Pathological and clinico-pathological studies on the protective effect of vitamin E against cadmium chloride toxicosis in male albino rats

By
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SUMMARY

The cytotoxic effect of cadmium chloride in albino rats and the possible protective effect of vitamin E against cadmium toxicity in rats were studied. The biochemical and histopathological structural changes of the livers, kidneys and testes sections were investigated.

Forty male albino rats were equally divided into four main groups as the following: control group, cadmium (Cd) treated group (1.5 mg/kg Bw/daily in drinking water), vitamin E treated group (20 IU in water/daily) and Cd + Vit E group (1.5 mg/kg Bw/daily + 20 IU in drinking water) for 15 days. The activities of plasma transaminases (AST & ALT), creatinine and urea were measured indicators for both liver and kidney functions. Levels of lipid peroxide (LPO) and glutathione (GSH) were determined using colorimetric methods.

The cadmium intoxicated group showed a significant elevation of plasma levels of transaminases, creatinine and urea that means dysfunction of both liver and kidneys. The levels of LPO were significant higher but the levels of GSH were significant lower than controls. The biochemical changes were supportive to the histopathological changes showed in the same group (Cd-treated group) in the form of severe degenerative as well as necrotic changes of the hepatocytes, renal tubular cells and the testicular cells besides edema, congestion and hemorrhage with mononuclear cells infiltrations. The cases treated with cadmium chloride and vitamin E showed marked improvement in both the biochemical and histological changes comparing to rats treated with cadmium alone as the following: A significant reduction in the levels of LPO, AST, ALT, creatinine and urea, while the levels of GSH were significantly increased in comparison with cadmium treated group. The histological changes of this group (Cd + vitamin E) were mild to moderate degenerative changes.

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(cloudy swelling, vacuolar and mild hydropic changes), and minute foci of mononuclear cell infiltration in the portal area.

The antioxidant properties of vitamin E associated with lipid peroxidation were effective in protection against cadmium toxicity.

**Keywords:** Rat; Vitamin E; Cadmium chloride; Liver; Kidney, Testes, **Running title:** cadmium toxicity and vitamin E.

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**INTRODUCTION**

The exposure to the toxic metals has become an increasingly recognized source of illness world wide (Patrick, 2003).

Cadmium is a heavy metal well known to be highly toxic to both human and animals. Some of toxic effects of cadmium exposure are testicular atrophy, renal tubular dysfunction, lung fibrosis, hypertension, osteoporosis, central nervous system injury, anemia and cancer (Barnhart, 1984; Tandon et al., 2003 and Chinnaaswamy and Jeyaprakash, 2005).

Cadmium may induce oxidative damage in different tissues by enhancing peroxidation of membrane lipids in tissues and altering the antioxidant system of the cell. The peroxidative damage to cell membrane may cause injury to cellular components due to the interaction of metal ions with cell organelles (Sarkar et al., 1995). The cadmium also induces damage via production of free radicals that alter mitochondrial activity and genetic information. The metabolism and excretion of cadmium depend upon the presence of antioxidants and cadmium metallothionein – binding-S- adenosyl L- methionine, lipoic acid, glutathione, selenium, alpha-tocopherol and ascorbic acid. Generally, the cellular damage (renal and hepatic cells) results from cadmium binding to SH-group in tissues, the production of lipid peroxides and the depletion of glutathione besides inhibits the activity of the antioxidant enzymes, which include cata-
lase, superoxide dismutase (Chambers et al., 1998).

Cadmium is detoxified in the liver through formation of metallothionein – cadmium complex, which is slowly released from that organ causing congestion, hemorrhage, apoptosis and necrosis. Also, cadmium metallothionein complex can be nephrotoxic as it accumulates in kidneys causing cloudy swelling of renal tubular cells as well as necrosis. Finally, testicular edema, atrophy and necrosis also detected (Gunn et al., 1963; Gibbiani, 1966; Gibbiani et al., 1974; WHO, 1992; Sumathi et al., 1996; Saucer et al., 1997; Brzoska et al., 2003; Karl et al., 2005; Jeyaprakash and Chinnaswamy, 2005).

Vitamin E, lipid–soluble antioxidant, keeps the maintenance of the cell membrane integrity via free radicals scavenging potential. Vitamin E prevents the process of lipid peroxidation by scavenging reactive oxygen species before they can damage cells. Furthermore, vitamin E is metabolized in the liver to glucuronides of tocopheronic acid and its Y-lactone and excreted principally in the bile (Aabcdefsies, 1997; Khan, 2003; Ahfs, 2004; Bansal et al., 2005; Anonymous, 2005 and Anonymous, 2007).

The aims of the present study are to detect the toxic effect of cadmium chloride in male albino rats and the protective role of vitamin E against cadmium toxicity in rats.

MATERIALS AND METHODS

I-Chemicals: All the chemicals utilized were fine. The Cadmium chloride, vitamin E, and reduced glutathione (GSH) were purchased from Sigma chemical company (St. Louis, Mo, USA).

II- Animals and treatment: Forty male albino rats (Wistar strain) weighing 150-175 gm were obtained from animal breeding center, College of Pharmacy, King Saud University, Riyadh and put in controlled – room temperature (27 ± 2 ºC), humidity (55±10%) and light 12:12 hrs L:D cycle. Animals were fed with standard pellets (Sawameh company, Burayda, KSA). They were given a week time to get acclimatized with laboratory condition. Ethical clearance for handling of experimental animals was obtained from the Committee Constituted for this purpose. After acclimatization the animals were divided into the following 4 groups of 10 rats each:

Group A: normal control;
Group B: The rats were treated with cadmium (1.5 mg/kg Bw./day) as cadmium chloride in drinking water for 15 days;
Group C: The rats were treated with vitamin E (20 IU) in water daily for 15 days.

Group D: The rats were administered Cadmium chloride 1.5 mg/kg BW for 15 days in drinking water + Vitamin E, 20 IU daily for 15 days also in drinking water.

At the end of the experimental period (15 days), the rats were deprived of food overnight and sacrificed by light ether anesthesia. Blood samples from all groups were collected from the orbital vein in heparinized tubes and were centrifuged at 5000 rpm for 10 min for plasma separation. The plasma sample was divided into aliquots and kept at -26 °C until biochemical analyses.

III- Biochemical studies:

The levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in plasma were estimated by the method of King, (1965). The plasma levels of creatinine and urea were estimated according to Fabiny and Ertingshausen (1971) and Chaney and Marbach (1962) respectively.

The plasma levels of lipid peroxides (LPO) were measured as thiobarbituric acid reactivity (TBARS). The product of the reaction between malondialdehyde and thiobarbituric acid was measured as described (Thayer, 1985). The plasma GSH levels were determined chemically as described by Ellman (1959).

IV-Histopathology:

Samples of liver, kidney and testes were fixed in 10% neutral buffered formalin solution, dehydrated in with 90% ethanol, cleared in xylol embedded in paraffin, thin section (5 µm in thickness), were prepared and stained with haematoxylin and eosin dye according to Bancroft and Stevens (1996).

V-Statistical analysis: The results are expressed as mean standard error (SE). Differences between groups were assessed by one-way analysis of variance (Bonferroni test) using the Prism version 4 software package for Windows. The level of significance was accepted with P <0.05.

RESULTS

I-Biochemical results: The plasma levels of transaminases, creatinine and urea in different studied groups are shown in Table (1). The Cd-group showed dysfunction of the liver and kidney as detected by significant elevation of plasma transaminases, creatinine and urea comparing with control group. In the Cd+Vit E group, the levels of GPT, and creatinine were significantly reduced in comparison with Cd-group. The plasma
levels of lipid peroxides and glutathione in different studied groups are shown in Table (2). The levels of LPO, were significantly higher but the levels of GSH were significantly lower in Cd-group than controls. In the Cd+Vit E group, the levels of LPO were significantly reduced but the levels of GSH were significantly increased in comparison with Cd-group. The mean plasma levels of the biochemical indicators of liver and kidney function tests and oxidative stress indices were presented in Fig. (1).

II-Histopathological results :

A- Gross Picture :

Severe congestion, hemorrhage, edematous swelling as well as focal to multifocal pale, white, depressed necrotic areas showed in liver, kidneys and testes of male albino rats intoxicated with cadmium chloride. The rats treated with CdCl₂ and vitamin E showed mild degree of degeneration and congestion of both central veins and hepatic sinusoids. Finally, no characteristic gross changes showed neither in control group nor in vitamin E. treated rats.

B- Histological changes :

1- Rats treated with Cd alone :

(Group B)

The liver tissues of rats intoxicated with CdCl₂ alone (group B) showed cloudy swelling, vacuolar, hydropic as well as fatty changes (microvesicular-form). Coagulative necrosis was observed and the necrotic hepatocytes were replaced by mononuclear cells mainly, lymphocytes. The portal triad showed biliary hyperplasia, and eosinophilic fluid infiltration besides congestion, hemorrhage and hyperactivation of Kupffer cells that lining the hepatic sinusoids (Fig. 2-5).

Concerning the kidney tissue, the epithelial cells lining the convoluted tubules showed hydropic degeneration, fatty change and hyaline droplets. Some of other renal tubular cells showed coagulative necrosis, that represented by nuclear hyperchromacia, karyorrhexis, karyolysis and cytoplasmic hypereosinophilia. Severe congested blood vessels with interstitial edema and necrosis were also appeared (Fig. 6-9).

The testicular tissues of the cases intoxicated with cadmium chloride alone showed diffuse thickening as well as hyalinization of the basement membrane of the seminiferous tubules. Diffuse testicular necrosis, damage and sloughing of the spermatogenic cells layers besides oligospermia or even in some cases asospermia. Diffuse distention of the interstitial spaces due to diffuse edema, con-
gestion, hemorrhage and necrosis of lydig cells and leukocytic cells infiltration. Some seminiferous tubules in some cases were dilated and others were atrophied. The sertoli cells were degenerated, necrotic and sloughed in the lumen with multinucleated cells aggregation (Fig. 10-11).

2- Rats treated with Cadmium chloride and vitamin E: (Group D)

The microscopic examination of the liver in rats treated with CdCl$_2$ + vitamin E showed mild to moderate reaction in comparison to the group B (CdCl$_2$ alone). The reaction represented by slight degree of cloudy swelling, hydropic changes and congestion (Fig. 12-13).

While, the renal tubular cells were responded to the protective effect of vitamin E in the form of mild hydropic changes of the renal tubular cells as well as slight congestion of the intertubular blood capillaries (Fig. 13).

Finally, the semineferous tubules of the male albino rats testes showed a little reaction in comparison to the destructive effects of CdCl$_2$ alone where showed mild degenerative changes, little interstitial edema and minute foci of apoptic necrosis (Figs. 14-15).

3- Control and vitamin E. treated rats: (Group A & C)

No characteristic histological changes except their normal architecture or pattern.
Table (1): Plasma levels of transaminases, creatinine and urea in different studied groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>(A) Control</th>
<th>(B) Cd</th>
<th>(C) Vit E</th>
<th>(D) Cd+Vit E</th>
<th>P-Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A vs B</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>57.47 ± 8.116</td>
<td>98.86 ± 12.08</td>
<td>51.34 ± 3.711</td>
<td>73.58 ± 8.601</td>
<td>&lt;0.0107 *</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>43.02 ± 3.643</td>
<td>117.5 ± 7.277</td>
<td>47.92 ± 4.564</td>
<td>89.72 ± 6.027</td>
<td>&lt;0.0001 ***</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.7284 ± 0.04103</td>
<td>2.106 ± 0.3281</td>
<td>0.7276 ± 0.05577</td>
<td>1.135 ± 0.1029</td>
<td>&lt;0.0006 ***</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>31.56 ± 2.217</td>
<td>84.07 ± 17.11</td>
<td>32.20 ± 2.523</td>
<td>67.61 ± 6.898</td>
<td>&lt; 0.0070 ***</td>
</tr>
</tbody>
</table>

Values are means ± SE for 10 rats (n= 10 for each group). Other details are given in materials and methods section.
Table (2): Plasma levels of lipid peroxides, nitric oxides and glutathione in different studied groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>(A) Control</th>
<th>(B) Cd</th>
<th>(C) Vit E</th>
<th>(D) Cd+Vit E</th>
<th>P-Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid peroxides (nmol/L)</td>
<td>1.484 ± 0.05108</td>
<td>2.888 ± 0.04540</td>
<td>1.385 ± 0.05611</td>
<td>2.175 ± 0.06139</td>
<td>&lt;0.0001 *** &lt;0.0001 *** &lt;0.0001 *** &lt;0.0001 ***</td>
</tr>
<tr>
<td>GSH (µM/ml)</td>
<td>7.501 ± 0.7180</td>
<td>5.027 ± 0.07344</td>
<td>7.129 ± 0.6162</td>
<td>5.553 ± 0.1755</td>
<td>&lt;0.0030 ** &lt;0.0168 * &lt;0.0033 ** &lt;0.0127 * &lt;0.0243 *</td>
</tr>
</tbody>
</table>

Values are means ± SE for 10 rats (n= 10 for each group). Other details are given in materials and methods section.
Fig. (1): The mean levels of the (A) AST; (B) ALT; (C) Creatinine; (D) Urea; (E) Lipid peroxidation and (F) Glutathione in all studied groups.
Fig. (2): Liver of rats, intoxicated with cadmium chloride alone, showing: Hydropic swelling, fatty changes and minute foci of necrosis and MNC infiltration (H & E .X.400).

Fig. (3): Liver of rats intoxicated with cadmium chloride alone; Notice the congestion, biliary hyperplasia and mononuclear cell infiltration (H&E.X.400).
Fig. (4): Liver of rats, intoxicated with cadmium chloride alone, showing: Biliary hyperplasia, diffuse eosinophilic infiltration as well as necrosis with mononuclear inflammatory cells (H&E.X.400).

Fig.(5): Liver of rats, intoxicated with cadmium chloride alone, showing: Necrotic hepatocytes that replaced by MNC infiltration (H&E.X. 200).
Fig.(6): Kidney of rat treated with CdCl₂ showing: severe degeneration of the renal cells (H&E. X. 400).

Fig.(7): Kidney of rat, treated with CdCl₂ showing: Intracellular hyaline droplets, and coagulative necrosis (H&E.X.400).
Fig. (8): Kidney of rat, treated with CdCl₂, showing tubular necrosis and congestion of the blood vessels (H&E. X.400).

Fig. (9): Kidney of rat, treated with CdCl₂, showing perivascular edema and mononuclear cell infiltration. Notice vacuolar degeneration of the tubular epithelial cells, necrosis with MNC infiltration (H&E. X. 400).
Fig. (10): Testes of rat, treated with CdCl₂, showing: Extensive widening of the interstitial spaces due to accumulation of edematous fluid, with mononuclear cells infiltration, (H&E.X. 400). Notice the necrosis of lydig cells.

Fig. (11): Testes of rat, treated with CdCl₂, showing: Complete testicular necrosis and sloughing of the spermatocytes (H&E.X.400).
Fig. (12): Liver of rat, treated with CdCl₂ + vitamin E., showing : Mild degree of vacuolation of the hepatocytes (H&E.X.400).

Fig. (13): Kidney of male albino rats, treated with CdCl₂ + vitamin E., showing : Mild degeneration and congestion (H&E.X.200)
Fig. (14): Testes of male rats, treated with CdCl₂ + vitamin E., showing: Mild interstitial edema and congestion (H&E .X . 200).

Fig. (15): Testes of male rats, treated with cdcl₂ + vitamin E., showing : Congestion and degeneration of sertoli cells (H&E. X.400).
DISCUSSION

Cadmium is a very toxic metal and an important environmental pollutant which is present in the soil, water, air, food and in cigarette smoke. Cadmium causes poisoning in various tissues (Liver, kidneys and testes) of humans and animals (Stohs et al., 2000).

In the present study, the cadmium induced oxidative damage by increased lipid peroxidation and inhibitions of enzymes required to prevent such oxidative damage. Similar results showed by (Kelley et al., 1999). Also cadmium chloride induces a significant elevation of the levels of transaminases, urea and creatinine that indicates dysfunction of both liver and kidneys in addition to testes. The damages caused by cadmium were histologically in the form of vacuolar, hydropic as well as fatty changes of the hepatocytes, renal tubular and testicular cells that finally showed sloughed. These results were coincide with those reported by many investigators (Gunn et al., 1963; Gibbiani, 1966; WHO, 1992; Saucer, 1997; Karl et al., 2005).

A possible explanations for cadmium toxicity are: cadmium induced damage via production of free radicals that alter mitochondrial activity and genetic information. The cellular damage showed in liver, kidneys and testes in the present study results from cadmium binding to SH-group in tissues, the production of lipid peroxides and the depletion of the glutathione besides inhibition of the activity of antioxidant enzymes (Sarkar et al., 1995). The another explanation is that cadmium detoxified in the liver through formation of metallothionein–complex, which is slowly released from that organ and causing congestion, hemorrhage, apoptosis and necrosis (Brazoska et al., 2003). Finally, the renal damaged in the present study may be due to that cadmium induced impairment to the glomerular infiltrations in rats causing renal tubular degeneration, necrosis and fibrosis (Uriu et al., 1998).

Marked improvement biochemically and histologically appeared in that rats treated with cadmium chloride and vitamin E comparing to that of cadmium chloride alone. The improvement occurred due to the antioxidant power of the vitamin E as scavenge the free radicals induced by cadmium chloride, inhibit prostaglandin F2 alpha production and enhance immunity response as well as prevent lipid peroxidation that cause damage to the cells (liver, kidneys and testes).

In conclusion, the vitamin E in the present study reduced the
free radicals generation and improved the antioxidant status in cadmium treated group which is confirmed histologically. Furthermore, vitamin E may be useful in decreasing the toxicity of cadmium. Finally, the only effective method of treatment for cadmium toxicity is the elimination or prevention of exposure.

REFERENCES


دراسات باثولوجية وباثولوجية اكلينيكية على التأثير الوقائي لفيتامين ه ضد التسمم بـكلوريد الكادميوم في ذكور فئران الألبينو

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الملخص العربي

هذه الدراسة تمت لمعرفة تأثير كلوريد الكادميوم الخلوي السام في ذكور فئران الألبينو وأيضاً دراسة الدور الوقائي المحتمل لفيتامين H وسوف يكون ذلك مدعوماً بالدراسات الكيميائية الحيوية والدراسات النسجية المرضية.

تم استخدام عدد 40 فئار ألبينو قسمت إلى أربع مجموعات (10/ مجموعة): المجموعة الأولى استخدمت كضابط للتجربة، المجموعة الثانية تم إحداث التسمم التجريبي باستخدام كلوريد الكادميوم بجرعة 1.5 مليجرام / كجم من وزن الجسم
مع مياه الشرب لمدة ١٥ يوم، المجموعة الثالثة استخدمت كلاً من كلوريد الكالسيوم وفيتامين هـ بجرعات ١٥ ملغ/جم من وزن الجسم + ٢٠ وحدة دولية فيتامين هـ لمدة ١٥ يوم أما المجموعة الرابعة والأخيرة فاستخدمت فيتامين هـ فقط بنفس الجرعة السابقة لفيتامين هـ لمدة ١٥ يوم. تم قياس الأنزيمات المسؤولة عن وظائف الكبد والكلي بالإضافة إلى قياس مستويات الدهون المؤكسد والجلوتاثيون لمراعفة تأثير الكالسيوم وفيتامين هـ.

في المجموعة الأولى التي عولجت بالكالسيوم فقط، شهد زيادة معنوية في مستويات أنزيمات الكبد والكلي، وهي الدالة على حدوث تغير في وظائفهما بالإضافة إلى زيادة ملموسة في مستوى الدهون المؤكسد ونقص في مستوى الجوتوثاثيون والكالسيوم وفيتامين هـ. التغيرات في الدهون المؤكسد ونقص في مستويات أنزيمات الكبد والكلي، بالإضافة إلى أنابيب الخصية وانتشار للخلايا الالتهابية، بينما الحالات التي تم علاجها باستخدام الكالسيوم وفيتامين هـ فلم تظهر تغيرات إيجابية في كلاً من الدراسات الكيميائية الحيوية والبهسبوتاكليلوجية، والتي ظهرت في صورة نقص مستوي الدهون المؤكسد وإنزيماً الكبد والكلي، أيضاً زيادة مستوي إنزيماً كود ملحة في الحيوان (تيل على تسخين الدهون) مقارنة بالمجموعة الأولى.

من الدراسة الحالية يمكن أن نتضح الآتي:

إن فيتامين هـ، وهو واقتي ضد التهيج بروفيريد الكالسيوم نتيجة خصائصه المضادة للأكسدة بالإضافة إلى منعها من أكسدة الدهون والتي تسبب تحميلاً بجسم الإنسان والحيوان مما سبب في حدوث تحسن واضح في الحيوانات التي عولجت باستخدام مقارنة بمجموعة الكالسيوم فقط.

المهمشون:

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