

## Assessment of protective effect of *thymus* on cadmium chloride that induced nephropathy and neuropathy in rats

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### Abstract:

This study included twenty seven rats were allowed to acclimate for 1 week in the animal house of veterinary medicine college. Rats were housed three per cage with dimension of (45\*35\*20). Rats were divided into three groups; the first group represent cadmium chloride (CaCl) treated group which include nine (9) animals denoted by (group A); second group represent thymus and cadmium chloride treated group which include nine animals (9) denoted by (B) and third group which represent control group which also include nine (9) animals denoted by (C). The CaCl was given by drinking water with dose rate (50 PPM) daily; the aqueous extraction of thymus also was given by drinking after adding of CaCl with dose rate (50 PPM) daily. Tissue samples were taken for histopathological study by killing (3) animals from each group at periods of (17, 24 and 31) days after exposure to CaCl. Routine histological technique ending by staining 5 µm with H&E and then examined by light microscope.

The histopathological results of group (A) revealed presence of pale area ( a” softening”) in the brain surrounding by edema. The tissue has vacuolated structure, also a number of astrocytes with small round basophilic nuclei and ill-defined cytoplasmic boundaries are present. Finding of hypertrophic reactive astrocytes and alterations of neurofibrils that clumped and twisted into odd shapes like tennis rackets or skeins of wool. The results of group (B) showed the protective effectiveness of *Thymus* extract against treatment with CACL and the brain tissue looks like normal regeneration occurs mainly at plaque margin and presence of normal white matter.

The histopathological results of kidneys of group (A) showed that the most prominent microscopic changes, in particular evident necrosis, especially in proximal tubules. There is atrophy of most of the tubules and the glomerular tuft shows marked increased in cellularity, with obliterated Bowman’s space . The most marked changes are the loss of tubules, and many of these remaining are small and lined by very atrophic epithelium. The results of Group (B) didn’t revealed any effectiveness of *thymus* extract against toxicity of kidney tissue with CACL.

The conclusion that the cadmium chloride may cause severe damage in brain

and kidneys of rats and thymus can used as a protective agent against toxicity with cadmium chloride and this protective effect occur obviously in protection of brain tissue against toxic effect of cadmium chloride.

## تقييم التأثير الواقي للزعر على الاعتلال الكلوي والعصبي الناتج من التسمم بكلوريد الكادميوم في الجرذان.

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### الخلاصة:

ضمّنت هذه الدراسة سبعة وعشرون جرذاً سُمِّحَتْ للتأقلم لأسبوع واحد في البيت الحيواني لكلية الطب البيطري. وأسكنت الجرذان ثلاثة لكل قفص ذو إبعاد (20\*35\*45). قُسمت حيوانات الدراسة إلى ثلاث مجموعات؛ تُمثّل المجموعة الأولى المجموعة المعاملة بمادة كلوريد الكادميوم (CaCl) وتضمنت تسعة حيوانات أُطلق عليها (المجموعة A)؛ تُمثّل المجموعة الثانية المجموعة المعاملة بمادة الزعر (thymus) ومادة كلوريد الكادميوم وتضمنت تسعة حيوانات أُطلق عليها (المجموعة B) والمجموعة الثالثة التي تُمثّل مجموعة السيطرة والتي تضمنت أيضاً تسعة حيوانات أُطلق عليها (المجموعة C). أعطى كلوريد الكادميوم بالماء الصالح للشرب بجرعة (50 جزء بالمليون) يومياً؛ المستخلص المائي للزعر أيضاً أُعطى عن طريق الشرب بعد إضافة CaCl بنسبة الجرعة (50 جزء بالمليون) يومياً. أُخذت عينات الأنسجة لغرض إجراء دراسة الفحص النسيجي المرضي بقتل (3) حيوانات من كل مجموعة في الفترات (17، 24 و 31) يوم من التعرض للكادميوم كلورايد. أُجريت التقنية الروتينية للفحص النسيجي المرضي والتي تضمنت اخذ عينة النسيج وتصيغها بصبغة الهيماتوكسولين الايوسين وبعد ذلك فحصها بالمجهر الضوئي لتقييم التغيرات النسيجية المرضية.

نتائج الفحص النسيجي المرضي للمجموعة (A) كُشفت وجود المنطقة الشاحبة في الدماغ محاطة بالوذمة مع وجود فراغات في النسيج vacuolated، مع عدد من الخلايا النجمية ذات انويه مستديرة صغيرة الحجم قاعدية الصباغ وسائتوبلازم غير محدد المعالم. مع وجود فرط تنسج تفاعلي للخلايا النجمية وتغيرات في الألياف العصبية التي تتجمع وتلتف لتصبح مشابهة لشكل مضرب التنس أو تُشَلَّل الصوف. نتائج المجموعة (B) أظهرت التأثير الوقائي للمستخلص المائي للزعر ضد التسمم بكلوريد الكادميوم خصوصاً لنسيج الدماغ.

نتائج الفحص النسيجي لكلي الجرذان للمجموعة (A) أظهرت بأن التغيرات المجهرية الأبرز، هو نخر واضح بشكل خاص، خصوصاً في الأنابيب الكلوية. هناك ضمور في أغلب الأنابيب الكلوية وفرط خلوي في الكبيبة الكلوية، مع تقلص المساحة بين الكبيبة الكلوية ومحفظة بومان. أكثر التغيرات التي لوحظت هي فقدان الأنابيب الكلوية، أما الأنابيب الكلوية المتبقية فكانت صغيرة الحجم وتعاني من قلة تنسج في الظهارة atrophic المبطنة للأنابيب الكلوية. نتائج المجموعة (B) لم تُكشَف أي تأثير للمستخلص المائي للزعر ضد تسمم نسيج الكلية بمادة كلوريد الكادميوم.

استنتجت الدراسة بان كلوريد الكادميوم يسبب أضراراً حادة في الدماغ وكلّي الجرذان بينما يلعب الزعر دوراً هاماً للوقاية من التسمم بمادة كلوريد الكادميوم و يُمكن ملاحظة هذا التأثير للزعر بصورة واضحة في حماية نسيج الدماغ.

**Introduction:**

Cadmium is a ubiquitous environmental pollutant (1) that has no known biological function in humans. Cadmium occurs in nature as a natural component of rock and sediment, soil and dust, air and water, and plant and animal tissues (2). The release of cadmium due to human activities is estimated at 4,000–13,000 tons/year, with mining and the burning of fossil fuel serving as the major contributors (3). Polluted industrial sites are continuous sources of environmental cadmium; leaching of cadmium into groundwater and distribution of metal-loaded soil particles by lateral wind erosion are two primary mechanisms (1). In the United States and Europe, the average uptake via ingestion in unpolluted areas has been estimated to be from 10–25  $\mu\text{g}/\text{day}$  (2). High fiber diets and a diet rich in shellfish increase the dietary intake substantially, although absorption may not increase proportionally. Even though the gastrointestinal absorption of cadmium is only a few percent, the absorption of cadmium in the lungs is 10–50%. As a consequence, cigarette smoking is a major route for nonoccupational lung exposure. One cigarette contains about 1–2  $\mu\text{g}$  of cadmium, roughly 10% of which is inhaled; based upon a 50% absorption rate, a person who smokes 20 cigarettes (1 pack) per day will absorb about 1–3  $\mu\text{g}$  of cadmium. Smokers, on average, have 4–5 times higher (~1.5  $\mu\text{g}/\text{L}$ ) blood cadmium (B-Cd)

levels than nonsmokers. Interestingly, despite the high cadmium content of cigarette smoke, there is little evidence for significant exposure from passive smoking (4,5). Major toxicities anticipated from cadmium exposure involve the renal, pulmonary, and, to a lesser extent, gastrointestinal systems. These include the development of renal proximal tubular dysfunction, glomerular damage with progressive renal disease, and respiratory symptoms including pneumonitis and emphysema (6). Low-level cadmium exposure has also been associated with increased urinary calcium excretion and direct bone toxicity, effects that recent research suggests may result in the development of osteoporosis (2,4). The body burden of cadmium, over half of which may reside in the kidneys, is most often measured through the use of urinary cadmium levels (6,7,8). Blood cadmium measurements generally reflect current or recent exposure and are especially useful in cases with a short exposure period and only minimal accumulation of cadmium in the kidneys (9). Both  $\beta_2$ -microglobulin and  $\alpha_1$ -microglobulin serve as organ-specific, early-effect biomarkers of tubular proteinuria and thus play a role in identifying early signs of cadmium-induced renal damage in those with potential exposures. (10). Recently extracts of plants have provoked interest as sources of natural products. They have been screened for their potential uses

as alternatives medicines for the treatment of many infectious diseases and also in preservation the toxic effects of oxidants. In modern days the antioxidants and antimicrobial activities of plant extract have formed the basis of many applications in pharmaceuticals, alternative medicines and natural therapy (11). Thyme (*thymus vulgaris*) belonging to the lamiaceae family, is a pleasant smelling perennial shrub, which grows in several regions in the world (12). It is grown native in Iraq, in the area between Al-mousl and Dhok districts (13). *Thymus vulgaris* (thyme) like some other wild *Thymus* species (wild thyme) possesses a wide range of biological activities including expectorant, spasmolytic, sedative (14), antibacterial (15), antifungal (16), antioxidant (17) etc. In general, investigations of biological activity in most of the studies were carried out on extracts, essential oils or pure compounds isolated from dried herbs of *Thymus* species. In the present work we aimed to determine protective effect of thymus extract against harmful effects of cadmium chloride on kidneys and brain in rats.

### Study design and methods:

#### Plant source

The leaves of *thymus vulgaris* plant was obtained from local market of Al-Diwanyia in 2009 during the month of September, identified by the national Iraqi institute for herbs, Baghdad – Iraq, then kept in to the sterile bottle until further use.

#### Preparation of aqueous extract of *thymus vulgaris*

aqueous extract of *thymus vulgaris* was prepared using method described by (18) and (19) the plant material (leaves) was allowed to air dry and afterwards pulverized in a grinder. The pulverized material 50 gm was extracted with 500 ml of distilled water in an electric blender left running for 15 minute, afterwards, the suspension was filtered twice, first through cheese-cloth (50% cotton, 50% polyester) and then through filter paper (Whatman No2). Water was evaporated in an over vacuum at 50 c. the extract was then filtered and preserved in sterile dark bottle at 4 c until further use.

#### In vivo study

This study included twenty seven rats were allowed to acclimate for 1 week in the animal house of veterinary medicine college. Rats were housed three per cage with dimension of (45\*35\*20). Rats were divided into three groups; the first group represent cadmium chloride (CaCl) treated group which include nine (9) animals denoted by (group A); second group represent thymus and cadmium chloride treated group which include nine animals (9) denoted by (B) and third group which represent control group which also include nine (9) animals denoted by (C). The CaCl was given by drinking water with dose rate (50 PPM) daily; the aqueous extraction of thymus also was given by drinking after adding of CaCl with dose rate (50 PPM) daily.

**Histopathological procedure:**

Tissue samples were taken for histopathological study by killing (3) animals from each group at periods of (17, 24 and 31) days after exposure to CaCl. Routine histological technique ending by staining 5  $\mu$ m with H&E and then examined by light microscope. (20).

**Histopathological study Results:****1) Neurological changes:**

Figure (1) represent control group (Group C) that showed normal brain tissue, normal neuron and normal astrocytes cells.

The results of group (A) at (17) day period revealed presence of pale area (a "softening") surrounding by edema (Figure 2). The tissue has vacuolated structure, also a number of astrocytes with small round basophilic nuclei and ill-defined cytoplasmic boundaries are present. At (24) day period the results revealed presence of large round cells (astrocytes) with foamy cytoplasm and the neuronal cell body may become tremendously distended (Figure 3). At (31) day period the results revealed finding of hypertrophic reactive astrocytes and alterations of neurofibrils that clumped and twisted into odd shapes like tennis rackets or skeins of wool (Figure 4).

The results of group (B) at (17) and (24) day periods showed the protective effectiveness of *Thymus* extract against treatment with CaCl and the brain tissue looks like normal (normal brain tissue, normal neurons and astrocytes). (Figure 5). At (31)

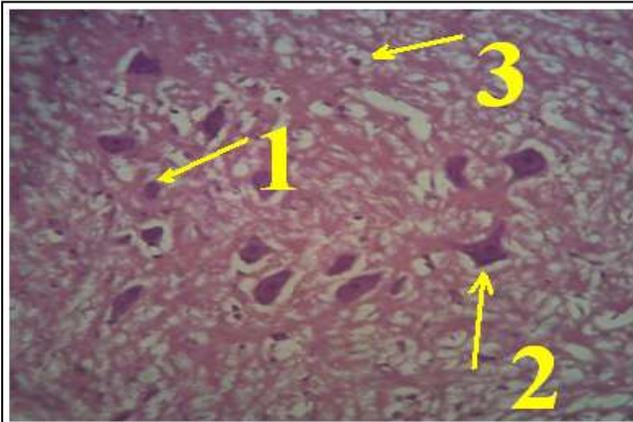
day revealed regeneration occurs mainly at plaque margin and presence of normal white matter (Figure 6).

**2) Renal Changes:**

The results of group (A) at (17) day period the histopathological studies of specimens of the rat kidneys showed that the most prominent microscopic changes, in particular evident necrosis, especially in proximal tubules. Only tubule contour was preserved with noticeable area where endothelium was attached, but there were no visible nuclei. Tubular endothelium delamination without necrosis was also observed, also the glomeruli are nearly all completely fibrosed and avascular. There is atrophy of most of the tubules which apart from a few very small tubules have particularly no lumen. The remaining tubules are dilated and containing densely eosinophilic (colloid) casts (Figure 7). At (24) day period the glomerular tuft shows marked increased in cellularity, as a result of proliferation of endothelial cells and infiltration with polymorph leukocytes. The increased number of swollen endothelial cells has blocked the lumen of many capillaries, and the swollen tuft has almost obliterated Bowman's space (Figure 8). At (31) day period the most marked changes are the loss of tubules, and many of these remaining are small and lined by very atrophic epithelium. The interstitial tissue is considerably expanded, severely fibrosed and heavily infiltrated by chronic inflammatory cells (Figure 9).

The results of Group (B) didn't revealed any effectiveness of *thymus*

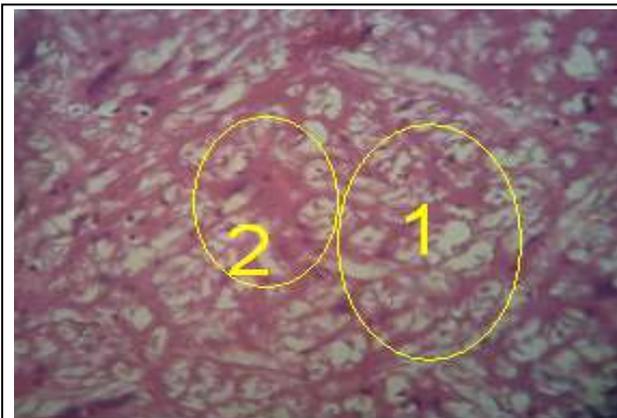
extract against toxicity of kidney tissue with CACL.



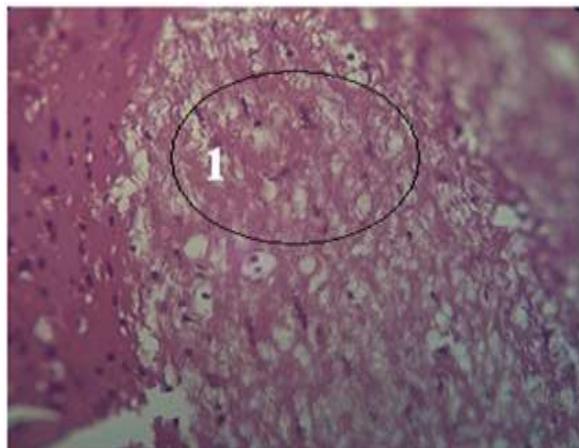
**Group (C) - Figure 1:-** Normal neuron: Astrocyte (1); neuron (2); oligodendrocyte (3). H&E (100 X).



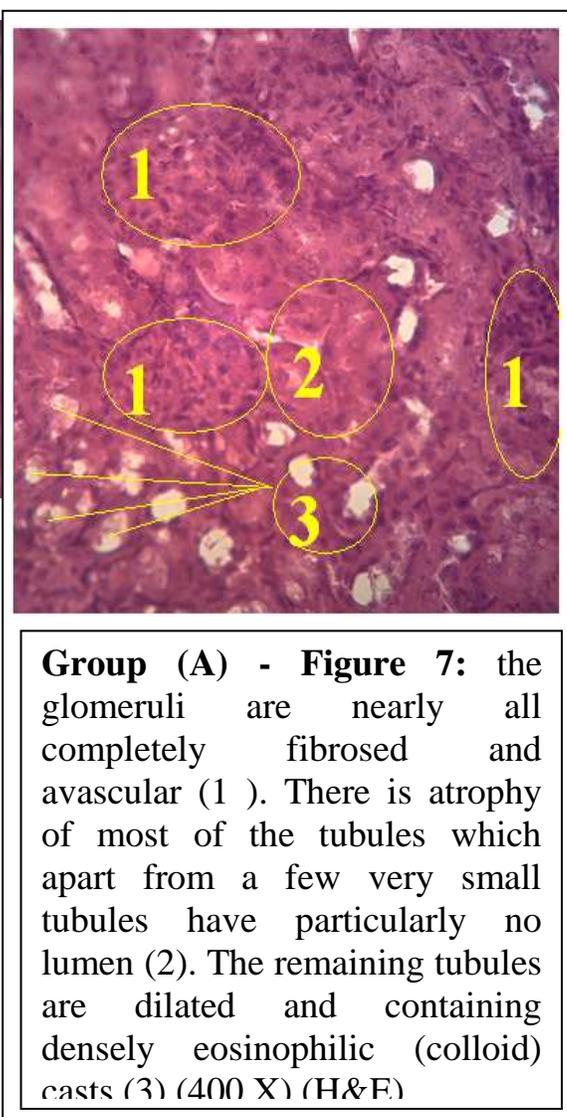
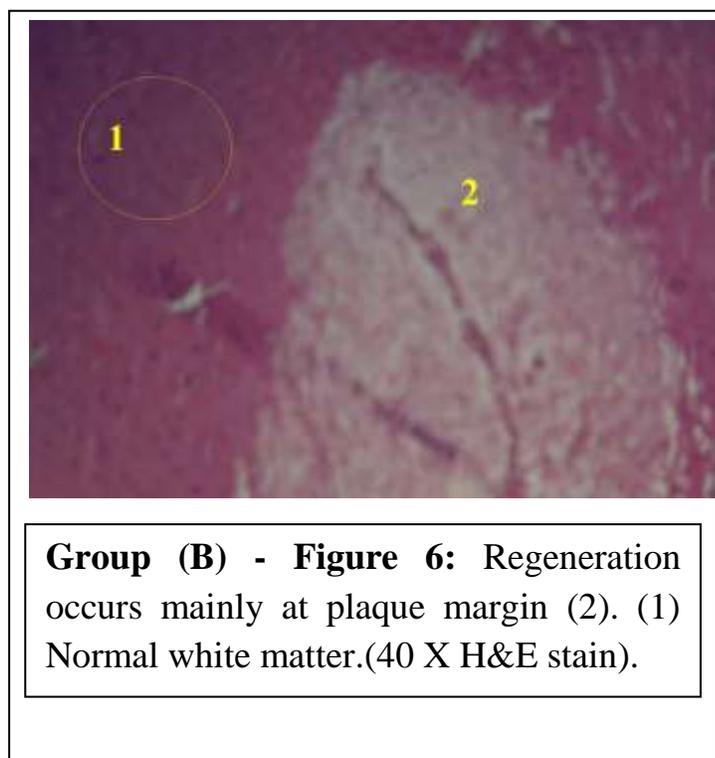
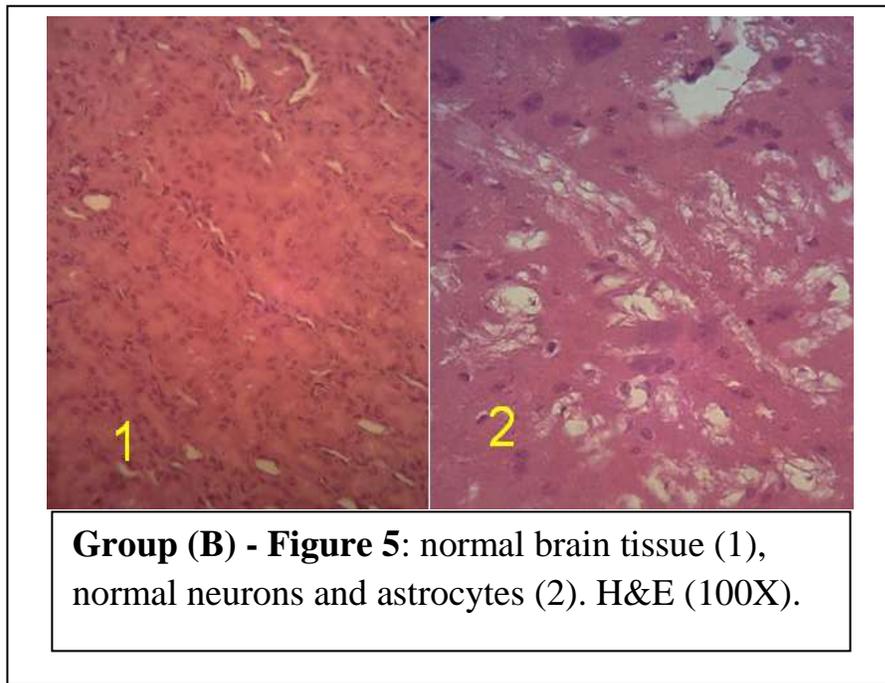
**Group (A)-Figure 2:-** showed extracellular cerebral edema (1). (100 X).

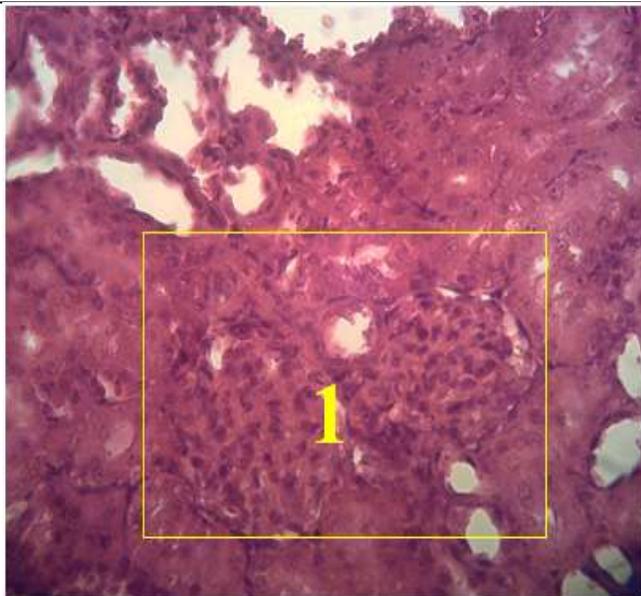


**Group (A)-Figure 3:** large round cells (astrocytes) with brain edema (1) and the neuronal cell body may become tremendously distended(2). H&E (400 X)

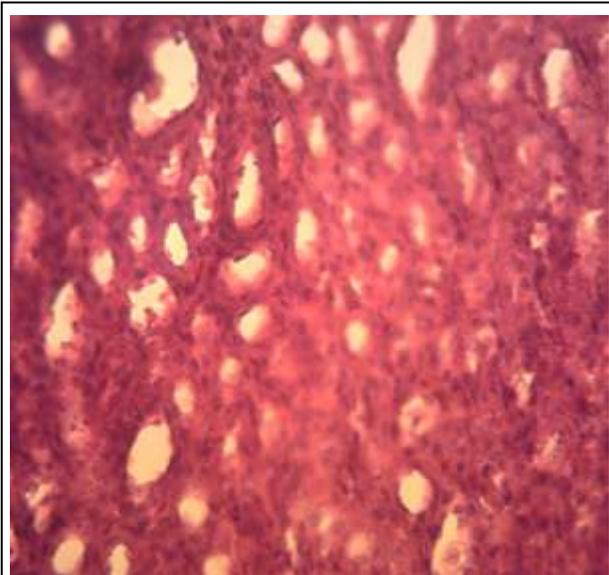


**Group (A) - Figure 4:-** hypertrophic reactive astrocytes(1).H&E (400 X).





**Group (A)- Figure 8:** the glomerular tuft shows marked increased in cellularity, as a result of proliferation of endothelial cells and infiltration with polymorph leukocytes(1). The increased number of swollen endothelial cells has blocked the lumen of many capillaries, and the swollen tuft has almost obliterated Bowman's space. (400 X) (H&E).



**Group (A) -Figure 9:** The most marked changes are the loss of tubules, and many of these remaining are small and lined by very atrophic epithelium. The interstitial tissue is considerably expanded, severely fibrosed and heavily infiltrated by chronic inflammatory cells. (400 X) (H&E).

### Discussion:

Absorption of cadmium from the gut appears to take place in two phases—uptake from the lumen into the mucosa, and transfer from the mucosa into the circulation. Cadmium is distributed throughout the body, but the major portion is found in the liver and kidney. The majority of absorbed cadmium is retained in the tissues. Renal concentrations far exceed hepatic concentrations following

part, involves in competition of calcium, iron and zinc binding sites on protein (21). Cadmium does not undergo metabolic conversion, but the cadmium ion can readily bind to anionic groups, especially sulfhydryl groups, in proteins and other molecules. Cadmium is bound to the protein metallothionein in the liver, which releases the metallothionein-cadmium complex, rather than free cadmium, into the bloodstream.

Metallothionein is a low-molecular-weight, sulfhydryl-rich protein that normally binds zinc. Metallothionein-bound cadmium is readily filtered by the renal glomerulus and reabsorbed from the glomerular filtrate by the proximal tubule cells, at which point the “exogenous” metallothionein is catabolized in tubular lysosomes, releasing free cadmium. The free cadmium stimulates the synthesis of metallothionein in the tubular cells, is then bound to the tubular metallothionein, and remains in the cells. Most of the absorbed cadmium is retained; some excretion of cadmium occurs through the urine, and urinary excretion increases with renal damage (3). In total, approximately one-third of the cadmium absorbed in the body is stored in the kidneys immediately after gastrointestinal or pulmonary absorption; cadmium circulates in the blood mainly bound to albumin and other high molecular weight proteins. These complexes are largely absorbed in the liver, and the uptake of cadmium by the kidney is limited. In chronic exposure or in situations long after a single exposure, however, much of the plasma cadmium is bound to metallothionein (MT). Due to its small molecular size, cadmium–MT, in contrast to the cadmium–albumin complex, is efficiently filtered through the glomerular membrane and reabsorbed by renal tubular cells through pinocytosis. (6,7)

Elinder *et al.*, (1983) and Pinot (2000) revealed that Cadmium-

induced renal injury initially presents as tubular proteinuria, with continued cadmium exposure, this tubular dysfunction progresses, and ultimately glomerular damage characterized by a decreased glomerular filtration rate may emerge this is in accordance with our results(2,4). This explanation of mechanism of action of cadmium and it's ability of inducing damage in glomeruli and renal tubules is interpreting our results that revealed progressive development of the lesions, according to the duration of exposure, from beginning of necrosis, especially in proximal tubules, and atrophy of most of the tubules which apart from a few very small tubules have particularly no lumen, while the remaining tubules are dilated and containing densely eosinophilic (colloid) casts at the first period (17) day. To marked increased in cellularity of the glomerular tuft and the swollen tuft has almost obliterated Bowman's space at the second period (24) day. While at third period (31) day, the most marked changes were the loss of tubules, and many of these remaining are small and lined by very atrophic epithelium. The interstitial tissue is considerably expanded, severely fibrosed and heavily infiltrated by chronic inflammatory cells. Our histopathological results revealed by our study were in accordance with others like; ATSDR (1999) that showed the most tissue damage concerned with environmental exposure to cadmium was kidney

damage(3), and Abdelghani *et al.*, (1995) that revealed renal damage appears to be a consequence of cadmium accumulation, such that the ability of the kidney to sequester cadmium through synthesis of metallothionein may be overwhelmed (22). Renal effects have been seen in humans and animals by both inhalation and oral exposure, and are the most sensitive effects of chronic oral exposure, occurring at intakes as low as 0.0078 mg/kg/day. Harris *et al.*, (1994) indicate that cadmium compounds must be toxic to cause renal damage, and several studies indicating that cadmium and cadmium compounds are human carcinogens, and although “a variety of soluble and insoluble cadmium compounds” were used in the cited animal studies, only cadmium chloride is explicitly named (23). OSHA, (2003) ensures that the acute health effects from inhaling cadmium include pneumonitis, pulmonary edema, and death (21). Regardless of the route of adsorption, approximately one half to one third of the body burden of cadmium is found in the kidneys, with the highest concentration in the renal cortex. Even if cadmium is stored in another compartment, it can be transported from there to the kidney where it causes renal damage. Therefore, OSHA considers all cadmium compounds to be toxic. Cadmium-induced renal injury initially presents as tubular proteinuria and with continued cadmium exposure, this tubular dysfunction progresses, and

ultimately glomerular damage characterized by a decreased glomerular filtration rate may emerge (2,4,24). Several studies have documented that in almost all cases, this cadmium-induced tubular proteinuria and damage is irreversible even if exposure ends (6,7,8,25). Akunts *et al.*, (1972) revealed that cadmium chloride inhibit alkaline phosphatase (APH) in kidneys of rats (26).

ATSDR, (1999) revealed that the cadmium chloride causes neurological effects which consisting of decreased motor activity, weakness and muscle atrophy, aggressive behavior, increased passive avoidance, and alterations in brain dopamine, 5-hydroxytryptamine, succinic dehydrogenase, and monoamine oxidase levels have been observed in rats exposed to 3.1–24 mg Cd/kg/day for an intermediate duration (3). In mice, necrosis of the choroid plexus epithelial cells have been observed following intermediate duration exposure to 1.4 mg Cd/kg/day as cadmium chloride in drinking water, but not after exposure to 0.2 mg Cd/kg/day (36). Chronic exposure to 3.6 mg Cd/kg/day as cadmium chloride in drinking water resulted in peripheral neuropathy in rats (27). Our study revealed that cadmium chloride cause neuropathological changes and these changes include presence of pale area ( a” softening”) surrounding by edema. The tissue has vacuolated structure, also a number of astrocytes with small round basophilic nuclei and

ill- defined cytoplasmic boundaries are present. Also large round cells (astrocytes) were present with foamy cytoplasm and the neuronal cell body may become tremendously distended. Finding of hypertrophic reactive astrocytes and alterations of neurofibrils that clumped and twisted into odd shapes like tennis rackets or skeins of wool. Many researchers were agreed with our results, like, (28) that indicate the cadmium burden of the liver, kidneys and brain tissue. Choudhuri *et al.*, (1996) confirm that cadmium is a potential neurotoxin in animals and humans, but some level of protection is provided by metallothionein in the brain (29). Kumar *et al.*, (1996) revealed that rats exposed to Cd (0.90 mg/kg, i.p.) For 30 days showed biomembrane changes in different brain regions (30). The effects of Cd were region specific and most, pronounced in olfactory bulb. Gupta *et al.*, (1994) showed that cadmium-induced blood brain barrier dysfunctions have been reported due to depletion of microvessel antioxidant substances and increased lipid peroxidation (31).

OSHA,(2003) indicate that the main symptoms of chronic cadmium intoxication can be attributed the “border” on teeth, hypochromic anemia, accelerated erythrocytes sedimentation rate, disorders of calcium metabolism, occurrence of pneumosclerosis, diseases of liver, kidneys, and neurostenic syndrome (21). One of the mechanisms of cadmium toxicity was Cadmium-

induced lipid peroxidation and this has been seen in animal studies in liver, kidney, brain, lung, heart, and testes (32).

The results of Group (B) didn't against toxicity with CACL in case of nephropathy, while the result revealed very important prophylaxis efficacy in protecting of brain against toxicity with CACL.

Disease prevention and management through the diet can be considered an effective tool to improve health and/or reduce the increasing health-care costs for these oxidation-linked chronic diseases, especially in low-income countries. Plant phenolics are an important subgroup of secondary metabolites, which have diverse functional and medicinal applications (33). Examples of plant phenolics with medicinal uses include lithospermic acid from *Lithospermum* sp. as anti-gonodotropic agent, salvianolic acid from *Salvia miltiorrhiza* as an antiulcer agent, proanthocyanidins from cranberry to combat urinary tract infections, thymol from *Thymus vulgaris* for anti-caries, and anethole from *Pimpinella* (34).The antioxidant activity of thymus extract may occur via various mechanisms such as the inhibitory effect on lipid peroxidation and by scavenging the radicals. In our results, the water *thyme* extract exhibit good radical scavenging activities against CACL and it was significant action specially in the first and second periods (17 and 24) , also at third period (31) which represent long

period of exposure the thymus cause regeneration in the meninges and mainly at plaque margin and presence of normal white matter, and that give indication for tow things , the first toxicity of CACL increased with the increase period of exposure and the second *Thymus extract* can protect the brain and also lead to regeneration in the meninges. Differently, Mata *et al.* (2007) have reported a low radical scavenging activity for wild thyme *Thymus serpyllum* and a high radical scavenging for *Mentha spicata* and *Rosmarinus officinalis* (35).

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