The protective effect of vitamin E on thallium acetate induced hepatic and renal toxicity in male laboratory rats

H. S. Shaheed, R. A. S. Al-Naimi and E. H. Al-Taee
Dep. Pathology and Poultry - College of Veterinary Medicine/University of Baghdad

Abstract

The present work was designed to make knowledge about the protective effect of vitamin E in case of thallium acetate toxicity. Determination of LD_{50} for thallium acetate was done according to probit method and the result was (LD_{50} 20 mg/ kg b.w.). A total of 90 experimentally male rats of approximately same age 6-7 weeks and body weight from 150-250 gms divided equally into 6 groups and treated for two months as follows: the first group was administered orally with thallium acetate at dose of 0.080 mg/ kg B.W., the second group was administered orally thallium acetate at dose of 0.160 mg \kg B.W., the third group was administered orally with vitamin E 40 mg/ k.g B.W. and thallium acetate 0.080 mg/ kg B.W., the fourth group was administered orally with vitamin E 40 mg/ k.g B.W. and thallium acetate 0.160 mg/ kg B.W, the fifth group was administered orally with vitamin E 40 mg/ k.g B.W, the sixth group served as a control was administered orally with distilled water. The main clinical signs observed in the toxic groups were alopecia, piloerection and ocular opacity in addition to vomiting (sometimes bloody), mild diarrhea, shortness of breath, aggression, hyperactivity and lack of appetite. The severity of the signs was increased with time of exposure. During the experimental periods (20, 40, 60 day) five animals from each groups were sacrified. Blood collection was done by direct cardiac puncture for hematological and biochemical examination. The results of biochemical parameters showed significant increased in ALT, AST, ALP, creatinine and urea. The pathological study showed that thallium acetate toxicity causes deleterious pathological changes with the formation of hyperplasic nodules in the liver. Supplemented groups with vitamins E showed improvement in all studied parameters with increase in the cellular immune response. It was concluded that oral exposure of thallium acetate in rat causes severe toxicopathological changes with precancerous lesions in the liver. Supplementation of vitamin E induced protective effects against thallium acetate toxicity in all of above studied parameters.

التأثير الاتقائي لفيتامين (E) على خلات الثاليوم المستحث للسمية الكبدية والكلوية لذكور الجرذان المختبرية

هبة صالح شهيد، راجحة عبد الستار النعيمي وإيمان هاشم يوسف الطائي
فرع الأمراض والدواجن– كلية الطب البيطري– جامعة بغداد

الخلاصة

صمم هذا العمل لغرض دراسة التسمم بخلات الثاليوم في ذكور الجرذان المختبرية ومعرفة التأثير الاتقائي لفيتامين E في حالة التسمم بخلات الثاليوم. تم تحديد الجرعة القاتلة للنصف LD50 لخلات الثاليوم بطريقة probit methods وكانت النتيجة هي (20 ملغم/ كغم) من وزن الجسم. استخدم في الدراسة 90 من ذكور الجرذان المختبرية بأعمار متقاربة من (6–7) أسابيع وتراوحت أوزانها بين 100–150 غم حيث قسمت إلى 6 مجاميع احتوت على مجاميع متساوية من الحيوانات وجرعت لمدة شهرين وكما يأتي: المجموعة الأولى: جرعت

0.080 ملغم من وزن الجسم من محلول خلات الثاليوم لكل كغم من وزن الجسم، المجموعة الثانية: جرعت 0.160 ملغم من وزن الجسم من محلول خلات الثاليوم لكل كغم من وزن الجسم، المجموعة الثالثة: جرعت 40 ملغم لكل كغم من وزن الجسم من فيتامين E و0.080 ملغم من محلول خلات الثاليوم لكل كغم من وزن الجسم، المجموعة الرابعة: جرعت 40 ملغم لكل كغم من وزن الجسم من فيتامين E و0.160 ملغم من محلول خلات الثاليوم لكل كغم من وزن الجسم، المجموعة الخامسة: 40 ملغم لكل كغم من وزن الجسم من فيتامين E، المجموعة السادسة: جرعت ماء مقطر وعدت مجموعة سيطرة. تم ملاحظة العلامات السريرية في المجموعات السامة طيلة فترة التجربة ووجد ان هنالك: الحاصة، انتصاب الشعر، عتامة العين بالإضافة إلى التقيؤ (الذي يكون دموي في بعض الأحيان)، إسهال خفيف، وضيق النفس، العدوانية، فرط النشاط وقلة الشهية وقد أزادت شدة الأعراض مع ازدياد فترة التعرض للتسمم. أجريت عملية سحب الدم مباشرة من القلب بعد كل (20، 40، 60 يوم) طيلة فترة التجربة وذلك لغرض إجراء الفحوصات الكيميائية الحياتية كما أخذت عينات من الكبد والكليتين لغرض إجراء الفحص المرضي التنسجي. أظهرت دراسات الفحوصات الكيمائية الحياتية زيادة معتدة في انظيمات الكبد (ALT, AST, ALP) والكيرياتينين واليوريا للكلية. بينت الدراسة وجود تغيرات مرضية مع فرط التنسج العقيدي للكبد. أما مجاميع التسمم المعاملة فيتامين E فقد أظهرت تحسنا في جميع المعالم التي ذكرت أعلاه مع زيادة في الاستجابة المناعية. وقد استنتج من الدراسة ان التسمم بخلات الثاليوم في الجرذان يؤدي إلى تغيرات مرضية سمية شديدة مع تكوين آفات مهياة للتسرطن في الكبد. وان إعطاء فيتامين E احدث تأثيرات اتقائية انعكست على جميع المعالم التي تضمنتها الدراسة.

Introduction

Thallium (TI) is a naturally occurring trace element, widely distributed in the earth's crust, but at very low concentration (1). It tends to be extremely toxic in a aqueous solution (2, 3). This soft gray element does not have a known biological use and does not appear to be an essential element for life, thallium poisoning is one of the most complex and serious toxicities known to man (4). While being a highly toxic element, TI has been studied to a much lesser degree than other toxic elements such as lead, cadmium or mercury, one of the reasons may be that classical analytical methods have poor sensitivity to thallium (5). The main threat to humans is through occupational exposure, environmental contamination, and accumulation in food, mainly in vegetables grown on contaminated soil. Increasing use in emerging new technologies, and demanding high-tech industry constantly raise concern about exposure risk to all living organisms (1). TI intoxication still occurs world wide (1, 6). The exact mechanism of thallium toxicity still remains unknown, although impaired glutathione metabolism, oxidative stress, and disruption of potassium-related homeostasis may play a role (7). Vitamin E is found naturally in some foods and is a very potent antioxidant that protects the body from free radical damage and oxidation. This is important during chelation because a heavy metal burden in tissues and bones creates oxidative stress on cell membranes and a general inflammatory conditions (8). The present work was designed to make knowledge about the protective effect of vitamin E in case of thallium acetate toxicity.

Materials and Methods

- **Determination of LD_{50} of Thallium acetate by probit method:** LD_{50} was determined according to Goyer and Clarokson (9). Twenty five male rats were divided into five groups, each group consists of five animals, administered orally by using of stainless steel stomach tube with the following doses of thallium acetate ($TlC_2H_3O_2$) according to the animal body weight in each group: 1. Group 1: 10

mg/kg.bw. 2. Group 2: 20 mg/kg.bw 3. Group 3: 30 mg/kg.bw 4. Group 4: 40 mg/kg.bw. 5. Group 5: 50 mg/kg.bw. The dose rate 1 ml/ 100 g.bw. The animals were monitored for 24 hrs. for the development of toxicity symptoms and death.

- **Experimental design:** Ninety albino male rats were divided into 6 groups, (n=15), they were treated as follow:

1. Group(1): animals were treated orally by stomach tube with thallium acetate at dose of 0.080 mg/ kg B.W.
2. Group(2): animals were treated orally by stomach tube with thallium acetate at dose of 0.160 mg/kg B.W.
3. Group(3): animals were treated orally by stomach tube with vitamin E 40 mg/ kg b.w. and thallium acetate 0.080 mg/ kg B.W.
4. Group(4): animals were treated orally by stomach tube with vitamin E 40 mg/ kg b.w. and thallium acetate 0.160 mg/ kg B.W.
5. Group(5): animals were treated orally by stomach tube with vitamin E 40 mg/ kg b.w.
6. Group(6): animals were treated orally by stomach tube with distilled water considered as control.

At 20, 40, 60 days of experimental period, 5 animals were scarified for pathological and biochemical studies.

- **Parameters of experiments:**
- **Clinical signs:** Clinical signs were checked continuously after thallium acetate treatment along the period of experiments (2 months), any change in activity or behavior of the animals was recorded.
- **Blood collection:** Blood was collected at 20, 40, 60 days of experiment via cardiac puncture technique. Blood samples were collected in plain tubes and used to separate serum which were stored at (-20c̊), till the time of assay for biochemical investigation.
- **Biochemical study:**
- **Serum Transaminaeses:** Alanine Aminotransaminase (AST) Asparate Aminotransminase (ALT). Colorimetric method for detection the activity the ALT and AST in the serum was done by using of AST and ALT kits (10, 11).

AST: Aspartete + α Keto glutarate $\rightleftharpoons$ Oxaloacetate + L- glutamate

ALT: Alanine + α Keto glutarate $\rightleftharpoons$ Pyruvate + glutamate

Alanine Aminotransaminase and Asparate Aminotransminase catalyzes the transfer of the amino group of glutomic to oxalacetic acid and pyruvic acid in reversible reaction. The transaminase activity is proportional to the amount of oxalate or pyruvat formed over a definite period of time and is measured by reaction with 2, 4-Dinitrophenhydrazin (DNPH) in alkalin solution at wave length of 510 nm by using visible spectrophotometer.

- **Alkaline Phosphatase (ALP):** Serum ALP concentration was enzymatically measured standard assay (ALP- Kit). Alkaline phpsphataes activity in serum was estimated spectrophotometrically by employing king Armstrong method, in which the disodium Phenyl Phosphate is hydrolysed with liberation of phenol and formation of sodium phosphate. The amount of phenol formed is estimated colorimetrically. The result was taken immediately at 510 nm. (10).
- **Blood urea**: (mMole/ liter) was calculated according to (Diacetyl manpxime) for estimate the level of urea in blood (11).
- **Creatinine** level (mMole/ liter) in blood serum was calculated according to Jaffemethod which described by (11).

- **Histopathological study:** At the end of the each period of experiment, five animals from each groups were sacrified under deep anesthesia and specimens from liver and kidneys were fixed in buffered formalin, sectioned (5 mm thickness) and stained with Hematoxylin and Eosin according to Luna and Lee (12).

Results and Discussion

- **LD_{50}:** LD_{50} was measured after plot the logarithm of doses against probit response from which LD_{50} was determined by vertical crosslink from the 5 probit response to the log number dose. The calculated LD_{50} was 20 mg/kg.bw. The present value of LD_{50} (20 mg/kg.bw) did not agreed with results of other studies in rats, that may be due to species and environmental adaptation.
- **Clinical signs:** The main clinical signs observed in the toxic groups were alopecia, (Fig. 1) piloerection and ocular opacity (Fig. 2), in addition to vomition (sometimes bloody), mild diarrhea, shortness of breath, aggression, hyperactivity and lack of appetite were in agreements with previous studies (13, 14, 15, 16).
- **Biochemical study:**
- **Liver parameters:** The results of ALP, AST and ALT values are shown in table (1). There was a significant increase with periods of experiments which reach (247.66 ± 0.005, 0.090 ± 0.003 and 0.110 ± 0.02) respectively at dose (0.080 mg/kg B.W.) while reach to (494.33 ± 0.005, 0.15 ± 0.005 and 0.203±0.03) at dose (0.160 mg/kg B.W.) at 60 day respectively as compared with control group (148.33±0.003, 0.048±0.003 and 0.040±0.03). In supplemented groups with vitamin E shown decrease in value of enzyme activity which reach to (178.66±0.005, 0.083 ± 0.003 and 0.059±0.03) respectively at dose (0.080 mg/ B.W.), while at dose (0.160 mg/kg.b.w.) reach (152.00± 0.005, 0.075 ± 0.001 and 0.068 ± 0.04) respectively at 60 days as compared with both toxic groups.

The result showed that thallium acetate ingestion induce a significant elevation of serum ALT, AST, and ALP levels in a dose and time dependent manner and that may be attributed to leakage of these enzymes from the liver cytosol into the blood stream when exsposed to thallium (17, 18). The results were in agreement with (19) who considered that high levels of aminotransaminase (ALT and AST) and ALP are crucial parameters in detecting liver damage with thallium exposure. The researchers suggest that the cause of increase serum activities of ALT and AST to the increased cellular basal metabolic rate, irritability and destructive changes of liver and skeletal muscles (20, 21). It has been reported that serum ALT was elevated significantly more than AST on thallium exposure which indicated liver damage and development of fibrosis (22), and that was compatible with the present study. Haman and animal studies reported the hepatoprotective effect of vitamins E (23, 24). In the present study the supplementation of vitamin E ameliorate the biochemical changes due to thallium acetate toxicity and that attributed to its antioxidant property buried in its structure. Structurally, the side chain in the 2- position facilitates the incorporation and retention of vitamin E in biomembranes, so that the 6- position is optimal for scavenging free radicals and terminating lipid peroxidation, furthermore, its oxidant effect is exhibited through protection of poly unsaturated fatty acid from oxidation by Reactive Oxygen Species, stabilization of membrane and breaking of antioxidant chains that prevent reactive oxygen species damage to the membranes (25). The ability of vitamin E to reveres or prevent chemical agents induced hepatotoxicity was demonstrated by (26), who showed that supplementation of vitamin E normalized AST and ALT levels elevated by carbon tetrachloride in rats. Similarly, the hepatoprotective effect of vitamin E in heavy metals toxicity was reported by(27).

Table (1) Effects of thallium acetate on the liver parameters of rats

Period / Group	ALP			AST			ALT		
	20 day	40 day	60 day	20 day	40 day	60 day	20 day	40 day	60 day
Thallium (0.080)	199.33 ± 0.005 C c	221.66 ± 0.005 C b	247.66 ± 0.005 B a	0.063 ± 0.002 B c	0.072 ± 0.003 B b	0.090 ± 0.003 B a	0.060 ± 0.01 D c	0.086 ± 0.01 B b	0.110 ± 0.02 B a
Thallium (0.16)	434.33 ± 0.004 A c	477.33 ± 0.004 A b	494.33 ± 0.005 A a	0.075 ± 0.003 A c	0.093 ± 0.005 A b	0.15 ± 0.005 A a	0.100 ± 0.02 A c	0.150 ± 0.03 A b	0.203 ± 0.03 A a
Thallium (0.080) + Vitamin E	267.00 ±0.003 B a	253.66±0.005 B b	178.66±0.005 C c	0.067 ± 0.002 B b	0.078 ± 0.003 B a	0.083 ± 0.003 B a	0.078 ± 0.01 C a	0.065 ± 0.03 D c	0.059 ± 0.03 D b
Thallium (0.16) + Vitamin E	156.00± 0.005 D a	154.30 ± 0.003 D b	152.00 ± 0.005 D c	0.054 ± 0.002 C c	0.066 ± 0.002 C b	0.075 ± 0.001 C a	0.093 ± 0.03 B a	0.077 ± 0.04 C b	0.068 ± 0.04 C c
Vitamin E	148.67 ± 0.003 E a	149.33 ± 0.003 E a	149.67 ± 0.003 E a	0.048 ± 0.003 C c	0.049 ± 0.003 D a	0.048 ± 0.003 D a	0.040 ± 0.01 B	0.041 ± 0.03 B	0.041 ± 0.03 E
Control	148.33 ± 0.003 E b	149.67 ± 0.003 E a	148.33 ± 0.003 E b	0.047 ± 0.003 C c	0.048 ± 0.003 D a	0.048 ± 0.003 D a	0.039 ± 0.02 E	0.040 ± 0.03 E	0.040 ± 0.03 E

Different small letters means significant ($p \leq 0.05$) results between groups

Different capital letters means significant ($p \leq 0.05$) results between periods

- **Kidney parameters:** The value of S. creatinin and urea concentration in both toxic groups were shown in table (2). There was a significant increase in values along the experiment which reached (4.280 ± 0.005 and 4.80 ± 0.005) respectively at dose of (0.080 mg/ B.W.), while at dose (0.160 mg/ kg.b.w.) reached (5.100 ± 0.002 and 5.12 ± 0.002) at 60 days respectively as compared with control groups (2.720 ± 0.005 and 3.34 ± 0.005). In supplemented groups with vitamin E it showed significant decrease in value of S. creatinin and urea with period of experiment which reached (2.950 ± 0.005 and 3.50 ± 0.003) at dose (0.080 mg/ B.W.) while at a dose of (0.160 mg/ kg.b.w.) it reached (3.040 ± 0.003 and 3.64 ± 0.003) respectively at 60 days as compared with both toxic groups. The kidney regulates plasma ionic composition including sodium, potassium, calcium, magnesium, chloride. It is also concerned with the removal of nitrogenous metabolic waste products such as urea, creatinin and uric acid (28). The elevation in urea and creatinin level in thallium acetate treated rats can be considered as a significant marker of renal dysfunction due to thallium (17, 29). The results are in agreement with (30) who stated that elevation of clinical chemistry parameters such as blood urea and ceratinin levels, indicating kidney damage with thallium exposure. The increase in urea concentrations in serum of animals treated with thallium acetate may be due to its effect on the liver function as urea is the end-product of protein catabolism. On the other hand, the elevated levels of urea might be due to the destruction of red blood cells (31, 32). And any malfunctioning in the glomarular filtration results in the retention of substances including urea (33). The nephroprotective effect of vitamin E may be due to the antioxidants effect of vitamin. Confirmation of the potency of vitamin E to enhance recovery from renal oxidative damages were reported by many workers (34, 35, 36).

Table (2) Effects of thallium acetate on the kidney parameters of the rats

Period / Group	S. ceartinin			Urea		
	20 days	40 days	60 days	20 days	40 days	60 days
Thallium (0.080)	3.140 ± 0.002 C c	3.850 ± 0.005 B b	4.280 ± 0.005 B a	3.94 ± 0.002 C c	4.16 ± 0.005 B b	4.80 ± 0.005 B a
Thallium (0.160)	3.540 ± 0.004 C c	4.260 ± 0.003 A b	5.100 ± 0.002 A a	4.50 ± 0.004 A c	4.80 ± 0.003 A b	5.12 ± 0.002 A a
Thallium (0.080) + Vitamin E	3.060 ± 0.003 D a	3.000± 0.005 D a	2.950 ± 0.005 D b	3.80 ± 0.003 D a	3.62 ± 0.005 D b	3.50 ± 0.003 D c
Thallium (0.160) + Vitamin E	3.440 ± 0.004 B a	3.220 ± 0.005 C b	3.040 ± 0.003 C c	4.06 ± 0.004 B a	3.98 ± 0.005 C b	3.64 ± 0.003 C c
Vitamin E	2.660 ± 0.004 E a	2.680 ± 0.004 E b	2.780 ± 0.005 E a	3.00 ± 0.004 E c	3.20 ± 0.005 E b	3.34 ± 0.005 E a
Control	2.802± 0.005 E a	2.720 ± 0.005 E b	2.720 ± 0.005 E b	3.28 ± 0.005 E b	3.34 ± 0.005 E a	3.34 ± 0.005 E a

Different small letters means significant ($p \leq 0.05$) results between groups
Different capital letters means significant ($p \leq 0.05$) results between periods

- **Pathological study:**
- **Liver:**
- **Thallium acetate: 0.080 mg/ kg B.W. orally. At 20 days period:** There is cellular degenerative changes of hepatocytes became hypertrophiod containing hyperchromic nuclei. The central viens and sinusoids are dilated and congested containing inflammatory cells in their lumina mainly neutrophils. **At 40 and 60 days period:** There is degeneration and necrosis of hepatocytes became disassociated from one another disrupts liver cell columns, increase in rate of apoptosis and severe hemorrhage (Fig. 3).

- **Thallium acetate: 0.0160 mg/ kg B.W. orally. At 20 days period:** The main microscopic pathological changes at this period were infiltration of inflammatory cells mainly mononuclear cells in the portal areas with severe congestion of portal blood vessels containing inflammatory cells in their lumina (Fig. 4). **At 40 days period:** In addition to the previous lesions there is mononuclear cells infiltration in the portal area with slight fibrosis (Fig. 5). In addition to fibrous thickening of the wall of central veins. **At 60 days period:** The main striking microscopic lesion at this period was the formation of hyperplastic nodules lacking the central veins causing pressure atrophy to the adjacent hepatic parenchyma (Fig. 6).
- **Kidneys:**
- **Thallium acetate: 0.080 mg/ kg B.W. orally. At 20 days period:** The main lesion at this period was diffuse coagulative necrosis of epithelial cells lining the proximal and distal convoluted tubules. In addition to atrophy of glomerular tufts with severe congestion of interstitial blood vessels. **At 40 days period:** In addition to previous lesions there is cortical multiple focal interstitial aggregations of mononuclear cells lead to pressure atrophy of proximal and distal convoluted tubules which showed diffuse necrosis of their epithelial lining cells (Fig. 7). **At 60 days period:** Deposition of hyaline droplets within the proximal and distal convoluted renal tubules appeared as deep eosinophilic rounded or oval in shape structures (Fig. 8).
- **Thallium acetate: 0.0160 mg\ kg B.W. orally. At 20 days period:** There is focal areas of severe cortical hemorrhage (Fig. 9) in addition there is a slight fibrosis of the organ capsule. **At 40 days period:** The capsule of the organ are greatly thickened due to fibrosis. The medullary collecting tubules undergo cystic dilation and contain eosinophilic hyaline casts (Fig. 10). **At 60 days period:** In addition to the previous lesions, tissue sections showed aggregation of mononuclear cells in the subcapsular area. Cortical region showed interstitial mononuclear cells infiltration with severe fibrosis (Fig. 11).
- **Supplemented groups:**
- **Liver:** The microscopic appearance was characterized by resolution of hepatocytes with kupffer's cells proliferation and the presence of mononuclear cells in the central veins (Fig. 12) especially after 40, 60, days of supplementation in both toxic groups. Other pathological lesions observed in the examined sections were the infiltration of lymphocytes in the dilated sinusoids at a dose of (0.080 mg/ kg B.W. administrated orally with vitamin E 40 mg/kg.B.W) begun at 20 days of supplementation and extend to the end of the experiment (Fig. 13). In 0.0160 mg/ kg B.W. administrated orally with vitamin E 40 mg/kg.B.W granuloma within parenchyma and beside the blood vessels with proliferation of kupffer's cells were the predominant microscopic picture especially at 60 days of supplementation (Fig. 14, 15).
- **Kidneys:** The severity of lesions are less in tissue sections exhibited by cloudy swelling of proximal and distal convoluted tubules instead of necrosis seen in toxic groups. These pathological changes are seen clearly in both supplemented groups especially in late stages of experiment (Fig. 16). In addition to perivascular lymphocytic cuffings of the interstitial blood vessels.
- **Toxic groups:**
- **Liver:** The significant pathological changes in the liver was the centri-lobular necrosis which begun centrally and progress peripheray in the lobule and it appeared aconstant feature for all examined sections. These changes may be due to accumulation of thallium in the mitochondria and lysosomes causing progressive hepatocyte organelles damage, cellular degeneration and necrosis, or it may result

from hypoxia in the perivenular region with increase in hepatic oxygen demand without an appropriate hepatic blood flow, the necrotic area formed gaps which lead to accumulation of blood as seen in the microscopic section (37). Necrosis with severe inflammatory reaction may account for an ischemic effect on hepatic cells. Ischemia causes loss of oxidative phosphorelation by mitochondria and the generation of ATP slow or stopped (38). ATP depletion is primary responsible for acute cellular degeneration and necrosis (39, 40). The heavy metal stress in all activity of living organisms often result in the production of reactive oxygen species ('ROS), which are relatively reactive as compared to molecular oxygen and thus potentially toxic (41, 42, 43). Consequent leakage of electrons from photosynthetic and mitochondrial electron transport chains to molecular oxygen enables higher production of ROS such as singlet oxygen, superoxide, hydrogen peroxide (H_2O_2), and hydroxyl radicals, which can also be formed in peroxisomes and plasma membranes. These cytotoxic ROS can disturb normal metabolic processes through oxidative damage of lipids, proteins, and DNA. The cytological changes of Gammarus pulex when exposed to thallium was examined with Transmission Electron Microscope. Due to thallium intoxication, degenerative changes were frequently present in the cellular membranes, there were changes in the mitochondria as partial or total loss of cristae, there was an increase in the number of lipid droplets, lysosomes and autophagic vacuoles that increase in hepatocytes and the nucleus showed significant shrinkage and deformation. Fragmentation and dilation of the rough endoplasmic reticulum (RER) and the number of lesion also increased in the inner and outer mitochondrial membranes and in the RER, a lot of lipid droplets were also observed in the hepatocytes (3). Tissue sections showed increase apoptosis of hepatocytes. Apoptosis is an active and highly regulated form of cell death responsible for the cellular default demise of the hepatocytes which occur due to the toxic effects of the thallium, this agreed with (44) who mentioned that thallium induces swelling in mitochondria and apoptosis in jurkat cells transition pore (MTP) in mitochondria. Furthermore; thallium acetate induced cell cycle arrest. The impairment of cell cycle progression may trigger the activation of a mitochondrial pathway and shifts the balance in Bcl-2 family toward the proapoptotic members, promoting the formation of the apoptosome and, consequently, apoptosis (45). Other important pathological changes especially at later stages of experiment was the formation of hyperplastic nodules. This is because the liver is regarded as the tissue of high regenerative capacity (46). According to (47) hepatocytes exhibit a very good regenerative response to several stimuli, including massive destruction of hepatic tissue by toxins, viral agents, or surgical extraction, this agreed with previous studies that noticed in young adult rats nearly all hepatocytes have the potential to re- enter the cell cycle (48, 49), or it might be due to mutagenic effect of the thallium acetate since contradictory results have been published by (50).

- **Kidneys:** Histopathological examination of kidneys sections showed diffuse coagulative necrosis of the epithelial lining of the proximal and distal convoluted tubules with atrophy of glomeular tufts. These changes begun earlier and became more severe at the late stages of experiment and this might be attributed to the damage caused by thallium acetate resulting from its concentration in renal tubules cells and reaction with proteins to cause necrosis (51). The other histopathological changes was the formation of hyaline droplets at a dose of (0.080 mg/kg B.W.) and hyaline casts at dose (0.0160 mg/kg B.W.) especially at the late stage of experiment. This result may be related to the toxic effect of thallium acetate leading to renal

insufficiency resulting in decreased execrtion of thallium acetate metabolites and their accumulation within the cortical tubules which leads to sloughing of tubular cells and formation of hyaline casts within the cortical tubules and can be attributed to sloughing of cell lining of these tubules and accumulate that leads to formation of hyaline casts (52).

- **Supplemented groups:** The present study demonstrates that the ingestion of vitamin E with thallium acetate in rats reducing or nullifying the injurious effects of thallium acetate as shown by histopathological findings of liver and kidney. Tissue sections of supplemented groups showed a marked proliferation of the kuffer's cells of the liver which is an indication of specialized highly activated macrophages (53). Furthermore, vitamin E has a hepatoprotective effect by the leakage of intracellular enzymes, reducing the oxidation of proteins and decreasing incidence of apoptosis (25, 54). The result of the present study showed resolution of hepatocytes this might be due to the efficiency of vitamin E in regeneration of the cellular and physiological status of liver tissue. These results coincides with the observation of others (55, 56). The cloudy swellings of the renal tubules confirmed the protective capacity of vitamin E.

Fig. (1) Macroscopic appearance of rat treated orally with (0.080 mg/kg.bw) of thallium acetate for 40 days showing focal alopecia ( ).

Fig. (2) Macroscopic appearance of rat treated orally with (0.160 mg/ kg bw) thallium acetate for 20 days showing ocular opacity ( ) and piloerection ( ).

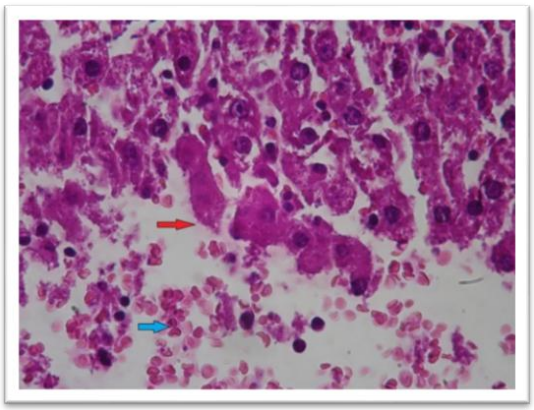

Fig. (3) Histopathological section of liver of rat treated orally with 0.080 mg/kg. bw/ day of thallium acetate for 40 days showing necrosis of hepatocytes ( ) with severe hemorrhage ( ) (H&E stain 400×)

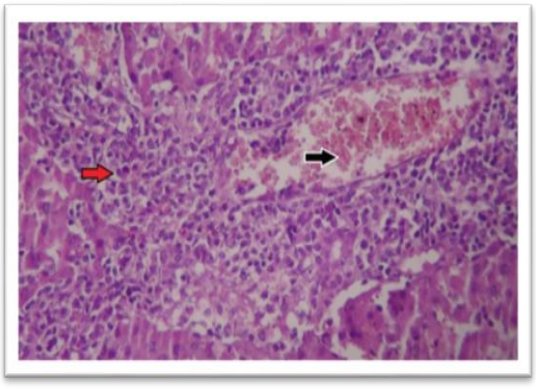

Fig. (4) Histopathological section of liver of rat treated orally with 0.160 mg/kg. bw/ day of thallium acetate for 20 days showing mononuclear cells infiltration in the portal area ( ) with severe congestion of portal blood vessels containing inflammatory cells in lumen ( )

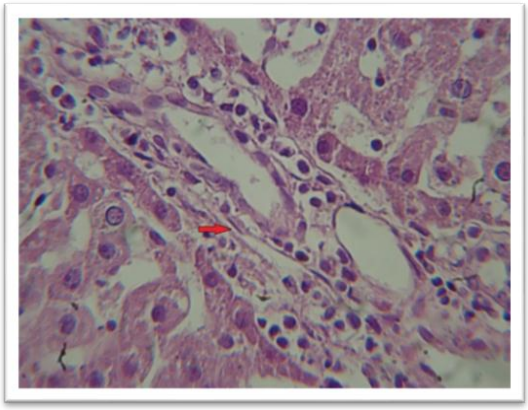

Fig. (5) Histopathological section of liver of rat treated orally with 0.160 mg/ kg. b.w./ day of thallium acetate for 40 days showing mononuclear cells infiltration in the portal area with slight fibrosis (➡) (H&E stain 400×).

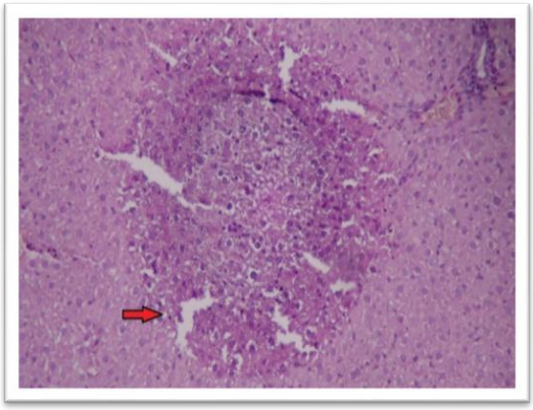

Fig. (6) Histopathological section of liver of rat treated orally with 0.160 mg/ kg. b.w./ day of thallium acetate for 60 days showing hyperplasic nodules lacking the central vein (➡) (H&E stain 400×).

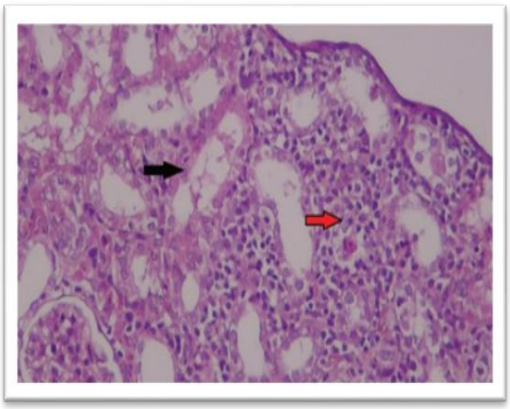

Fig. (7) Histopathological section of kidney of rat treated orally with 0.080 mg/ kg. b.w./ day of thallium acetate for 40 days showing focal aggregation of mononuclear cells leads to pressure atrophy of cortical renal tubules (➡) which shows diffuse necrosis of their epithelial lining cells (➡) (H&E stain 400×).

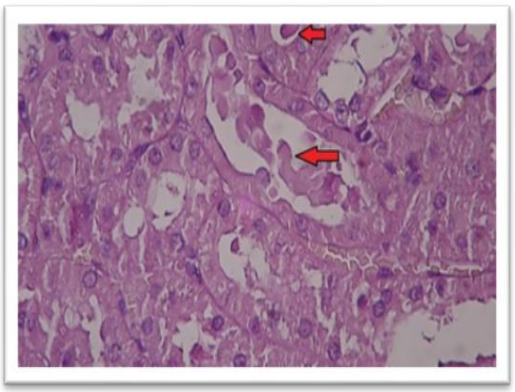

Fig. (8) Histopathological section of kidney of rat treated orally with 0.080 mg/ kg. b.w./ day of thallium acetate for 60 days showing hyaline droplets within the cortical renal tubules appeared as deep eosinophilic rounded or oval in shape (➡) (H&E stain 400×).

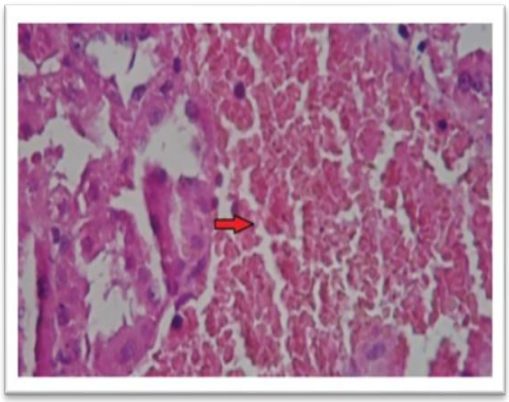

Fig. (9) Histopathological section of kidney of rat treated orally with 0.160 mg/ kg. b.w./ day of thallium acetate for 20 days showing severe hemorrhage (➡) of the cortical area (H&E stain 400×).

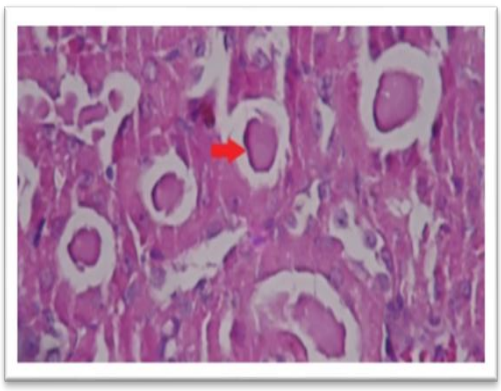

Fig. (10) Histopathological section of kidney of rat treated orally with 0.160 mg/ kg. b.w./ day of thallium acetate for 40 days showing eosinophilic hyaline cast (➡) (H&E stain 400×)

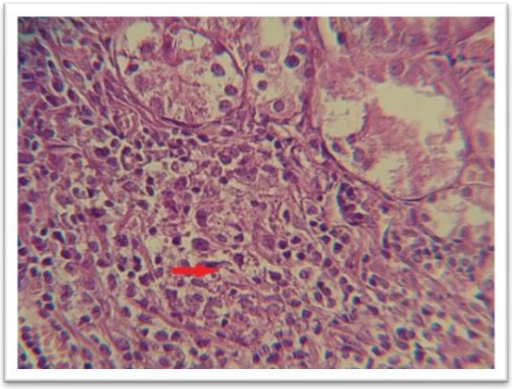

Fig. (11) Histopathological section of kidney of rat treated orally with 0.160 mg/kg. bw/ day of thallium acetate for 60 days showing interstitial mononuclear cells infiltration and marked fibrosis of cortical area (➡) (H&E stain 400×)

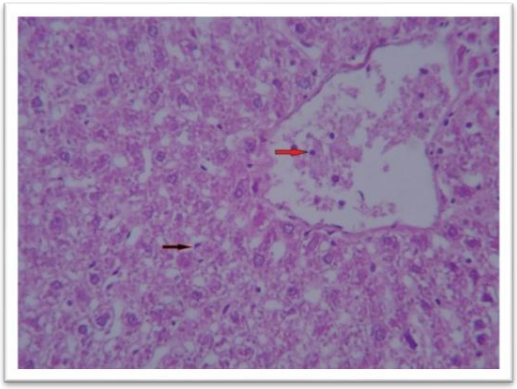

Fig. (12) Histopathological section of liver of rat treated orally with (0.080 mg/ kg. bw/ day of thallium acetate with vitamin E 40 mg/ kg b.w.) for 60 days showing resolution of hepatocytes with kupffer's cell proliferation (➡) and mononuclear cells in the central vein (➡) (H&E stain 400×)

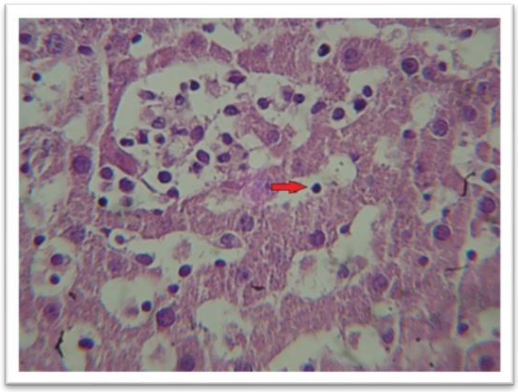

Fig. (13) Histopathological section of liver of rat treated orally with (0.080 mg/kg. bw/ day of thallium acetate with vitamin E 40 mg/ kg b.w.) for 40 days showing infiltration of mononuclear cells in the dilated sinusoids (➡) (H&E stain 400×).

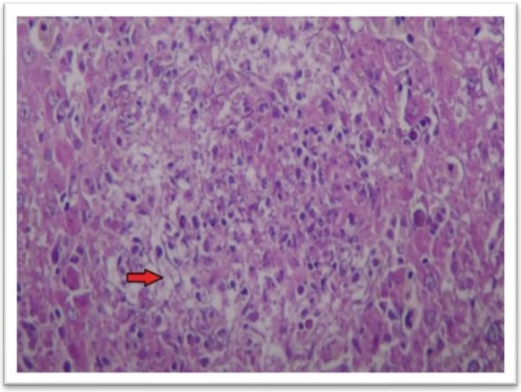

Fig. (14) Histopathological section of liver of rat treated orally with (0.160 mg/kg. bw/ day of thallium acetate with vitamin E 40 mg/ kg b.w.) for 60 days showing formation of early granuloma (➡) (H&E stain 400×).

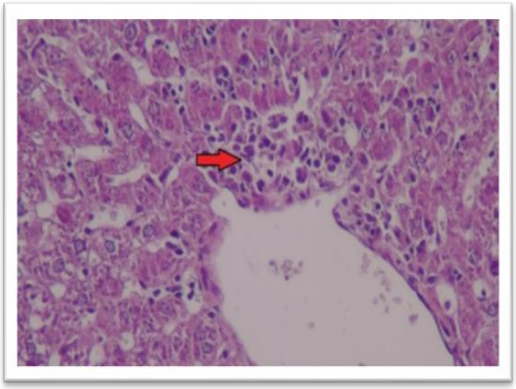

Fig. (15) Histopathological section of kidney of rat treated orally with (0.160 mg/kg. bw/ day of thallium acetate + vitamin E 40mg/ kg b.w.) for 60 days showing granuloma beside blood vessel (➡) (H&E stain 400×).

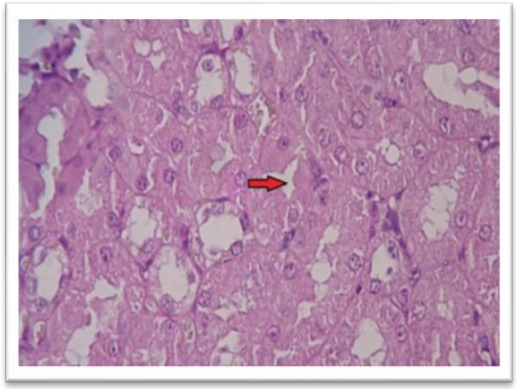

Fig. (16) Histopathological section of kidney of rat treated orally with 0.160 mg/kg. bw/ day of thallium acetate with vitamin E 40 mg/ kg b.w.) for 60 days) showing cloudy swelling of the renal cortical tubule (➡) (H&E stain 400×).

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