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Ameliorative effect of aqueous leaves extract of *Rosemarinus officinalis* on cadmium - induced kidney injury in albino rats

El-Morsy*, A.M., Sakr, S.A., and Bayomy, M.F

Zoology Department & Faculty of Science Menoufia University, Shebin El-Kom, Egypt
*Corresponding author e.mail: Semsem_72@ymail.com

Abstract

Cadmium (Cd) is one of the heavy metals causing risks for living organisms and induced high toxicity to different biological system. Rosemary (*Rosemarinus officinalis*) is plant widely used in food. It has antimicrobial, antioxidant, anti-carcinogenic properties. The present work studied the effect of rosemary leaf extract on Cd induced kidney injury in rats.Cadmium chloride (30 mg/kg b.w, 5 consecutive days/week for 8 weeks) administration increased renal MDA but decreased GSH, CAT and SOD activities versus control. In parallel, serum urea and creatinine increased. Cadmium chloride caused histological alternations involved shrinkage in glomeruli, congestion in blood vessels, dilation in renal tubules and leucocytic infiltrations. Ultrastructural observations showed abnormality in nucleus, endoplasmic reticulum, brush border, proximal and distal convoluted tubules. In the contrary, administration of aqueous extract of rosemary restored these changes. The results suggested that rosemary ameliorative these changes due to its antioxidant properties by scavenging free radicals resulting from cadmium chloride.

Keywords: Kidney; Cadmium chloride; Rosemary; Biochemistry; Histology.

1 Introduction

Heavy metals are important toxicants known to show adverse effects in humans causing public health risks. The risk of heavy metal exposure is still a main concern in developing countries (D'Souza et al., 2003).The concentration of heavy metals increased in the environment and was proved to be carcinogenic (Valverde et al., 2000). Heavy metals act as a threat to living organisms since they are highly toxic and accumulated in their body tissues (Sardar et al., 2013).Cadmium (Cd) is one of the most important toxic heavy metals causing risks for living organisms and human inducing high toxicity to different biological systems (Singh et al., 2011).Cd is unique among the metals because of its combination of toxicity in low dosages, long biological half-life (of about 30 years in humans), its low rate of excretion from the body and the fact that it is stored predominantly in the soft tissues like liver and kidney (Jones et al., 1990). It also exerted toxic effects on reproductive systems, development of the embryo (Simoniello et al., 2010), immune system (Waalkes et al., 1999) and considered as a respiratory toxicant (Hollis et al., 1999). Cd is known as a non - essential heavy metal causes oxidative stress (Szollosi *et al*., 2009). According to Wang et al. (2014), Cd was found to generate free radicals. Cadmium is considered as nephrotoxic and hepatotoxic metal (Prozialeck et al., 2009, Mahran et al. 2011).

Natural herbs are widely consumed by humans on a daily basis, these natural products have many biologic and pharmacologic properties (Hosseinimehr, 2014). Herbs are naturally rich in bioactive plant products with food value to keep energy balance in the body and substantial therapeutic value in several diseases (Sharma, 2010). Among these herbs is rosemary (*Rosemarinus officinalis*), which is one of household herbs, used as spices in foods, and employed in traditional medicine (Nabavi et al., 2015). Rosemary is effective in treatment of headache, musculoskeletal pains, and seizures (Boroushaki et al., 2002). It is useful for memory (Moss et al., 2003), a hair growth stimulator

(Murata et al., 2013), and acts as antispasmodic, smooth muscles relaxant, memory booster (Machado et al., 2012). Rosemary has anticarcinogenic, anti-inflammatory and chemopreventive action (Razavi-Azarkhiavi et al., 2014). Extracts ofrosemary contains flavonoids and phenols which showed antioxidant properties (Nabavi et al., 2015). Antioxidants reverse the effects of free radical and may prevent the body from several diseases (Gupta et al., 2006). The present work planned to discuss the possible ameliorative effect of rosemary on cadmium chloride induced toxicity on kidney of albino rats.

2 Materials and Methods

Cadmium chloride ($CdCl_2$)

It is a chemical substance obtained from Raheja Centre, Mumbai, India. Cadmium chloride was dissolved in distilled water and was administrated orally to rats at a dose level 30 mg/kg b.w. for 5 consecutive days per week for 8 weeks according to Ohta et al. (2000).

Preparation of rosemary extract

Rosemary (*R. officinalis*) leaves was collected from greenhouse in Faculty of Science, Menoufia University, Shebin El-Kom, Egypt. Rosemary extract was prepared according to Dorman et al. (2003). 50 g of the powdered herb was dissolved in 500 ml distilled water in a quick fit round bottom flask connected to a hydrodistillation apparatus. It was then left to slowly boil for 120 min. The water in flask was removed and replaced by another 300 ml of fresh distilled water and boiled for another 60 min, then filtered. The filtrate was subjected to lyophilization process by a freeze dryer under pressure 0.1-0.5 mbar and temperature -35 to - 41 °C. The dry extract was stored at 4 °C until used.

Animals and treatments

Male albino rats (*Rattus norvegicus*) weighing 120 ± 5 g were obtained from Veterinary Sciences Institute, Helwan, Egypt. They kept in standard laboratory condition for at least one week before initiation of the experiments, being maintained on standard rodent diet, and were given free access to food and water. The animals were housed in especially designed plastic rodent cages in animal house in Faculty of Science, Menoufia University, Shebin El-Kom, Egypt. This study and all procedures were approved by the Animal Care and Bioethics of the Egyptian Committee, and the animal work was done at Faculty of Science, Menoufia University. The animals were divided into four groups:

Group 1: Control group.

Group 2: Rats were orally administrated rosemary extract at a dose of 220 mg/kg b.w for 5 consecutive days per week for 8 weeks (Dorman et al., 2003).

Group 3: Animals were orally administrated with $CdCl_2$ at a dose level 30 mg/kg b.w for 5 consecutive days per week for 8 weeks according to Ohta et al. (2000).

Group 4: Rats were administrated $CdCl_2$ and rosemary extract for 5 days per week for 8 weeks.

Light and electron microscopic examination

The treated animals and their controls were anesthetized and dissected after 4 and 8 weeks of treatment. Kidneys were removed and fixed in 10% neutral formalin for 24 h, washed in running tap water for 24 h, and dehydrated in ascending grades of ethanol and two changes, cleared in two changes of xylene and embedded in paraplast and sections of 5 micrometer thickness were cut. Slides were stained with haematoxylin and eosin for histological examination. For ultrastructural examination very small pieces of kidney were fixed in glutaraldehyde then rinsed in phosphate buffer, post fixed in buffered solution of 1% osmium tetroxide for 3 h at 4 °C, then processed with the standard steps: dehydration, infiltration, embedding and polymerization. The ultrathin sections were examined by using JEOL electron microscope (Karnovsky, 1965).

Biochemical assays

For biochemical study, urea was measured in sera according to Tabacco et al. (1979) and creatinine was measured according to Young and Friedman, (2001). Kidneys were removed and homogenized in normal mammalian saline (0.9% NaCl) solution (1 mg tissue in 10 ml saline), using ultrasonic homogenizer. Tissue homogenate was kept in -20 °C deep freeze for one week to allow enzymes to liberate in the homogenate. Samples were centrifuged by cooling centrifuge and the supernatant was taken for biochemical analysis of enzymes. Glutathione (GSH) was estimated using the method of Buetler and Kelly (1963). Catalase (CAT) was determined according to the method of Goth (1991). Superoxide dismutase (SOD) was determined according to Beauchamp and Fridovich (1971). Lipid peroxidation was measured according to Ruiz-Larrea et al. (1994).

Statistical analysis

The data were expressed as mean± standard error. Data were analyzed by using Student's t -test and homogeneity of variances (Levene test) using statistical program of social science (SPSS) software for windows.

3 Results

Histological results

Light microscope observations

The kidney of control rat had normal renal structure of both cortex and medulla. The cortex showed a normal structure of renal glomeruli. The proximal convoluted tubules are lined with typical thicker cubic epithelium with apical brush border. The distal convoluted tubules show considerably lower cubic epithelium. The tubules have a relatively regular distinct lumen. The glomeruli capsule is lined with a flat epithelium and contains inside glomerular tuft of blood capillaries (Fig. 1)

Animals administrated $CdCl_2$ for 4 weeks showed that the kidney tissue was injured. The glomeruli were shrinkage, renal tubules became dilated and proximal tubules showed hemorrhage (Fig. 2). Cytoplasmic vacuolation appeared in the cells of the tubules and blood congestion appeared in the renal vessels (Fig. 3). Intertubular leucocytic infiltration was observed (Fig. 4). After 8 weeks of treatment with $CdCl_2$, these changes increased. Leucocytic infiltrations were increased (Fig. 5). Edematous spaces and fragmented glomeruli were observed (Fig. 6). After treatment with $CdCl_2$ and the rosemary extract, an improvement in kidney tissue was observed, the glomeruli appeared normal with capsular space and the renal tubules showed normal appearance (Fig. 7).

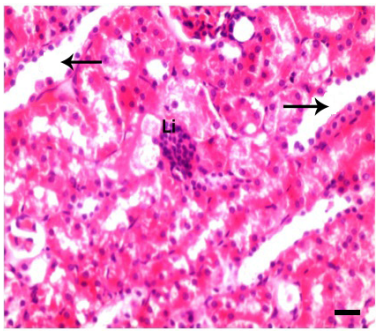

Fig (4): Kidney section of a rat treated with $CdCl_2$ for 4 weeks showing leucocytic infiltrations (Li) and disorganization of renal tubules (arrows)(H&E, scale bar = 0.02mm)

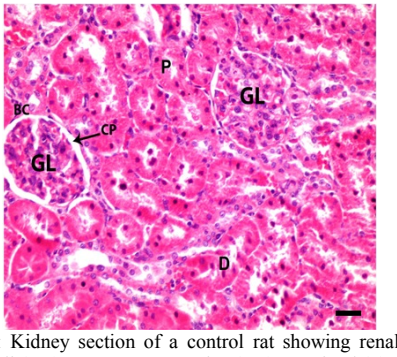

Fig (1): Kidney section of a control rat showing renal capsule, normal glomeruli (GL), Bowman's capsule (BC), proximal (P) and distal (D)

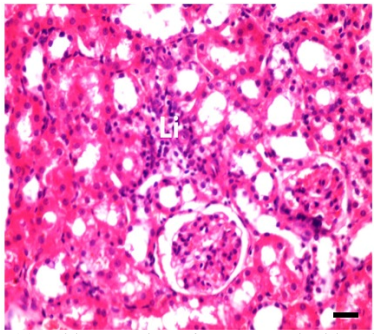

Fig (5): Kidney section of a rat treated with $CdCl_2$ for 8 weeks showing leucocytic infiltrations (Li) (H&E, scale bar = 0.02mm).

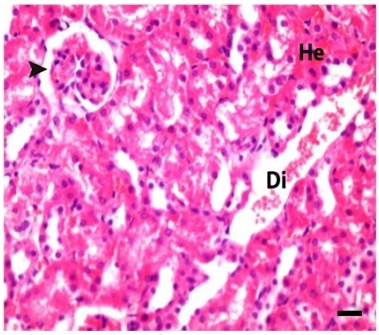

Fig (2): Kidney section of a rat treated with $CdCl_2$ for 4 weeks showing shrinkage in glomeruli (arrowhead), dilated and congested blood vessel (Di), blood hemorrhage (He)(H&E, scale bar = 0.02mm).

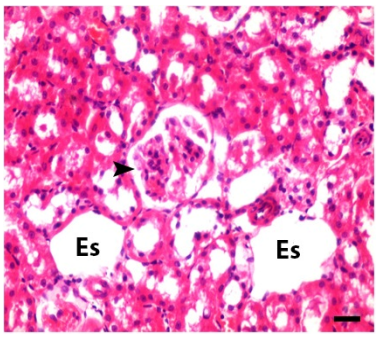

Fig (6): Kidney section of a rat treated with $CdCl_2$ for 8 weeks showing Edematous spaces (Es) and fragmented glomeruli (arrowhead) (H&E, scale bar = 0.02mm).

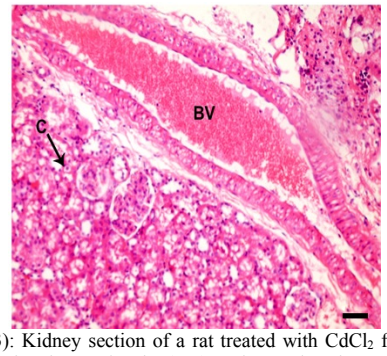

Fig (3): Kidney section of a rat treated with $CdCl_2$ for 4 weeks showing congestion in renal vein (BV) and cytoplasmic vaculation of the renal tubular cells (C) (H&E, scale bar = 0.03mm).

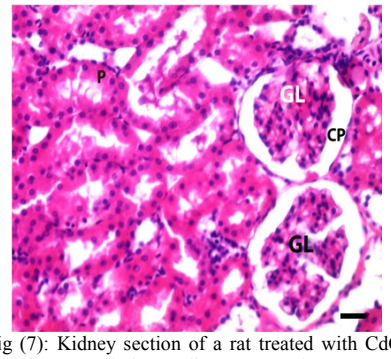

Fig (7): Kidney section of a rat treated with $CdCl_2$and rosemary extract showing normal glomeruli (GL), capsular space (CP) and normal proximal (P) convoluted tubules (H&E, scale bar = 0.02mm).

Ultrastructural observations

Kidney sections of epithelial cells of proximal convoluted tubule of control rat examined with electron microscope revealed normal nucleus, with nucleolus. The chromatin is divided to heterochromatin and euchromatin. The cytoplasm contains numerous mitochondria of various sizes, rough endoplasmic reticulum (Fig. 8). The distal renal tubules composed of tall cuboidal epithelial cells with densely packed, long mitochondria between deep infoldings, basal lamina, apical nucleus and small apical cytoplasmic microprojections (Fig. 9). The glomeruli are arterial capillary tufts, located throughout the renal cortex. The capillary tufts are encased by a fibrous structure known as Bowman's capsule that is lined with a single epithelial cell layer known as parietal epithelial cell.The glomerular capillary formed of the endothelial cells line the blood side of the capillaries and are perforated by fenestrae. The visceral epithelial cells, or podocytes, are located on the filtrate side of the capillary loops. These cells have complex cytoplasmic extensions known as primary and secondary foot processes (Fig.10).The sections obtained from animals treated with rosemary extract showed normal structure.

Many alternations were observed in rats treated with $CdCl_2$. The nucleus of the proximal convoluted tubular cells appeared irregular with abnormal shape. Increased number of lysosomes, vacuoles and dilated rough endoplasmic reticulum. The basal lamina appeared thick. Mitochondria appeared swollen with districted cristae. Ribosomes appeared detached from the rough endoplasmic reticulum (Fig. 11). Apoptotic nucleus, degenerated brush border and thickening in basal lamina were observed (Fig. 12). Shrinkage nucleus, swollen mitochondria, thickening in the basement membrane appeared in distal tubular cells (Fig. 13). Most of the foot process of podocytes appeared irregular with complete disappearance of their slit membranes (Fig.14). After treatment with $CdCl_2$ and rosemary extract, kidney sections appeared with normal nucleus, brush border. Few Lysosomes and swollen mitochondria were noticed (Fig. 15). Regular podocytes with normal podocytes cytoplasmic process were seen (Fig.16).

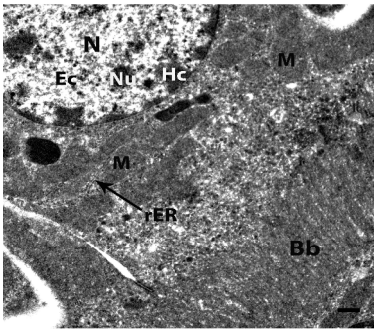

Fig (8): TEM micrograph of a normal kidney showing epithelial cell of proximal convoluted tubule with normal nucleus (N), nucleolus (Nu), heterochromatin (Hc), euchromatin (Eu), mitochondria (M), rough endoplasmic reticulum (rER) and brush border (Bb) (scale bar =500nm).

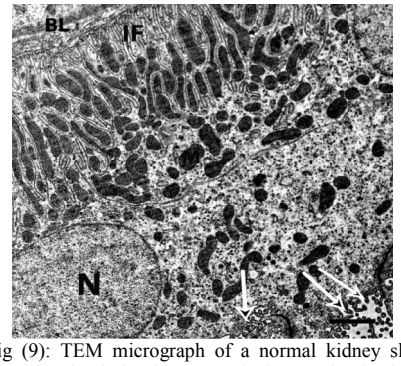

Fig (9): TEM micrograph of a normal kidney showing normal distal convoluted tubules has normal elongated mitochondria (M), numerous foldings (F), basal lamina (BL), cytoplasmic microprojections (arrows) (scale bar =200µm).

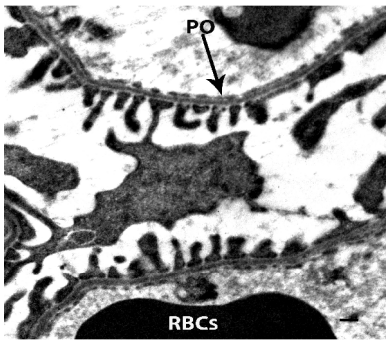

Fig (10): TEM micrograph of a normal kidney showing Malpighian corpuscle with normallly arranged podocytes (PO) process.

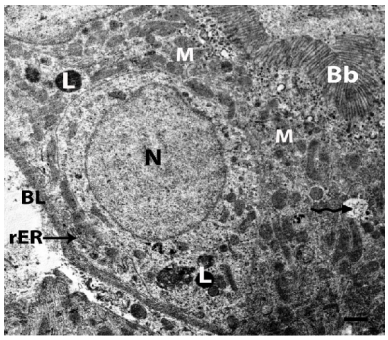

Fig (11): TEM micrograph of epithelial cells of proximal convoluted tubule of a rat treated with CdCl2 for 4 weeks showing irregular nucleus (N), dilated rough endoplasmic reticulum (rER), vacuole (irregular arrow), thick basal lamina (BL), abnormal mitochondria (M), lysosomes (L) and brush border (Bb)(scale bar =200µm).

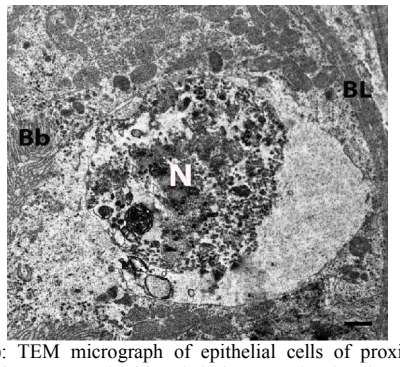

Fig (12): TEM micrograph of epithelial cells of proximal convoluted tubule of a rat treated with $CdCl_2$ for 8 weeks showing cell of proximal tubules with apoptotic nucleus (N), thickening in basal lamina (BL), and degeneration in brush border (Bb) (scale bar =200µm).

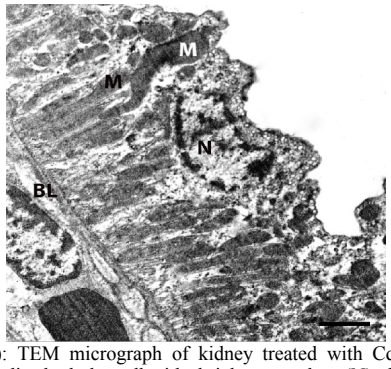

Fig (13): TEM micrograph of kidney treated with $CdCl_2$ for 8 weeks showing distal tubular cell with shrinkage nucleus (N), thickening in basal lamina (BL), and swollen mitochondria (M) (scale bar =200µm).

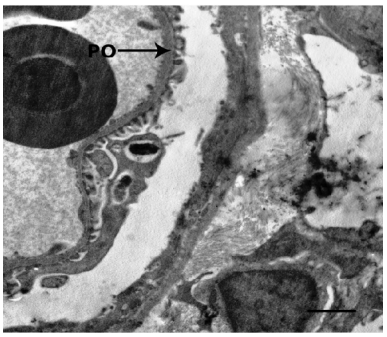

Fig (14): TEM micrograph of kidney treated with $CdCl_2$ for 8 weeks showing Malpighian corpuscle with irregular cytoplasmic process of podocytes (PO) (scale bar =200µm).

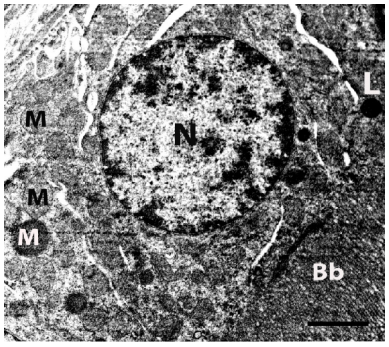

Fig (15): TEM micrograph of kidney treated with $CdCl_2$ and rosemary extract showing normal nucleus (N) and brush border (Bb). Lysosomes (L) and swollen mitochondria (M) (scale bar =200µm).

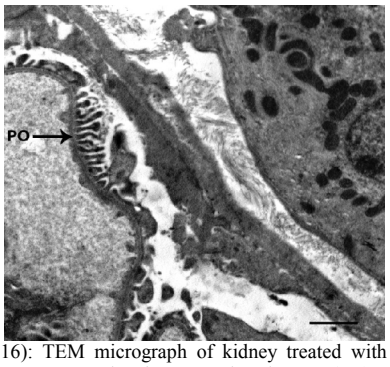

Fig (16): TEM micrograph of kidney treated with CdCl2 followed by rosemary extract showing normal podocytes (PO) arrangement,) (scale bar =200µm).

Biochemical results

Animals received rosemary extract alone showed no significant differences in the whole set of biochemical parameters compared with the control group. Data in Figs. 17, 18 revealed that there are highly significant increase ($P<0.001$) in serum urea and creatinine in animals treated with $CdCl_2$ after 4 and 8 weeks. A significant decrease was recorded in serum urea and creatinine after 8 weeks treatment with $CdCl_2$ and rosemary extract. Animals treated with $CdCl_2$ showed that levels of GSH, CAT and SOD were decreased significantly ($P<0.001$) in comparison with control group (Figs. 19-21). On the other hand, these enzymes increased significantly ($P<0.001$) after 8 weeks of treatment with rosemary extract when compared with $CdCl_2$ group. MDA marker, the lipid peroxidation, was increased in animals treated with $CdCl_2$, but after treatment with $CdCl_2$ and rosemary extract MDA decreased (Fig. 22).

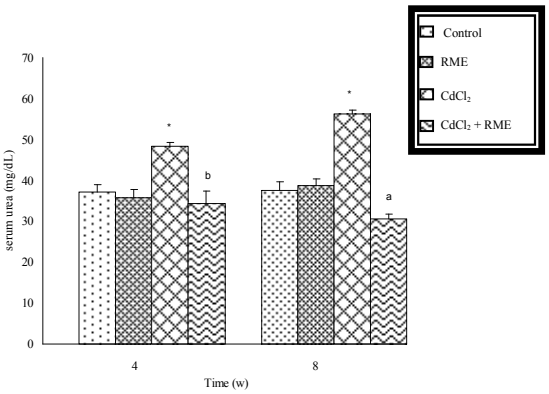

Fig (17): Effects of cadmium and rosemary extract on serum urea.

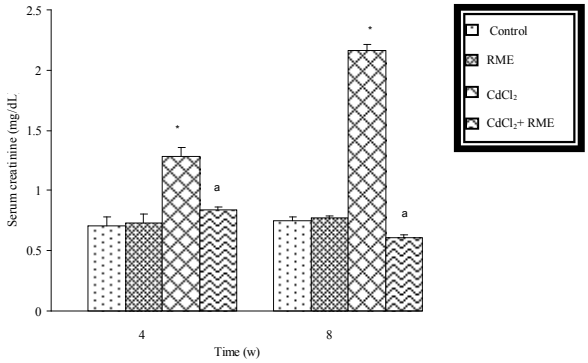

Fig (18): Effects of cadmium and rosemary extract on serum creatinine.

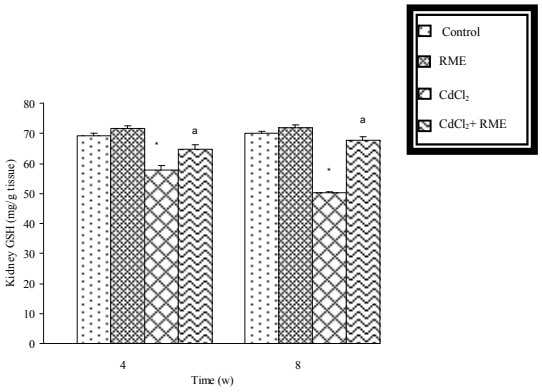

Fig (19): Effects of cadmium and rosemary extract on Kidney GSH.

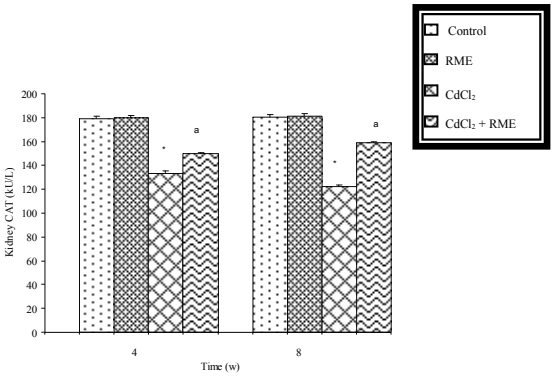

Fig (20): Effects of cadmium and rosemary extract on Kidney CAT.
Where:
(*): highly significant (P<0.001) in comparison with control group
(a): highly significant (P<0.001) in comparison with $CdCl_2$ group.
(b): very significant (P<0.01) in comparison with $CdCl_2$ group.
(c): significant (P<0.05) in comparison with $CdCl_2$ group.

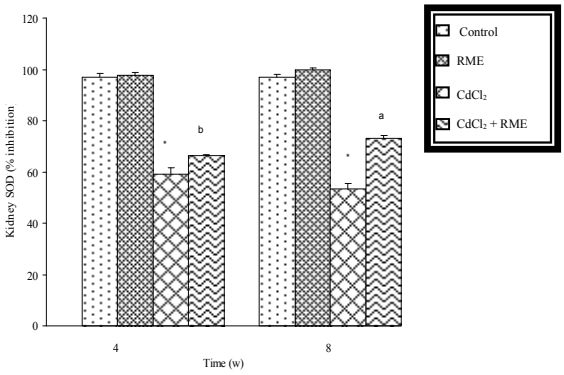

Fig (21): Effects of cadmium and rosemary extract on Kidney SOD.

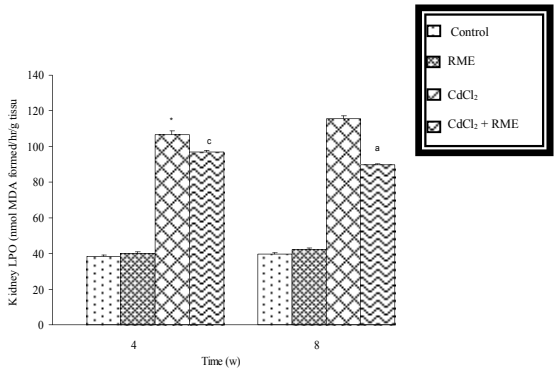

Fig (22): Effects of cadmium and rosemary extract on Kidney MDA.

4 Discussion

The present results showed that $CdCl_2$ administration caused nephrotoxicity in rats. Histological and ultrastructural observations showed many changes. The effect of cadmium on the kidney was studied by many investigators. Brzóska et al. (2003) observed enlargement in the renal glomeruli besides, mononuclear infiltration in rats treated with $CdCl_2$. After rats treated with $CdCl_2$ for 8 weeks, glomeruli became enlarged. The renal tubules were vacuolated and their nuclei appeared densely stained after exposed to $CdCl_2$ (Abdel-Moneim and Ghafeer, 2007). When $CdCl_2$ given to rats in drinking water, the renal tubules became degenerated and congested blood vessels noticed (Mehana, 2008). Degeneration in proximal and distal tubules, apoptotic cells in proximal tubules observed after rats treated with $CdCl_2$ (Kukner et al., 2007).

Mahran et al. (2011) reported that $CdCl_2$ caused loss of brush border, nuclear membrane damage, chromatin condensation, swelling mitochondria, increased number of lysosomes and degranulation and disintegration of rough

endoplasmic reticulum in kidney of rats. Kidney of cadmium treated rats showed glomerular swelling, tubular dilation and degenerate in both glomeruli and renal tubules (Tripathi and Sirvastav, 2001). Inflammationin in certain region of the kidney cortex of female rats was observed after exposure to $CdCl_2$ (Mohammad et al., 2013). Wide spread of necrosis was observed in both tubules and glomeruli, and no nuclei identified after rats received daily $CdCl_2$ (Hussain and AL-Taee, 2014). Thickening of basement membrane in distal tubules and elongated mitochondria observed in kidney of rats treated with $CdCl_2$ (Saber et al., 2013). Cadmium given tofemale rats for 4 weeks revealed dilation of renal tubules with hemorrhage and degeneration (Muhammed, 2014). Among the histological observations in the present work is the cytoplasmic vacuolization of the tubular epitlelia. Tripathi and Srivastav (2011) explained that the tubular vacuolization might be an indicator of hydrolytic changes in the renal tissue and indicated that toxicant might cause a failure in the ion pump transport of tubular cells which caused swelling of epithelium and degeneration of tubules. These alterations also suggested incapability of renal cells to cope with functional disturbance provoked by toxicants. The results obtained in this work revealed increased in serum urea and creatinine of $CdCl_2$-treated rats. These results in agreement with results obtained by Gaurav et al. (2011), who found that serum urea and creatinine increased after rats treatment with $CdCl_2$. Urea and creatinine increased in serum of rats treated with $CdCl_2$ (Koriem et al., 2013, Hussein et al., 2014, El-Boshy et al., 2015).Increased in urea and creatinine may be attributed to that cadmium boned to metallothionein in the liver and released into plasma then filtered in the glomerular and taken by the proximal tubules of the kidney. Cadmium damages the proximal tubular cells of the kidney (Sudo et al., 1996). The Non- enzymatic antioxidant GSH decreased in this study. This similar to results showed by Hagar and Al Malki, (2014) and El-Boshy et al., (2015) who found that $CdCl_2$caused decline in renal GSH in rats. The reduction in GSH might due to its consumption in the scavenging of free radicals generated by cadmium (Nigam et al., 1999). Also may be consumed in the detoxification of Cd. It has been reported that the sulfhydryl group of cysteine moiety of GSH has affinity for metals such as Cd, forming thermodynamically stable mercaptides complexes which as inert and excreted *via* the bile (Mohanpuria et al., 2007). Lipid peroxidation marker MDA increased, while antioxidant enzymes CAT and SOD decreased in kidney of $CdCl_2$-treated rats. These results run parallel with those obtained by Bekheet et al. (2011), who found that $CdCl_2$ caused decreased in renal CAT and SOD, and increased in MDA in rats. Also, significant increase in MDA and significant decrease in CAT and SOD was showed in kidney of rats treated with $CdCl_2$ (Renugadevi and Prabu, 2010, Virk et al., 2013).These changes in antioxidant systems may be due to generation of free radicals by cadmium toxicity (Bagchi et al., 1996). Over-production of ROS induces oxidative stress and lipid peroxidation, this over production may attribute to the direct action of cadmium on peroxidation reaction and iron- mediated peroxidation (Pillai and Gupta, 2005). Injury of cells resulting from binding of cadmium to sulphydral groups found in mitochondria leading to inactivation of sulphydryl group causes oxidative stress (Adikwu et al., 2013). Decrease in CAT and SOD activity might be a result of metals deficiency. Cadmium caused decrease in iron (Jurczuk et al., 2004), which act as active site of CAT. Aqueous extract of rosemary ameliorated the toxic effects of $CdCl_2$ on the kidney. These results are similar to that obtained by Rasha and Abdella (2010), who found that rosemary leaf aqueous extract reduced all histological changes in kidney caused by doxorubicin. Streptozotocin caused histological changes such as hemorrhage and damage in glomeruli, but after injection with rosemary all changes decreased (Ayaz, 2012). Gentamicin caused degeneration in proximal tubules, disruption in brush border and extensive leucocytic infiltration, after treatment with rosemary these changes restored (Azab et al., 2014). El-Mougy and Youssef, (2011) reported that rosemary extract prevents the increase in urea and creatinine caused by azathioprine. When rosemary extract received before CCl_4, urea and creatinine decreased (Metwally et al., 2012). Pretreatment with rosemary extract before aspartame caused decline in serum urea and creatinine (Hozayen et al., 2014). Rosemary extract caused increase in renal antioxidant enzymes and decreased MDA. These observations are in agreement with the result of Rasha and Abdella (2010), who found that aqueous leave extract of rosemary increased renal GSH, CAT and SOD, and decreased renal MDA after administration of doxorubicin. Rosmarinic acid is one of important component of rosemary caused increased in renal GSH, CAT and SOD, and decreased renal MDA when given with gentamicin sulphate to rats (Tavafi and Ahmadvand, 2011). Rosemary given with lead acetate caused a decrease in renal MDA, increased in GSH, CAT, SOD (Abdel El Kader et al., 2012). An increase in renal GSH, CAT and SOD and a decrease in MDA was recorded in rats treated with aspartame and rosemary extract (Hozayen et al., 2014).The major proposal for action of rosemary is to intercept the free radicals and protect cellular molecules from oxidative damage (Sancheti and Goyal, 2007).Many constituents in rosemary such as rosmarinic and caffeic, phenolic compounds and flavonoids has been reported as antioxidant, anti-inflammatory, anti-microbial, anti- tumor and anti- mutagenic (Capecka et al., 2005). In this work, aqueous leaf extract of rosemary ameliorated the toxic effects in the kidney caused by $CdCl_2$ and this may be a result of its antioxidant properties.

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