

PROTECTIVE EFFECT OF THYMUS VULGARIS EXTRACT AGAINST CADMIUM INDUCED NEPHROTOXICITY AND TESTICULAR DAMAGE IN ALBINO RATS

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ABSTRACT

40 male albino rats were used for these experimental studies. They were divided equally into 4 groups: the first group was used as control group, the second group was given thyme extract (300 mg/kg b.w) by gastric intubation, the third group was given CdCl₂ (3mg/kg b.w) intraperitoneally while the fourth group were treated by both agents. Cadmium chloride treated rats exhibited microcytic hypochromic anemia and a significant ($P \leq 0.05$) increase in total leucocytic count as well as a significant increase in serum urea, creatinine and malondialdehyde. Moreover a significant decrease in serum total proteins, albumin, globulin, A/G ratio and catalase were detected. Histopathologically, marked degenerative changes were observed in renal and testicular tissues in CdCl₂ treated rats along with diminished expression of Bcl-2 (B-cell lymphoma2) in renal tissues. However, thyme extract treatment ameliorated the deleterious effects of CdCl₂ on biochemical parameters, enhanced the endogenous antioxidant status, reduced lipid peroxidation and the lesions in renal tissues. Thyme extract restored the immunohistochemical expression of Bcl-2. However, hematological parameters and sever testicular degeneration were not alleviated by thyme extract. Our findings also demonstrated that thyme extract can alleviate CdCl₂ nephrotoxicity via its potent antioxidant and anti-apoptotic activities.

Key words: Cadmium, nephrotoxicity, testicular, protective, oxidative stress.

INTRODUCTION

Cadmium (Cd) is one of the most serious environmental and occupational contaminants exhibiting serious health hazards to human as well as animals. People chronically exposed to low doses of Cd, possess a wide range of tissue damage with increase incidences of cardiovascular diseases (Tellez-Plaza *et al.*, 2013), renal damage (Wang *et al.*, 2016), loss of nutritional elements and minerals which later resulting in diabetes and osteoporosis (Satarug *et al.*, 2011), induce apoptosis in rat tissues including testes (Zhao *et al.*, 2015) and reduced sperm count or even infertility (Li *et al.*, 2016). Reactive oxygen species (ROS) and free radicals are implicated in Cd toxicity inducing oxidative damage in various tissues (Liu *et al.*, 2009). Liver and kidneys are considered the most sensitive tissues to Cd toxicity (Ognjanovic *et al.*, 2010). Cadmium nephrotoxicity was resulted from generating free radicals and consequently inducing cell necrosis and

apoptosis (Reyes *et al.*, 2002; El-Sharaky *et al.*, 2007). To date there is no effective medication to Cd toxicity (Matovic *et al.*, 2011). Medicinal plants are considered a new resource for future medications (Cordero-Perez *et al.*, 2013). Many studies have highlighted the antioxidant properties of numerous natural products against many toxicants (Shatti *et al.*, 2011). *Thymus vulgaris* (thyme) is one of Lamiaceae family which has a potent natural antioxidants fighting several diseases even cancers (Cerdeira *et al.*, 2013). Thyme is cultivated worldwide for numerous medical purposes (Abu-Darwish *et al.*, 2009). It is used in the treatment of gastroenteric disorders, bronchopulmonary disorders and also is used as anthelmintic, antispasmodic, sedative, antimicrobial and antifungal (El Nekeety *et al.*, 2011). Therefore, the present study aimed to assess whether *Thymus vulgaris* extract could antagonize the deleterious effects of Cd on the hematological, biochemical, oxidative stress, histopathological criteria in kidney as well as immunohistochemical expression of Bcl-2 in renal tissue and its effect on the histological structure of testes.

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MATERIALS AND METHODS

1. Collection of Thyme and preparation of the extract

The plant material was dried at ambient temperature. The air dried plant materials were grounded to fine powder. This fine powder was soaked in adequate volume of 70 % ethyl alcohol. Extraction was carried out by intermittent shaking at room temperature for 3 days. After filtration through filter paper (Whatman No. 4), the residue was re-extracted twice, then the extract was evaporated under reduced pressure using a rotatory evaporator and dried to obtain a constant weight. The residue was stored at 4°C until use according to Williamson *et al.* (1996).

2. Cadmium chloride (CdCl₂): was purchased from (Sigma Aldrich Corporation, St. Louis, Missouri, USA).

3. Animals and treatments:

Forty male Albino rats 4 weeks old weighing (90-100) grams, obtained from the Animal House Colony of the National Research Center, Giza, Egypt, were used in the present study. Rats were acclimatized for one week to laboratory conditions, and provided with commercial balanced diet and tap water ad-libitum throughout the experiment. Rats were randomly divided into 4 groups each consisting of 10 animals and were treated as shown in Table (1).

Table 1: Experimental groups and their treatments.

Groups		Thyme extract			Cadmium chloride		
No	Name	Route	Dose	Duration	Route	Dose	Duration
1	Control	–	–	–	–	–	–
2	Thyme	Intragastric	300mg/kg b.w.*	21 days	–	–	–
3	CdCl ₂	–	–	–	Intraperitoneal	3mg/kg b.w.	5 times/week for 3weeks
4	Thyme/Cd	Intragastric	300mg/kg b.w.	3days before CdCl ₂ treatment and continued daily for 3 weeks	Intraperitoneal	3mg/kg b.w.	5 times/week for 3weeks

*b.w. means body weight

At the end of the experimental period, two blood samples were withdrawn from the medial canthus of the eye under light ether anesthesia and immediately divided into aliquots one with anticoagulant for hematological examination and the other left to clot, then centrifuged for 10 minutes at 3000 r.p.m. Serum was separated and stored in eppendorf tubes at –20°C to be used for biochemical analysis. After blood collection, the rats were sacrificed by decapitation. Kidneys were collected and rapidly excised from each animal, trimmed and divided into two parts; the first part was washed free of blood with 0.9% NaCl solution and distilled water to assess tissue oxidative status and antioxidant indices. The second part was used for histopathological examination and immunohistochemistry. Also tissue sections from the testes were taken for histopathology.

4. Hematological parameters:

The aliquot contained EDTA (1mg/ml) were used for assessing red blood cells (RBCs), hemoglobin content (Hb), and packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total leucocytic count (TLC) and differential leucocytic count according to Feldman *et al.* (2000).

5. Biochemical parameters:

Total proteins and albumin were estimated according to Burtis and Ashwood (1999) and Dumas and Biggs (1972) respectively. Globulin concentration (Glob) in serum was measured by subtracting albumin concentration from total proteins and consequently albumin to globulin ratio (A/G) was calculated. Serum urea and creatinine were estimated spectrophotometrically (Biomerieux, Egypt) according to Fawcett and Soctt (1960) and Szasz *et al.* (1979) respectively.

6. Estimation of antioxidants and lipoperoxidation markers in renal tissues:

Only the renal tissues were used but the testicular tissues were not used. The dissected renal tissues were washed with 50 mM sodium phosphate-buffered saline (100 mM Na₂ HPO₄/NaH₂PO₄, pH 7.4) in an ice-containing medium, with 0.1 mM EDTA to remove any RBCs and clots. Then tissues were homogenized in 5-10 ml cold buffer/gm tissue and were centrifuged at 5000 rpm for 30 min. The resulting supernatant was transferred into an eppendorf tube and was preserved at –80°C into aliquots for the spectrophotometric estimation of tissue malondialdehyde (MDA) Mihara and Uchiyama (1978) and catalase (CAT) Aebi (1984).

7. Histopathology:

Tissue specimens from the kidneys and testes of each animal were excised, directly fixed in 10% formalin solution, dehydrated in alcohols, cleared in xylene and embedded in paraffin blocks. Sections of 5 μm thickness were obtained and stained with haematoxylin and eosin (H&E) according to Bancroft *et al.* (1991).

8. Immunohistochemistry of Bcl-2 expression in renal tissues:

Immunohistochemical labelling of Bcl-2 was performed on the kidneys of all tested animals using 4- μm thick paraffin-embedded sections. The sections were dewaxed in xylene and rehydrated in graded ethanol. For antigen retrieval, the sections were immersed in (EDTA solution, pH 8). Endogenous peroxidase was blocked by incubation in 0.3% hydrogen peroxide in methanol for 20min at room temperature (RT). The sections were incubated with Protein Block Serum Free to prevent the binding of non-specific proteins. Immunolabelling of Bcl-2 was performed on all samples, using anti-Bcl-2 (Santa Cruz). Sections were incubated with primary antibodies Bcl-2 1:100 overnight at 4°C. Then, the sections were washed 3 times with phosphate-buffered saline (PBS), and incubated with anti-mouse IgG secondary antibodies (EnVision + System HRP; Dako) for 30 minutes at RT. The sections were washed with PBS and visualised with EnVision+ System, HRP-labelled polymer anti-rabbit (Dako®). After washing 3 times with PBS, 3,3'-diaminobenzidinetetrahydrochloride (Liquid DAB + Substrate Chromogen System, Dako®) was added to

the sections. The sections were then washed in distilled water, counterstained with Mayer's haematoxylin, dehydrated in an alcohol gradient, cleared with xylene and mounted for examination under light microscope.

9. Statistical analysis:

A one way ANOVA with Duncan multiple comparison tests were used to compare significance between different experimental groups. Means and standard error for each variable were estimated. Differences between means of different groups carried out using dissimilar superscript, which showed a significance at ($P \leq 0.05$).

RESULTS

Hematological findings:

The effects of CdCl_2 toxicity and the preventive effects of thyme extract on the hematological parameters are shown in Table (2). Thyme extract sole treated group revealed a significant ($P \leq 0.05$) decrease in erythrogram parameters (RBCs, HB, PCV, MCV, MCH). A significant ($P \leq 0.05$) decreases in blood parameters (RBCs, HB, PCV, MCV and MCH) in CdCl_2 intoxicated rats compared to the control group were detected. This picture has not been improved in rats group received both CdCl_2 and thyme extract. Contrary to these results, a significant ($P \leq 0.05$) increase in TLC, neutrophil, lymphocyte and monocyte counts were observed in both CdCl_2 and CdCl_2 simultaneously supplemented with thyme extract compared to control group.

Table 2: Hematological parameters in different experimental groups.

Group	Control	Thyme	CdCl_2	Thyme+ CdCl_2
Parameters				
RBCs ($\times 10^6/\mu\text{l}$)	7.55 \pm 0.03 ^a	6.24 \pm 0.08 ^b	6.08 \pm 0.15 ^b	5.83 \pm 0.22 ^b
Hb (gm/dl)	13.5 \pm 0.15 ^a	11.06 \pm 0.19 ^b	10.0 \pm 0.31 ^c	9.9 \pm 0.30 ^c
PCV (%)	40.4 \pm 0.49 ^a	33.5 \pm 0.79 ^b	30.2 \pm 1.11 ^{bc}	28.3 \pm 0.9 ^c
MCV (fl)	53.5 \pm 0.75 ^a	53.7 \pm 0.51 ^a	49.6 \pm 0.71 ^b	48.5 \pm 0.91 ^b
MCH (pg)	17.9 \pm 0.2 ^a	17.7 \pm 0.2 ^a	16.4 \pm 0.2 ^b	16.9 \pm 0.3 ^b
MCHC (%)	33.4 \pm 0.2	33 \pm 0.2	33.11 \pm 0.3	34.9 \pm 0.3
TLC($\times 10^3/\mu\text{l}$)	7.88 \pm 0.64 ^b	6.81 \pm 0.75 ^b	19.3 \pm 1.12 ^a	18.5 \pm 1.78 ^a
Neutrophils ($\times 10^3/\mu\text{l}$)	4.24 \pm 0.45 ^b	3.57 \pm 0.18 ^b	7.84 \pm 0.82 ^a	6.74 \pm 0.64 ^a
Lymphocytes ($\times 10^3/\mu\text{l}$)	2.1 \pm 0.28 ^b	2.02 \pm 0.48 ^b	8.23 \pm 0.82 ^a	8.9 \pm 1.11 ^a
Monocytes ($\times 10^3/\mu\text{l}$)	1.53 \pm 0.17 ^b	1.1 \pm 0.18 ^b	3.26 \pm 0.3 ^a	2.86 \pm 0.5 ^a

The data are presented as means \pm S.E. (n = 10). Cadmium chloride (CdCl_2), red blood corpuscles (RBCs), hemoglobin (Hb), packed cell volume (PCV), Total leucocytic count (TLC). Different superscript letters within a row indicate significantly different mean values ($p \leq 0.05$).

Biochemical parameters:

The adverse effects of CdCl_2 and the ameliorative effects of thyme on serum protein profile (total proteins, albumin, globulin and A/G) in control and treated rats are shown in Figure (1). Rats treated with thyme extract alone showed a non-significant change in total proteins, albumin and globulin concentrations

while a significant ($P \leq 0.05$) decrease in A/G was detected when compared to control rats. On the other hand, CdCl_2 treated group represented a significant ($P \leq 0.05$) decline in serum total proteins, albumin, globulins and A/G compared with control rats. Pretreated rats with thyme extract together with CdCl_2 administration showed a significant ($P \leq 0.05$)

increase in serum total proteins, albumin and A/G with a non-significant change in globulin concentration compared to CdCl₂ treated groups. The deleterious effects of CdCl₂ and the preventive effects of thyme extract on serum urea and creatinine in control and treated rats are shown in Figure (2). Thyme extract sole treated rats showed a non-significant change in urea and creatinine

concentrations when compared to control rats. In addition, CdCl₂ treated group revealed a significant ($P \leq 0.05$) increase in urea and creatinine concentrations indicating nephrotoxicity compared to control rats. Pretreated rats with thyme extract along with CdCl₂ administration showed a significant ($P \leq 0.05$) decrease in urea and creatinine compared to CdCl₂ treated groups.

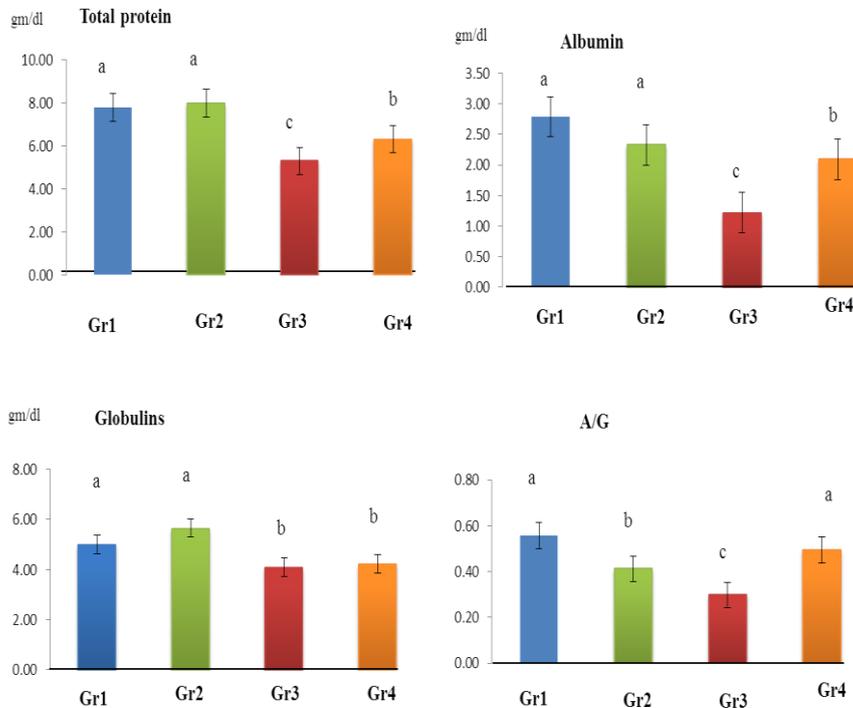


Figure 1: Serum protein profile (total protein, albumin, globulin and A/G) in control and treated groups. Data are expressed as mean ± standard error (SEM). Different letters with each column indicate significant differences when compared to the control group at $p < 0.05$. A/G = (albumin/globulin ratio). Gr1= control group, Gr2=Thyme extract treated group (300mg/ kg b.w) intragastric daily for 21 days, Gr3=Cadmium chloride CdCl₂ treated group and Gr4=(Thyme (300mg/ kg b.w) intragastric for 3days before CdCl₂ injection and continued along with CdCl₂ injection (3 mg/kg b.w) 5 times per week for 3 weeks

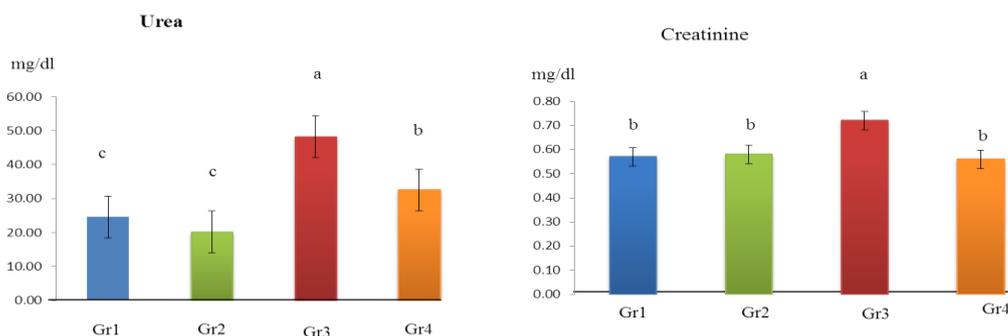


Figure 2: Serum urea and creatinine. Data are expressed as mean ± standard error (SEM). Different letters with each column indicate significant differences when compared to the control group at $p < 0.05$. Gr1= control group, Gr2=Thyme extract treated group (300mg/ kg b.w) intragastric daily for 21 days, Gr3=Cadmium chloride CdCl₂ treated group and Gr4=(Thyme (300mg/ kg b.w) intragastric for 3days before CdCl₂ injection and continued along with CdCl₂ injection (3 mg/kg b.w) 5 times per week for 3 weeks.

Oxidative stress and antioxidant biomarkers:

The adverse effects of CdCl₂ and ameliorative effects of thyme extract on renal lipid peroxidation and antioxidant parameter catalase (CAT) are shown in Figure (3). CdCl₂ treated rats revealed a significant ($P < 0.05$) increase in renal MDA and a significant

($P < 0.05$) reduction in renal CAT compared to control rats. While, thyme pretreated rats along with CdCl₂ administration revealed a significant ($P < 0.05$) reduction in MDA level and a significant ($P < 0.05$) enhancement of CAT level compared to Cd group.

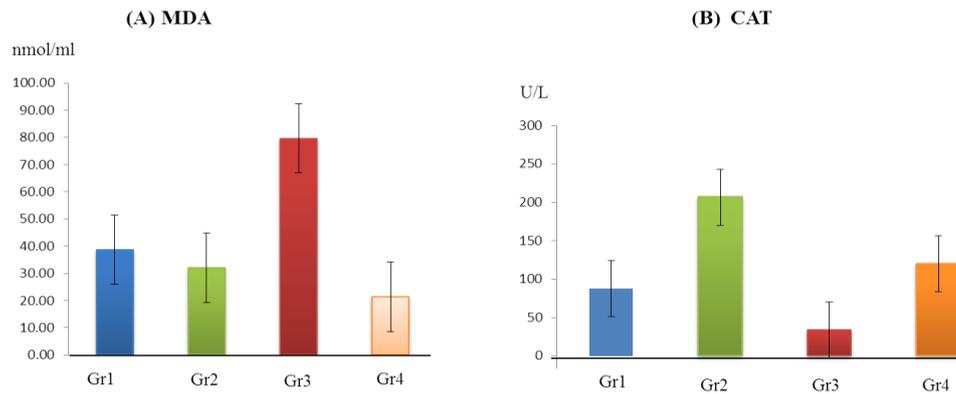


Figure 3: Renal lipid peroxidation and antioxidant parameter in control and treated groups; (A) MDA: malondialdehyde, (B) CAT catalase. Data are expressed as mean \pm standard error (SEM). Different letters with each column indicate significant differences when compared to the control group at $p < 0.05$. Gr1= control group, Gr2=Thyme extract treated group (300mg/ kg b.w) intragastric daily for 21 days, Gr3=Cadmium chloride CdCl₂ treated group and Gr4=(Thyme (300mg/ kg b.w) intragastric for 3days before CdCl₂ injection and continued along with CdCl₂ injection (3 mg/kg b.w) 5 times per week for 3 weeks

Histopathology:

a. Kidneys:

Healthy control rats showed normal appearance of glomeruli and renal tubules (Fig4. A). No obvious histopathological changes were noticed in the kidneys of thyme supplemented rats. Kidneys of CdCl₂ treated rats revealed marked congestion of inter-tubular blood vessels and glomerular capillaries (Fig4. B), hyaline casts (Fig4. C), together with marked vacuolar degeneration of tubular epithelium with nuclear pyknosis in both

cortical (Fig4. D) and medullary regions (Fig5. A). In addition, focal areas of tubular coagulative necrosis with mild mononuclear cells infiltration (Fig5. B) were observed. Also, focal regenerative changes were detected as prominent nucleoli and basophilic cytoplasm (Fig5. C). Combined administration of thyme/Cd resulted in marked restoration of normal cortical and medullary renal parenchyma with minimal vacuolar degenerative changes of tubular epithelium (Fig5. D, E).

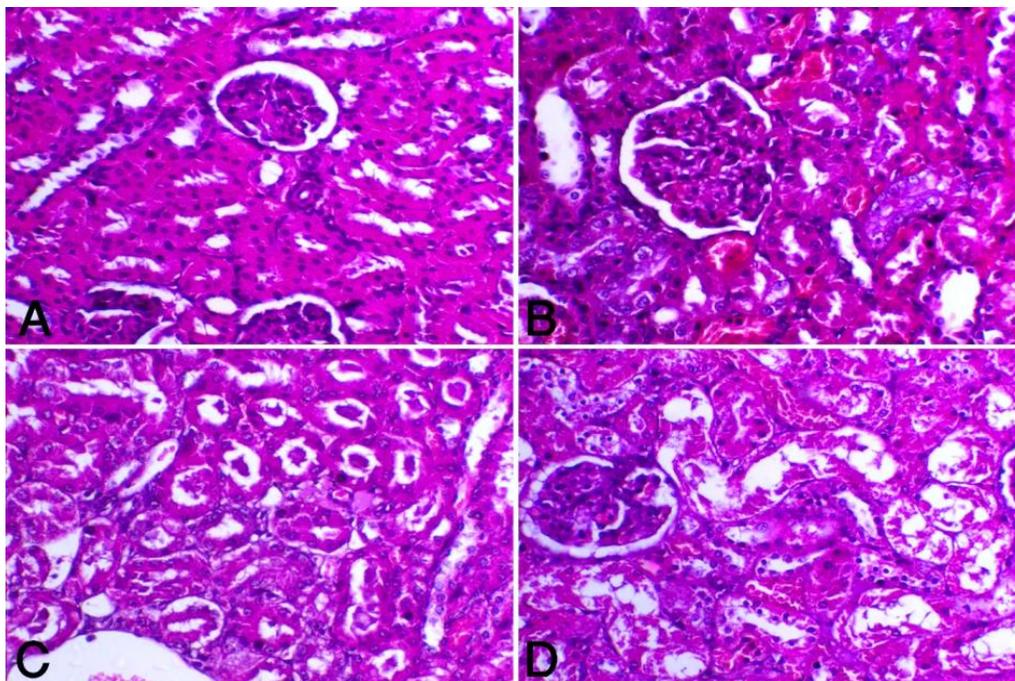


Figure (4): Histopathological changes in the kidneys of cadmium intoxicated rats.

(A): Kidney of control rats showing normal glomeruli and renal tubules (HE, 10 x20). (B): Kidney of Cd treated group showing marked congestion of inter-tubular blood vessels (HE, 10x20). (C): Kidney of Cd treated group showing marked congestion of glomerular and inter-tubular blood vessels (HE, 10x20) (D): Kidney of Cd treated group, renal cortex showing extensive vacuolar degeneration of the renal tubular epithelium with pyknotic nuclei and congestion of inter-tubular blood vessels (HE, 10x20).

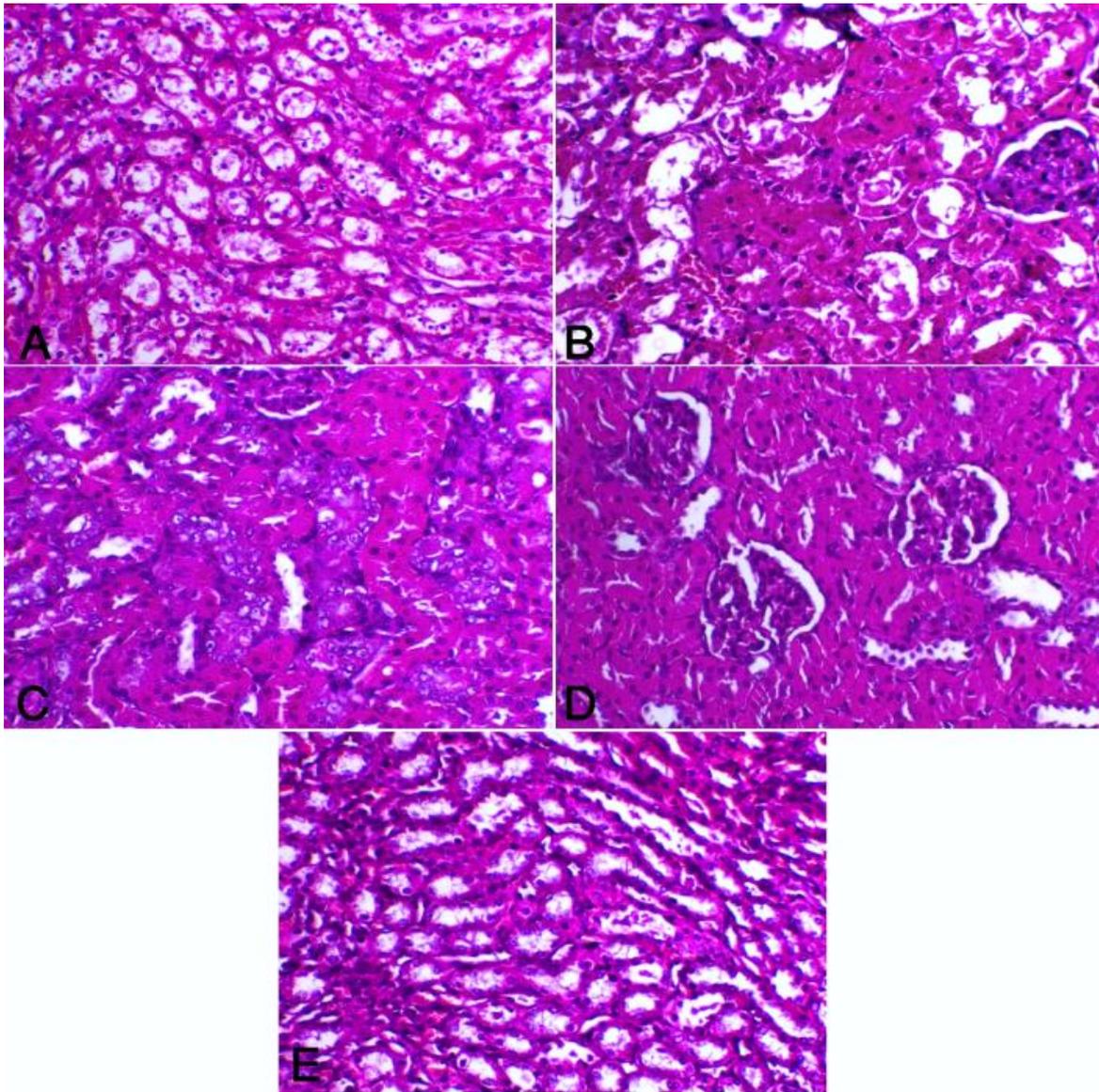


Figure (5): Histopathological changes in the kidneys of cadmium intoxicated rats and the protective effect of thyme.

(A): Renal medulla of Cd treated group, showing extensive vacuolar degeneration of the tubular epithelium with pyknotic nuclei (HE, 10 x10). (B): Kidney of Cd treated group showing focal area of coagulative necrosis with mononuclear cells infiltration (HE, 10x20). (C): Kidney of Cd treated group showing tubular regeneration with prominent nucleoli and basophilic cytoplasm (HE, 10x20) (D): Kidney of thyme/Cd treated group showing restoration of histological architecture of renal cortex with mild vacuolar degeneration of some tubules (HE, 10x20). (E): Renal medulla of thyme/Cd treated group showing restoration of histological architecture of renal medulla (HE, 10x20).

b. Testes:

Histological examination of the testes of the control group, showed normal testicular histology of seminiferous tubules, with all stages of spermatogenic cells and normal clusters of leydig cells (Fig6. A). No obvious histopathological changes were noticed in the testes of thyme supplemented rats. By contrast, the rats treated with CdCl₂ showed reduction in testicular size on gross examination and numerous histopathological alterations in the form of: marked

coagulative necrosis and the tubular epithelium are totally disorganized and necrotic with loss of lining cells (Fig6. B), vacuolation of the seminiferous tubular cells, associated with impaired spermatogenesis and dilatation of the interstitial spaces with degeneration of leydig cells (Fig6. C). However, combined administration of thyme/Cd didn't showed any improvement in the testicular architecture or restoration of normal histological structure when compared with control rats (Fig6. D).

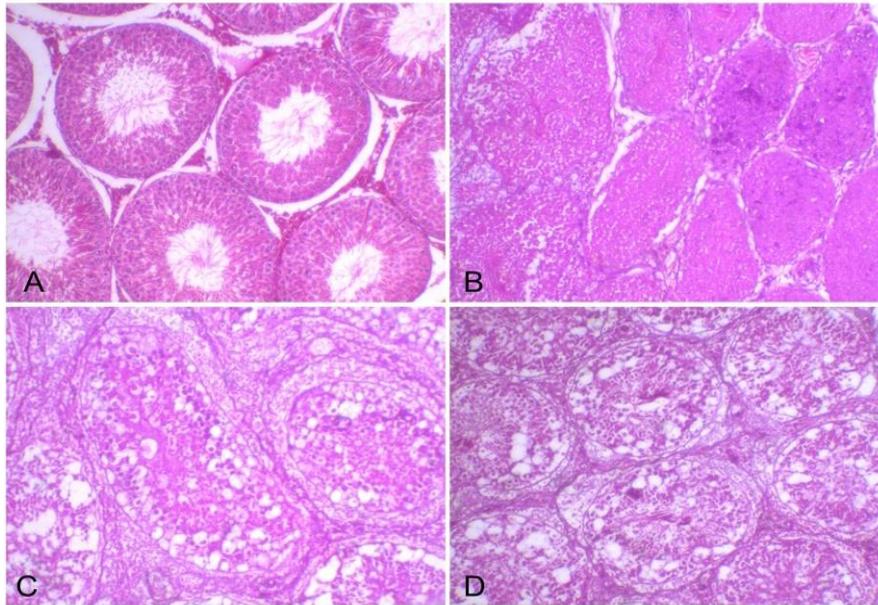


Figure (6): Histopathological changes in the testes of cadmium intoxicated rats and the protective effect of thyme. (A): Testicular architecture in the control normal rats showing well-organized distribution of seminiferous tubules with active spermatogenesis and normal clusters of Leydig cells (HE, 10 x10). (B): Testes of Cd exposed group showing extensive coagulative necrosis of the seminiferous tubules (HE, 10x10). (C): Testes of Cd exposed group showing marked vacuolation of germinal epithelium with degeneration of Leydig cells (HE, 10x20) (D): Testes of thyme/Cd treated group showing marked vacuolation of germinal epithelium (HE, 10x20).

Immunohistochemistry:

Immunolabelling of Bcl-2 was observed in the cytoplasm of renal tubular epithelial cells of control rats (Fig7. A). Very weak expression of Bcl-2 was observed in the cytoplasm of few tubular cells in both cortical and medullary regions in Cd intoxicated rats

(Fig7. B, C). However, most of renal tubular epithelial cells in the cortical and medullary regions were strongly expressed Bcl-2 in rats received thyme/Cd when compared with CdCl₂ intoxicated rats (Fig7. D, E).

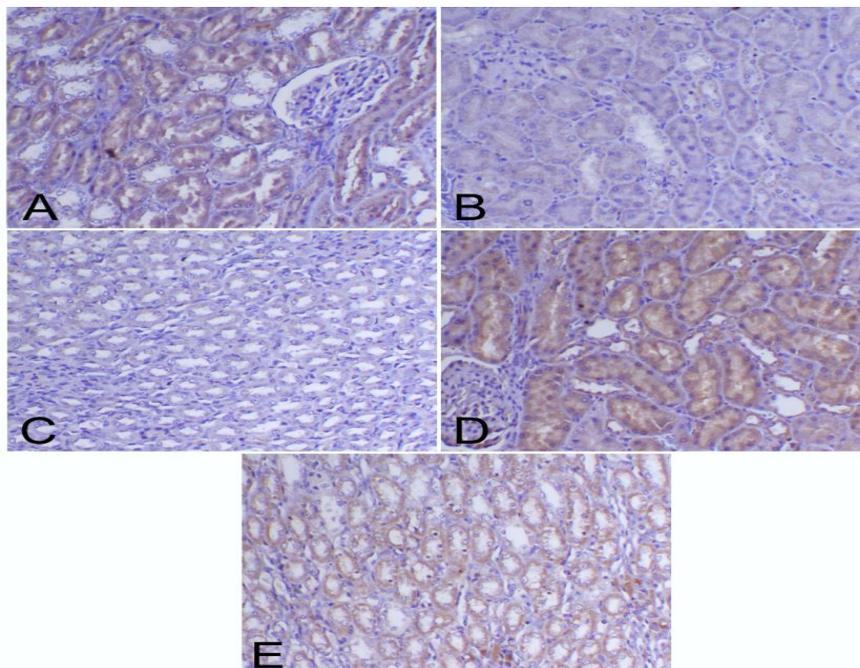


Figure (7): Immunohistochemical expression of Bcl-2 in the kidneys of Cd intoxicated rats. (A): Kidney of control group showing immunoreactivity of Bcl-2 in the cytoplasm of renal tubular cells (IHC, 10x20). (B): Renal cortex of Cd treated group, showing very weak immunoreactivity in the cytoplasm of few renal tubular cells (IHC, 10x20). (C): Renal medulla of Cd treated group, showing very weak immunoreactivity in few of renal tubular cells (IHC, 10x20). (D): Kidney of thyme/Cd treated rats, renal cortex showing strong cytoplasmic immunoreactivity of Bcl-2 in all renal tubular cells. (IHC, 10x20). (E): Renal medulla of thyme/Cd treated rats, showing strong cytoplasmic immunoreactivity of Bcl-2 in all renal tubular cells (IHC, 10 x20).

DISCUSSION

Cadmium (Cd) is a naturally occurring heavy metal causing severe risks to human health (Krichah *et al.*, 2003). Acute Cd exposure produced toxicity to the lung, liver, testes, and brain (Shimaa and Emad, 2014). Cd is unique among other heavy metals because of its ability to induce toxicity at a very low dosage, long biologic half-life and its low excretion rate from the body (Kaoud and Mekawy, 2011). About one-third of the absorbed Cd is stored in the kidneys directly after gastrointestinal or pulmonary absorption (Jarup *et al.*, 1998b). Hematopoietic system is one of the most sensitive system to evaluate the toxicity of environmental pollutants and drugs in humans as well as animals (Yuan *et al.*, 2014). The results of the existent study revealed a significant ($P < 0.05$) reduction in the erythrogram parameters (RBCs, Hb, PCV, MCV and MCH) in only thyme treated group, CdCl₂ intoxicated group and rat group received both of them. It is likely that microcytic hypochromic anemia developed during the period of CdCl₂ exposure due to decrease of the intestinal iron (Fe) absorption by Cd in line with countless studies showing the reduction of Fe levels in the liver and other organs, when animals are exposed to nutritional Cd loading (Kozłowska *et al.*, 1993 and Elsenhans *et al.*, 1994). Several studies have provided indication that Cd hinders Fe absorption through the intestine, probably by competing with Fe in the absorption process (Schafer and Elsenhans, 1985 and Kozłowska *et al.*, 1993). Additionally, the anemia induced by Cd may be due to its accumulation in kidney, spleen and liver which might suppress the action of these hematopoietic tissues (Ashour, 2014). On the other hand, the anemia developed during the period of exposure to either thyme alone or CdCl₂ with thyme extract can be explained by presence of tannins which are a complex group of polyphenolic compounds that are existing in many species usually consumed by ruminants (Fruto *et al.*, 2004). It is well known that thymol (as a naturally occurring phenolic monoterpene) found in thyme oil extracted from *Thymus vulgaris* is rich source by Tannins which reduces Fe absorption when involved in the diet at extraordinary levels (South and Miller, 1998). Regarding to the leukogram in the present work, CdCl₂ exposure led to leukocytosis with absolute neutrophilia, lymphocytosis and monocytosis which is in the agreement with (Djokic *et al.*, 2014) who approved that Cd intoxicated rats exhibited neutrophilia and monocytosis which is as a result of induced bone marrow neutrophils release and mobilization as well as relocation from tissues into circulating neutrophils under inflammatory conditions. Also, Cassatella (1999) referred that granulocytes are also a source of cytokines that might modulate local tissue inflammatory reaction. The observed lymphocytosis are in harmony with the investigations of Oshawa and Kawai (1981) who explained lymphocytosis as an enhanced

lymphopoiesis and/or an efflux of lymphocytes from the lymphopoietic loci after s.c injection of sub-lethal doses of CdCl₂ in mice (0.0625 to 2 mg/kg) for 1-7 weeks and daily inhalation of cadmium oxide fumes in rats (0.1 and 1 mg /m³) for 4 weeks. Similarly, Harada *et al.* (1979) reported that people occupationally exposed to Cd have possessed an increase in the large lymphocyte count. This was referred to the cytological shift correlated to a concomitant decrease in the number of small lymphocyte. This cytological shift was due to transformation of lymphocytes by Cd as shown by in vitro lymphocyte culture (Shenker *et al.*, 1977), and or by the compensatory supply of blast-like lymphocytes from lymphoid tissues resulting from primary destruction of peripheral lymphocytes by Cd. The current biochemical findings demonstrated that thyme sole supplemented group in significantly changed serum total protein and albumin are in line with (Toghiani *et al.*, 2010) who verified that thyme powder didn't affect serum protein and albumin of broiler chicks. On the other hand, the reduction in serum total proteins in CdCl₂ sole treated group which may indicate disorders in protein synthesis and metabolism as a result of individual actions or interactions of the complex metal constituents (Olivier *et al.*, 2005). It has been similarly described that Cd exposed rats showed reduced serum total proteins with increased serum urea and creatinine due to functional impairment to kidney (Morowati, 2001). Also, it has been reported that renal injury induced by Cd is initially presents as tubular proteinuria, which progresses on continuous exposure (Pinot *et al.*, 2000). The decrease in serum total proteins, seems to be attributed to a decrease in albumin, which also indicates that Cd induced liver and kidney damage. This is consistent with the work of Shatti, (2011) who reported a rise in creatinine level which indicated renal tubular damage due to Cd induced nephrotoxicity. Several studies have documented that the Cd induced tubular proteinuria and damage is permanent even though exposure ends (Jarup and Elinder, 1993 and Jarup *et al.*, 2000). Also, the significant increase in serum urea and creatinine levels ($P < 0.05$) in rats treated with Cd which agreed with Nasim *et al.* (2015) who intraperitoneally administered Cd 1 mg/kg for 21 days where creatinine and protein levels were decreased while levels of urea, Na and K were increased as a result of Cd exposure. Moreover, CdCl₂ treatment mediates oxidative stress which evidenced by elevated renal MDA levels and depleted enzymatic antioxidant defenses, including CAT. The increased renal lipid peroxidation (LPO) in Cd intoxicated rats may be due to a significant ($P \leq 0.05$) inhibition of the activities of free radical capturing enzymes such as CAT. Our results are supported by the findings of Schauder *et al.* (2010) who identified that CAT is a crucial antioxidant enzyme having heme as an active location prosthetic group, Cd is known to diminish iron integration and hinder with heme biosynthesis.

Similarly, Ansari *et al.* (2017) who reported that Cd that has been accumulated in the kidney generates ROS that causes oxidative stress, inflammation, apoptosis and glomerular dysfunction. On the same line Elkhadragy and Abdel Moneim, (2017) detected that the deleterious effects of Cd may be related to its capacity to generate ROS that injure the kidney via two pathways; including its capability to increase LPO and deplete ROS scavenging capability. LPO is a noticeable marker of oxidative stress and it is known to rise cellular content of epoxides, hydroperoxides, and MDA which in sequence can react with cellular proteins, DNA and RNA initiating renal tissue damage (Kehrer and Klotz, 2015). Our findings revealed that the improvement of renal functional parameters to be closer to control values indicates that thyme extract could alleviate the plasma membrane and induce cellular healing. The highest values of free radical scavenging activity of thyme extract could be due to its higher content of phenolic components (Lu and Foo, 2001). Our results indicate that thyme extract can safeguard the ordinary capacity of the kidney and protect against Cd induced injury. In the current study, CdCl₂ treated rats showed marked congestion of inter-tubular blood vessels and capillaries of glomerular tufts, hyaline casts as well as marked vacuolar degeneration of tubular epithelium with nuclear pyknosis. In addition to, focal areas of tubular coagulative necrosis accompanied with slight mononuclear cells infiltration. Also, multiple focal areas of tubular regeneration with aprominent nucleoli and basophilic cytoplasm were detected. Combined administration of thyme/Cd, thyme resulted in normalize kidney function tests with marked restoration of renal parenchyma to normal histological appearance when compared with control rats which supported by significant decline in MDA and significant increase in CAT levels. Subsequent regeneration of proximal tubular cells happens after Cd exposure in rats and dogs (Iwai and Matsuno, 1991). Moreover, (Liu *et al.*, 2000) validated that Cd exposure induces apoptosis and subsequent renewal of the renal tubular epithelium in chicken kidneys. The observation of multiple foci of tubular regenerating foci in the existing study were in bargain with Tanimoto *et al.* (1993) who noted that proliferating proximal tubular cells (tubular regeneration) is a part of the response to apoptotic injury. Additionally, exposure to CdCl₂ leads to a decrease in the activities of antioxidant enzymes, such as superoxide dismutase (SOD) and CAT (Jurczuk *et al.*, 2004). The current observed lesions were supported by increasing renal MDA and depletion of CAT which is in agreement with Fouad and Jersat, (2011) who also reported a kidney damage, with increased MDA levels, and a decline in GSH and CAT activities after Cd exposure. B-cell lymphoma 2 (Bcl-2) family protein members are importantly well-known to adjust the release of apoptosis activating factors that Bcl-2 to Bax ratio controls cell survival or death (Oltval *et al.*, 1993). Subsequently, changes in

the Bax and Bcl-2 expression activates caspase-3 initiating apoptotic processes (Rana, 2008). In the current study, Cd administrated group showed very weak immunoreactivity of Bcl-2 in the glomerular and tubular epithelium which indicates the apoptotic effect of Cd. On the other hand, thyme/Cd combined administration resulted in restoring Bcl-2 expression where almost all of renal tubular epithelial cells showed intense cytoplasmic immunoreactivity with some reactivity in glomerular cells which strongly support the antiapoptotic properties of thyme. To the best of our knowledge, currently there is no report about the effects of thyme/Cd on the expression of Bcl-2 in renal tissues. There is a stout indication suggests that the harmful effect of Cd on testicular function, even though very minimal concentrations, making testes the most vulnerable organ to Cd toxicity (Blanco *et al.*, 2010). Also CdCl₂ caused sterility in adult rats, mice, and hamsters via wide spread degeneration of spermatogenic epithelium (De Souza Predes *et al.*, 2010; Ponnusamy and Pari, 2011). In the current study, histopathological examination of testes in Cd treated rats showed extensive necrosis and vacuolation of the seminiferous tubular epithelium, associated with impaired spermatogenesis. These findings are strongly agreed with Adel *et al.* (2016) who administered CdCl₂ (5 mg/kg b.w) which induced congestion, edema in the interstitium, irregularities in arrangement of the epithelial lining of the seminiferous tubules, and accumulation of the degenerated and sloughed spermatogenic cells in the center of the seminiferous tubules. The observed testicular damage in the current study may due to that the plasma membrane of spermatozoa contains large amounts of polyunsaturated fatty acid, which is more liable to the ROS arising from Cd toxicity (Alvarez and Storey, 1995). However, combined administration of thyme/Cd showed no improvement in the testicular tissue when compared with control rats. From these findings, supplementary studies are needed to precisely assess the protective role of thyme against testicular damage induced by Cd by using grading doses of thyme extract where the current used dose of thyme, showed no protective effect, which may be small to counteract the testicular damage induced by Cd.

CONCLUSION

Cadmium had a deleterious effect on the renal hematological, biochemical, anti-oxidative activities beside it cause toxic nephritis and degenerative orchitis. Thymus vulgaris extract is protecting against Cd nephrotoxicity as it had antioxidant and anti-apoptotic activities. This is the first study to exhibit the effect of Thymus vulgaris on the expression of Bcl-2 in the renal tissue. In the current study, thyme couldn't alleviate the induced testicular damage which may be due to complete spermatogenesis in the adult rat is approximately 40 days which isn't existing in the current study duration. So further studies by

carrying out experimental design of long duration sufficient for completing spermatogenesis along with using grading doses of thyme are needed to assess its protective effect against testicular damage together with estimation of testosterone level, acid and alkaline phosphatase activities and oxidative stress markers. Finally, we recommend eating diets containing amounts of thyme to counteract the nephrotoxic effect of the possibly exposed Cd.

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تقييم التأثير الوقائي للزعر على الاعتلال الكلوي وتلف الخصية الناتج عن التسمم بالكاديوم في الجرذان البيضاء

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اجريت التجربة على 40 جرذاً أبيضاً، تم تقسيمها الى أربع مجموعات ، بكل مجموعة 10 جرذان: المجموعة الاولى: استخدمت كمجموعة ضابطة. المجموعة الثانية: تم تجريعها بالزعر. المجموعة الثالثة: تم حقنهم بكلوريد الكاديوم. المجموعة الرابعة: تم تجريعها بالزعر وحقنهم بكلوريد الكاديوم. وقد أظهرت نتائج الفحوصات المعالجة بالكاديوم صورة فقر الدم ميكروسيتيك هيپوكروميك وزيادة معنوية ($P \leq 0.05$) في إجمالي عدد الكريات البيض، العدد المطلق للخلايا متعادلة الصبغة والخلايا اللمفاوية والخلايا وحيدة الانوية. وكذلك زيادة معنوية ($P \leq 0.05$) في البارامترات الكيميائية الحيوية المرتبطة بالإصابة الكلوية، بما في ذلك اليوريا والكرياتينين وعلوة على ذلك، تم الكشف عن معنوية ($P \leq 0.05$) في البروتينات الكلية في الدم، الألبومين، الجلوبيولين و A / G. بالنسبة لتقييم كفاءة بعض الأنزيمات المضادة للأكسدة مثل (الميلانوداي الدايم، الكاتاليز) في النسيج الكلوي لوحظ زيادة معنوية في مستويات الميلانوداي الذهب مع انخفاض الكاتاليز في المجموعة المعاملة بالكاديوم مقارنة بالمجموعة الضابطة ومع ذلك لوحظ ان استخدام الزعر قد ساهم في تحسين الآثار الضارة لسمية كلوريد الكاديوم على المعاملات الكيميائية الحيوية وتعزيز حالة مضادات الأكسدة الذاتية، وانخفاض بيروكسيد الدهون في حين لم تحسن المعاملات الدموية مقارنة بالمجموعة الضابطة الغير المعالجة وقد تأكدت هذه التغييرات من قبل فحص الهستوباثولوجي لأنسجة الكلى بالمجموعة المعاملة بالكاديوم حيث أظهر الفحص: وجود احتقان ملحوظ في الأوعية الدموية وضمور الكبيبات والانابيب الكلوية مع نخر في الخلايا وتغييرات تكيفية مع زيادة انتشار الخلايا وحيدة الانوية اما المجموعة المعالجة بالزعر مع الكاديوم أظهرت تحسن ملحوظ لهذه التغييرات في النسيج الكلوي. اما عند فحص انسجة الخصية ظهرت المجموعة الضابطة المظهر النسيجي الطبيعي للانابيب المنوية مع الحيوانات المنوية النشطة بينما لوحظ في المجموعة المعاملة بالكاديوم ضمور في حجم الخصية وضمور في الخلايا الانبوبية المنوية المرتبطة بضعف الحيوانات المنوية وفي المجموعة المعاملة بالزعر مع الكاديوم لم يلاحظ اي تحسن في انسجة الخصية بالمقارنة بالمجموعة الضابطة. أظهرت نتائج التحليل الهستوكيميائي قلة تعبيرات Bcl-2 في كلى المجموعة المعاملة بالكاديوم مقارنة بالمجموعة الضابطة، اما المجموعة المعالجة بالزعر مع الكاديوم لوحظ عودة التعبيرات الى أعلى من معدلاتها. نستخلص من الدراسة أن مستخلص الزعر كعشب طبيعي له تأثير وقائي فعال يحمي ضد الآثار السامة للكاديوم على نسيج الكلى عن طريق وجود مضادات الأكسدة ومضادات موت الخلايا المبرمج .