oxidative stress **(Bahar *et al*., 2017)**. Our observation regarding administration of aqueous extract of *P.crispum* only at dose level 40 mg/kg bw is in line with the previous publication of **Awe and Banjoko (2013)** who reported that there was no significant difference in ALT and blood BUN levels at the doses of 10 -100mg/kg compared to the control group and suggests that the ethanol extract of *P. crispum* is not toxic when administered orally at doses of 10-100 mg/kg for 8 weeks in rats. On the other hand the same author noticed that administration of *P. crispsum* extract at dose level1000 mg/kg body weight for 8 weeks caused significant increase in ALT and blood BUN levels and suggests that the extract at this oral dose level would cause renal damage. Supplementation of diets with fresh parsley leaf can increase antioxidant capacity of rat plasma and decrease oxidative stress in humans **(Nielsen *et al*., 1999)**. Similarly, aqueous and ethanol extracts of fresh parsley leaf strongly inhibit lipid oxidation **(Wong and Kitts,2006)**. Above mentioning data may be explain the improvement and protective effect of parsley against gentamicin nephrotoxicity.

The kidney is a common target for toxic xenobiotics due to its capacity to extract and concentrate toxic substances by highly specialized cells and also, due to its large blood flow (about 21% of cardiac output) **(Salgado *et al*.,2007 and Choi *et al*., 2011)**. Therefore, histological examination of the kidney was done in the present work and was found to be supported the results obtained from the serum biomarker assay of kidney functions. In the current study no visible change was observed in kidney histological sections of mice treated orally with aqueous extract of *P. crispum* (Parsley) only. In the present work it was found that kidney sections of mice injected intramuscular with gentamicin showed marked deleterious histological changes whereas intramuscular injection of gentamicin (80 mg/kg, i.m.) for 7 consecutive days to mice caused signs of nephrotoxicity manifested by elevation in serum urea, creatinine and total protein. As well as examination of kidney sections of gentamicin injected mice revealed a marked deleterious in the tubules and renal corpuscle in form necrosis and degenerated of renal tubules with stenosis or complete occlusion of their lumen which were more prominent in proximal convoluted tubules, shrinkage of the glomeruli with widening or occlusion capsular space, dilated and congested intertubular blood vessels and hemorrhage within interstitial tissue in cortex and medulla regions associated with leucocytes infiltration. Similarly, increase serum creatinine, eosinophilic casts and leukocyte infiltration in nephrotoxic rats were previously reported by other investigators **(Ademuyiwa *et al*.,1990; Elliott *et al*.,2000; Cuzzocrea *et al*.,2002; Salgado *et al*.,2007and Tavafi *et al*.,2012)**. Also, the tubular necrosis and degenerative changes seen in the present work were in accordance with the findings of previous investigations **(Can *et al*., 2000; Kumer *et al*., 2000 a & b; Al-Majed *et al*., 2002; Ekor *et al*., 2006; Nitha and Janardhanan, 2008; Saleemi *et al*., 2009; Ali *et al*., 2011 and Dehghani *et al*., 2011).** Inaddition, **Elgazar and AboRaya (2013)** noticed that treatment with gentamicin induces nephrotoxicity manifested by biochemical and histological changes in rats. Moreover, earlier report by **Kacew (1989)** noticed that gentamicin caused tubular necrosis and loss of brush borders. Similar findings also recorded by other investigators who reported that kidney of gentamicin treated rat's (80 mg/kg for 10 days by intraperitoneal route) illustrated presence of inflammatory depositions and cell necrosis, blood vessels congestion and interstitial inflammation **(Ramhariya *et al*., 2015)**. **Azab *et al*. (2014)** reported that the gentamicin (rats injected with gentamicin 100 mg/kg b. wt /day for 10 days) had adverse effects on the kidney structure mainly on the proximal convoluted tubules, renal corpuscle, vessels, and interstitial tissues. The proximal tubules showed degeneration of the epithelial lining cells with disruption of their brush borders and presence of debris in the lumen. The renal corpuscles showed degeneration in the glomeruli and disrupted Bowman's capsule. The vessels showed thickening of the wall and degeneration of the endothelial lining cells. The interstitial tissues showed perivascular infiltration of inflammatory cells and interstitial massive hemorrhage. Our findings are also in agreement with previous studies reported by **Noorani *et al*. (2011), Nale *et al*. (2012) and Ullah *et***

***al*. (2013)** who was found that gentamicin administration caused marked changes in kidney tubules may be due to gentamicin reabsorption in proximal convoluted tubules, causing degeneration and necrosis of the epithelial cells of the tubules. These changes are manifested by dilated tubules, loss of brush border, severe leucocytic infiltrations, tubular degeneration and presence of tubular casts. **Alarifi *et al*. (2012)** reported that gentamicin (injected to rats at dose 80 mg/kg/day and 150 mg/kg/day for four consecutive weeks) produced outstanding tubular, glomerular and interstitial alterations that included degeneration, necrosis, cytolysis and cortical tubular desquamation together with mesangial hypercellularity, endothelial cell proliferation and blood capillary congestion. Also, **Alarifi *et al*. (2012)** showed that the cortex of the kidney was more affected than the medulla as a result of long-term treatment with gentamicin. This might indicate that a relatively higher concentration of gentamicin reaches the cortex via the bloodstream than that entering the medulla. This is in agreement with the findings of **Karahan *et al*. (2005)** which showed that most of the gentamicin accumulates in the renal cortex. Similar to our finding also **Alarifi *et al*. (2012)** showed that tubular damage was more prominent in proximal convoluted tubules than distal tubules. This could be due to the fact that proximal convoluted tubules are the primary sites of reabsorption and active transport. This leads to a higher concentration of gentamicin in the epithelial lining of these tubules and suggests that the gentamicin toxicity is related to its accumulation in the proximal tubules **(Alarifi *et al*.,2012 and Noorani *et al*.,2011)**. This predominant toxicity in the proximal tubules is caused by taking up the aminoglycosides into the epithelial cells of the renal proximal tubules and stay for a long time which lead to nephrotoxicity **(Nagai and Takano,2004).** Also, the accumulation of gentamicin in proximal renal tubules leads to brush border network damage **(Whiting *et al*.,1996)**. The above mentioning data may be explained the prominent in proximal convoluted tubules histopathological alterations in the present work. Our finding concerning leukocytes infiltration was in harmony with previously published reports who reported that the level of leukocytes infiltration was significantly (23.87-fold) higher in rats treated i.p. with gentamicin at dose 100 mg/kg for 12 days than that of the control animals **(Ahmadvand *et al*., 2016)**. One of the reasons behind this manifestation includes increased oxidative stress **(Dufour and Loonis,2007)**. The mechanism of nephrotoxicity caused by gentamicin was attributed to stimulation of generation of reactive oxygen species (ROS) causing tissue oxidative stress **(Sha and Schacht, 1999; Cuzzocrea *et al*., 2002 and Tavafi *et al*., 2012). Balakumar *et al*. (2008), Tavafi. (2013), Ahmadvand *et al*. (2014) and Moreira *et al*. (2014)** stated that nephrotoxicity is one of the most important side effects of the use of gentamicin resulted in reactive oxygen species generation such as superoxide anion, hydrogen peroxide and hydroxyl radicals in the kidney. Also, the gentamicin induces renal tubular damage via energy depletion in renal tubular cells beside inducing of oxidative stress **(Tavafi,2013)**. In addition, it induces cellular injury and necrosis by reducing the efficiency of antioxidant enzymes in the kidney such as superoxide dismutase, catalase, glutathione peroxidase and glutathione **(Balakumar *et al*.,2010)**. Gentamicin binds to the phospholipids of the cell membrane of the renal tubules and enters inside the cells, then it binds to subcellular organelles, alters the mitochondrial respiration and small amount may be taken up by lysosomes **(Erdem *et al*.,2000)**. Free radicals released from mitochondria of renal tubular cells were found to be the main factor in induction of gentamicin induced nephrotoxicity **(Kumer *et al*., 2000 a & b; Al-Majed *et al*., 2002 and Abdel-Raheem *et al*., 2010)**. Drug-induced nephrotoxicity is an important cause of renal failure. Moreover, both lysosomes and mitochondria have been shown to send death signals through the activation of specific stress sensors. Also, lysosomes membrane rupture and release of acid hydrolases contribute to apoptosis and necrosis of proximal tubular cells **(Servais *et al*.,2005)**. Aminoglycosides throughout the endocytic pathway are taken up into the epithelial cells of the renal proximal tubules and stay there for a long time, which leads to nephrotoxicity **(Nagai and Takano, 2004).** Acidic phospholipids, broadly distributed in the plasma membranes in various tissues, were considered to be the binding site of aminoglycosides in brush-border membrane of proximal tubular cells **(Nagai and Takano, 2004).** The proximal convoluted tubules in cortex were dilated and showed patchy necrosis, loss of brush border, presence of cellular debris and accumulation of inflammatory exudates within their lumen. The epithelial cells of proximal convoluted tubules showed hydropic changes with cytoplasmic vacuolations at some places. Some of the tubules exhibited desquamated epithelial cells in their lumina. The nuclei of these cells were swollen and karyolitic in the male mice treated intraperitoneally with gentamicin at dose level 80 mg/Kg/day for fifteen days **(Qdiar *et al*.,2011).** Nephrotoxicity caused by gentamicin seemed to be attributed to the oxidative stress caused by generation of reactive oxygen species **(Cuzzocrea *et al*., 2002; Tavafi *et al*., 2012).** However, **Sha and Schacht (1999)** suggested that amino glycoside antibiotics can stimulate formation of reactive oxygen species (ROS) and cause oxidative

stress. ROS scavengers and antioxidants can be used to alleviate gentamicin induced nephrotoxicity **(Mazzon *et al*., 2001 and Maldonado *et al*., 2003)**. Also, **Tavafi (2013)** confirmed that gentamicin induced nephrotoxicity can cause renal injuries due to ROS generation. An excess production of reactive oxygen species (ROS) is harmful to cells, which is likely to exert toxic effects in the cells involved in the pathogenesis of certain diseases and aging. To scavenge and neutralize these free radicals, the cells are endowed with the antioxidant defense system of enzymes such as superoxide dismutase, catalase and glutathione peroxidase. But an imbalance between reactive oxygen metabolites and antioxidant defense mechanisms of the cells, leading to excessive production of free radicals, creates a condition termed as oxidative stress **(Schroeder, 1984).** The oxidative stress in the cells leads to lipid peroxidation, inactivation of enzyme activities including antioxidant enzymes and DNA breakage **(Wiseman and Halliwell, 1996).** Furthermore, the depletion of glutathione (GSH) may lead to lipid peroxidation whereas glutathione is one of the essential compounds for maintenance of cell integrity and participation in cellular metabolism **(Moazedi *et al*.,2007)**. It was observed in many oxidative stress states that the reduction of GSH induces the elevation of lipid peroxidation.

In the current study Co-treated with aqueous extract of *P. crispum* and gentamicin showed marked improvement in the histological structure of kidney in comparison to gentamicin only treated group. In the present work, oral administration of aqueous extract of *P. crispsum* extract (Parsley) caused nephroprotective effects as they reversed the biochemical and histological alterations induced by gentamicin in mice. **Fejes *et al.* (1998)** reported that the nephroprotective effect of *Petroselinum* was attributed to its *in vitro* antioxidant activity (free radical scavenger activity) due to its high content of flavonoids. Most researchers against gentamicin nephrotoxicity focused on the use of various antioxidants. Usage of antioxidants improved histological injuries such as tubular necrosis, tubular cell edema and apoptosis in gentamicin-injected rats **(Baradaran and Rafieian-kopaei,2013; Tavafi *et al*., 2012 & Tavafi and Ahmadvand,2011).** Our results showed that *P. crispum* has some beneficial effects in decreasing the biochemical alterations, tubular necrosis and can probably decrease some nephrotoxic complication of gentamicin for inhibition progression of kidney damage complications in mice. The protective effect of *P. crispsum* extract (Parsley) can be explained that aqueous extract of *P. crispum* has a high scavenging capacity of reactive oxygen species and free radicals, as thought to be one of the main mechanisms of the antioxidant action exhibited by phenolic phytochemicals **(Moreno *et al*.,2006).** The antioxidant activity of parsley has been reported previously. Antioxidant therapy is one of the most important treatment strategies for kidney damage patients for the prevention and slowing of kidney damage complications progression **(Ahmadvand *et al*., 2016).** This has raised the possibility that antioxidants could acts as prophylactic agents against many pathological conditions **(Maodaa *et al*.,2016)**. This biological activity may be attributed to its constituents obtained from plants, mainly phenolic compounds such as flavonoids. Flavonoids are well-known antioxidant possessing free radical scavenging and metal chelating activity **(Perron and Brumaghim, 2009).** Flavonoids and other antioxidant constituent of medicinal plants have been reported to inhibit xenobiotic induced nephrotoxicity in experimental animal models due to their potent antioxidant effects **(Devipriya and Shyamaladevim, 1999).** Moreover, natural antioxidants strengthen the endogenous antioxidants defenses and restore the optimal balance by neutralizing reactive species **(Ho *et al*.,1994).** The protective role of parsley may be attributed to its higher content of flavonoids and vitamine C which either scavenge free radical which aid to remove damage from liver and kidney **(Jassim, 2013). Ozsoy-Sacan *et al*. (2006)** concluded that protective role of parsley extract probably, due to its antioxidant property, potential free radical scavenging and membrane protective effects. The evidence for the potential role of the oxidants in the pathogenesis of many diseases suggests that antioxidants may be of therapeutic use in these conditions. Polyphenols in plants are a versatile group of antioxidants that protect against oxidative damage by directly neutralizing reactive oxygen species. A large amount of polyphenols is present in asparagus, radish, carrot, onion, beet, cabbage, lettuce and parsley in the form of flavonol glycosides **(Ames *et al*., 1993).** Polyphenols are excellent antioxidants, it has ability to prevent oxidation *in vivo*. This depends on their absorption, transportation and incorporation into appropriate tissues and cellular sites **(Vora *et al*., 2009)**. Flavonoids are natural antioxidants derived from plants and commonly found in foods, such as fruits and vegetables, with the ability to sequester free radicals. The leaves of parsley contain several flavonoids in the form of quercetin, apiin, luteolin and apigenin **(Chenard *et al*., 2005 and Pattison *et al*., 2004)**. Flavonoids are dominant compounds of this plant (**Pápay *et al*.,2012)**. One of the most abundant natural flavonoids present in a large number of fruits and vegetables is **quercetin** (3,5,7,3`,4`, pentahydroxyflavone) which prevents oxidative injury and cell death by scavenging free radicals, donating

hydrogen compound, quenching singlet oxygen, and preventing lipid peroxidation or chelating metal ions **(Lee *et al*.,2010).** Quercetin has significant cytoprotective effect in cisplatin-induced renal tubular damage in vivo in rats **(Devi and Shyamala, 1999)**. **Liu *et al.* (2010)** suggested that quercetin could protect rat kidney against lead-induced injury by improving renal function, attenuating histopathologic changes, reducing ROS production, renewing activities of antioxidant enzymes, and decreasing DNA oxidative damage and apoptosis. Multiple lines of experimental evidence suggest a positive association between quercetin intake and improved outcomes of inflammatory risk **(Russo *et al*.,2012)**. Flavonoids such as quercetin, may help to delay oxidant injury and cell death, by scavenging oxygen free radicals **(Jovanovic *et al*.,1994)** and protect cells from lipid peroxidation **(Dechameux *et al*., 1992)** present in parsley. According to **Hirano *et al.* (2001)** parsley has strong antioxidant properties, as it contains glycosides, quercetin, myristicin. The leaves of parsley was found to be contain several flavonoids tannins, sterols and triterpenes **(Pattison *et al*., 2004; Chenard *et al*., 2005 and Vora *et al*.,2009).** Also, **Patil *et al.* (2008) and Pattison *et al.* (2004)** stated that parsley (*P. crispum*) is considered to be the rich source of antioxidants that can help to break the chain reactions of free radical formation**.** Parsley is considered to be one of the highest sources of flavonol glycosides, including quercetin, apiin, luteolin and apigenin **(Patil *et al*., 2008**). Apigenin, one of the main flavonoids in parsley showed strong antioxidant effects, increasing the activities of antioxidant enzymes and, in turn, decreasing the oxidative damage to tissues **(Kolarovic *et al*.,2010).** Moreever, parsley have diuretic effect that aid remove excretion of toxic material from body tissue **(Adnan *et al*., 2013).** It has been showed that ethanolic extract of parsley leaves helped in maintaining the balance of oxidants and antioxidant defense enzyme activities in the brain of mice under D- galactose- induced oxidative stress in the brain regions of mice **(Vora *et al*., 2009)**. Parsley supplement also restored glutathione (GSH) balance and decreased lipid peroxidation and peroxidase activity **(Vora *et al*., 2009)**. Besides having significant nutritional value, parsley also exhibits antioxidant and neutralizing properties **(Mahmood *et al*.,2014)**. As well, **Tunali *et al*. (2000)** confirmed that components of fresh parsley leaf and the methanol extracts of parsley scavenge free radical and protecting against membrane oxidation. **Al-Howiriny *et al.* (2003) and Ozsoy-Sacan *et al.* (2006)** reported that administration of parsley aqueous extract (2 g/kg b.wt. for 45 days) significantly attenuates the oxidative stress and improves heart tissue of diabetic rats. Also, oral administration of parsley aqueous extract to diabetic rats caused a significant improvement in whole blood glutathione. The same authors add that this finding reflects the ability of parsley aqueous extract to quench free radicals and up regulate the synthesis of glutathione (GSH). This antioxidant property of parsley referred to its content of polyphenols, vitamins E and C **(Vora *et al*.,2012).** Taken together, our data revealed that aqueous extract of *P.crispum* have a greater potential to delay or lessened the oxidative stress and nephrotoxicity of gentamicin**.**

5. Conclusion:

Administration of aqueous extract of *P. crispum* (Parsley) for one week succeed to lessened nephrotoxicity of gentamicin in male mice and ameliorating of oxidative stress and degenerated of renal tubular cells. The improvement in biochemical and histopathological findings may related to its flavonoids and other antioxidant constituents in this plant. However further studies concerned with aqueous extract of *P. crispum* with different doses and durations are needed to elucidate the protective role of this plant. Finally, it is concluded that treatment with gentamicin for a week at dose used in the present work has marked but not sever effects on the renal tissue and consumption of aqueous extract of *P.crispum* (Parsley) should be recommended to lessened nephrotoxicity of gentamicin**.**

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