Evaluation of the Curcumin role against cytotoxicity of cadmium chloride in mice

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ABSTRACT

The present study was aimed to evaluate the role of curcumin (CMN) against cytotoxicity of cadmium chloride. Thirty adult male albino mice 3 months old and 20-25 grams body weight, were caged into six equal groups. Mice were injected, i.p with 50 mg CMN/kg and/or 0.67 mg CdCl₂/kg b.w for 96h, separately and alternated. The alternated trials were continued for consecutive 8 days.

Results appeared that mice injected with cadmium had significant higher frequency of chromosomal aberrations as fragments, centric fusion, gaps, stickiness and aneuploidy. Also, CdCl₂ appeared sperm hummer, without hook, banana and amorphous abnormalities. The administration of CMN improved the frequency of the chromosomal aberrations and sperm abnormalities induced by CdCl₂. The administration of CMN after CdCl₂ protects the body from cytotoxicity arising from CdCl₂ as well as normal sperm values were recorded. The administration of curcumin before CdCl₂ injection did not improve the CdCl₂ effect. It could be concluded that the employed dose of CMN protects the body from cytotoxicity arising.

Keywords: Cadmium, Curcumin, sperm abnormalities, chromosome aberrations.

INTRODUCTION

Cadmium is known as one of the most toxic heavy metals. International Agency for Research on Cancer (IARC) has classified cadmium as, Group I, carcinogen (IARC, 2004 and De Sousa Viana et al., 2011). Human exposure to Cd can occur by food (the highest average of concentrations are found in Molluscs, kidneys, liver, cereals, cocoa and leafy vegetables), water, smoking cigarette and uses of cadmium and cadmium-based products such as television phosphors, a pigment in paints, plastics and plasters (Singh et al., 2012; Ashraf, 2012 and Ogungbe and Lawal, 2008). Cadmium is known to be the most harmful heavy metal it causes various diseases such as arteriosclerosis, osteomalacia, hypertension, osteoporosis, cancer and anemia.
The possible mechanism of cadmium induced toxicity is oxidative stress, generation of reactive oxygen species (ROS) resulting in oxidative deterioration of lipids, proteins and DNA consequently, initiating various pathological conditions in humans and animals (Cuypers et al., 2010). So, to relieve Cd adverse effect the antioxidants induction is considered as an important therapeutic approach (Renugadevi and Prabu, 2010).

Curcumin, a polyphenolic substance derived from Curcuma longa In India since times immemorial the powered form of dried Curcuma. It is a mandatory spice present in every Indian kitchen. Curcumin has been shown to have a wide spectrum of biological actions, these includes anti-inflammatory, anti-carcinogenic, anti-mutagenic and antibacterial activities and given the designation of super antioxidant and in some experiments, it has been shown to be 300 times more potent than vitamin E. (Singh et al., 2007).

**MATERIALS AND METHODS**

**Animals**
In this study thirty adult Swiss albino mice (Mus musculus) were used, varying from 20-25gm in weight and aged three month old. Mice were obtained from the National Research Center (N.R.C.), (Dukki, Cairo, Egypt) were caged individually under standard conditions of light, temperature, humidity and fed with standard pellet diet and water ad libitum.

**Chemical and natural:**
Cadmium was used in the formed of cadmium chloride (CdCl$_2$) (Oxford Laboratory, Mumbai, India), with concentration of 0.67mg/ kg or 1/10 LD$_{50}$ according to Bench et al. (1999). Curcumin (CMN) (common curcumin powder) which was purchased from local market was used in concentration of 50 mg CMN/kg b. w. according to Mohammed (2013). Curcumin crystalline: $C_{21}H_{20}O_6$, Molecular weight = 368.39. Minimum assay (acidimetric) 99%. Melting point= 170-180°C.

**Dosage and treatments**
The experimental animals were categorized in 6 equal groups, 5 animals each. Group I was used as untreated negative control. Group II, (control +), was i.p injected with 0.5 ml saline solution daily for 96hs. Group III, was i.p injected with 0.67 mg CdCl$_2$/kg b.w. dissolved in 0.5 ml saline solution as a single dose and give for 96hs. Group IV, was injected i.p, with 50 mg CMN/kg b.w. dissolved in 0.5 ml saline solution daily for 96hs. Group V, was injected i.p with CMN daily for 96hs, then single dose of CdCl$_2$ for 96hs (as a protective trial), Group VI, was i.p, injected with CdCl$_2$ for 96hs as single dose then followed by 4 consecutive Curcumin doses, for 96hs, (as a treatment trial).
Cytogenetically study
Colchicine was injected intraperitoneally 2-3 hrs. before sacrificing animals. Bone-marrow was extracted from femur bone and metaphases were shown according to the method of Yosida and Amano (1965). The preparations of mitotic chromosome were made according to Ford and Hamerton (1956). Giemsa stain (7%) in phosphate buffer (pH 6.8) was used for slides. Hundred spreads metaphases per animal were investigated for chromosomal aberration analysis.

Sperm head morphology assay
Cauda epididymides were excised and both epididymides were minced together in isotonic medium then filtered to exclude large fragments. The cells' Smears were prepared and 5% Eosin Y stained (aqueous). Light microscope (100x), green filter was used to examine smears. Thousand sperms were assessed for each animal to investigate the morphology of sperm abnormality according to the criteria of Wyrobek and Bruce (1975). Any overlay or contact sperms or heads without tails were ignored.

Statistical analysis
Mean ± SE was expressed to all values where 5 animals were evaluated, n=5, in each group. Statistical analysis of cytogenetic was performed on SPSS software (version 18) using one-way ANOVA test. Significance was considered when P values less than 0.05.

RESULTS
Various chromosomal aberrations were observed in the bone marrow cells of albino mice injected with cadmium. These were of structural and numerical type, were identified and quantitated relative to non-treated control. Structural aberration included chromatid deletions (Fig. Ib); fragments (Fig. Ic); centric fusion (Fig. Id); centromeric attenuation (Fig. If); end to end (Fig. Ig); rings (Fig. Ih); polyploidy (Fig. II); stickiness (Fig. Ij). A chromatid was considered to have a Gap when it had an unstained area shorter than its diameter or equal to it, and a break was also scored when a chromatid was markedly shorter than its sister chromatid. End to end association was scored when two chromatids of different chromosomes appeared attached this could result from reciprocal translocation or stickiness. The stickiness is considered as assort of chromosomal agglutination of unknown nature which resulted in a pycnotic or sticky appearance of chromosome.

Figure (2) shows shaped sperms (a) Normal sperm, (b) Hummer sperm, (c) Without hook, (d) Banana shape.
As shown in table (1), the mean number nuclei with chromosomal aberration in mice treated with cadmium appear high significant of chromosomal aberrations in all animals compared with control. The more types of aberration appeared with chromosomal fragments, centric fusion, gaps,
stickiness and aneuploidy. Also, the mitotic activity shows decrease in the treated groups with cadmium chloride when compare to control.

Table (2) incidence of total abnormal shaped sperms per thousand treated with Cd and CMN which include Hummer sperm, without hook, amorphous, Banana shape.

Table (1): Average of chromosomal aberration and mitotic index in bone marrow cells of male mice treated with Cd and CMN.

<table>
<thead>
<tr>
<th>Group</th>
<th>Deletion</th>
<th>Fragment</th>
<th>Centric fusion</th>
<th>Centromeric attenuation</th>
<th>End to end</th>
<th>Gap</th>
<th>Stickiness</th>
<th>Trisomy</th>
<th>Total % Mitotic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.8±0.4</td>
<td>3.0±0.5</td>
<td>8.2±0.7</td>
<td>4.2±0.4</td>
<td>1.6±0.5</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.2±0.8</td>
<td>24±3.3  292.6±12.5</td>
</tr>
<tr>
<td></td>
<td>b,c,d,f,</td>
<td>b,c,d,f,</td>
<td>d,e</td>
<td>d,i</td>
<td>e</td>
<td>e,f,i</td>
<td>e,g</td>
<td>e,i</td>
<td>e,g,h,i</td>
</tr>
<tr>
<td></td>
<td>g,h,i</td>
<td>g,h,i</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control+</td>
<td>1.6±0.5</td>
<td>5.8±0.7</td>
<td>8.6±1.0</td>
<td>5.0±0.7</td>
<td>0.2±0.2</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.2±0.6</td>
<td>21.4±3.7  263±17.1</td>
</tr>
<tr>
<td></td>
<td>a,c,d,g,i</td>
<td>a,c,d,g,i</td>
<td>C,d,e</td>
<td>i</td>
<td>C,e,f,g,i</td>
<td>e</td>
<td>e,f,i</td>
<td>e,g</td>
<td>e,g,h,i</td>
</tr>
<tr>
<td>CMN</td>
<td>2.6±1.0</td>
<td>5.0±0.7</td>
<td>4.6±0.9</td>
<td>6.6±0.5</td>
<td>1.0±0.4</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>2.0±0.7</td>
<td>21.8±4.2  263.2±8.2</td>
</tr>
<tr>
<td></td>
<td>a,c,i</td>
<td>a,b,c,e,i</td>
<td>a,c,f,g</td>
<td>e</td>
<td>e,f,i</td>
<td>e,g</td>
<td>e</td>
<td></td>
<td>e,g,h,i</td>
</tr>
<tr>
<td>Cd</td>
<td>5.6±0.6</td>
<td>6.8±0.4</td>
<td>11.8±1.5</td>
<td>5.0±0.5</td>
<td>2.2±0.4</td>
<td>0.4±0.2</td>
<td>1.0±0.3</td>
<td>1.4±0.3</td>
<td>34.2±4.4  174±12.1</td>
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<td>a,b,c</td>
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<td>a,b,c,d</td>
<td>a,b,c,d</td>
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<td>g,h,i</td>
<td>g,h,i</td>
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<tr>
<td>CdCMN</td>
<td>3.4±1.1</td>
<td>3.2±1.0</td>
<td>7.5±0.9</td>
<td>4.2±0.9</td>
<td>2.2±0.9</td>
<td>0.0±0.0</td>
<td>0.6±0.4</td>
<td>1.4±0.9</td>
<td>22.5±6.1  209±14.5</td>
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<td>a,b,c,e,i</td>
<td>a,b,c,e,i</td>
<td>a,b,c,d,f,g,h,i</td>
<td>i</td>
<td>b</td>
<td>e</td>
<td>a,b,c,d</td>
<td></td>
<td>a,b,c,d,f,g,h,i</td>
</tr>
<tr>
<td>CMNCd</td>
<td>4.4±0.5</td>
<td>3.2±0.9</td>
<td>8.0±1.1</td>
<td>8.0±1.3</td>
<td>1.8±0.4</td>
<td>0.2±0.2</td>
<td>0.8±0.4</td>
<td>1.0±0.2</td>
<td>27.4±5.0  194.6±10.2</td>
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<td>a,b,c,d</td>
<td>a,b,c,d,f,g,b,c</td>
<td>b</td>
<td>a,b,c,d</td>
<td>a</td>
<td>a,b,c,d</td>
<td></td>
<td>a,b,c,d</td>
</tr>
</tbody>
</table>

a; significant with control, b; significant with control +, c; significant with CMN, d; significant with Cd, e; significant with Cd CMN, f; significant with CMN Cd. Significant means P<0.05.
Table (2) incidence of total abnormally shaped sperms per thousand treated with Cd and CMN.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Normal sperm</th>
<th>Hummer</th>
<th>Banana</th>
<th>Amorphous</th>
<th>Without hook</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sig</td>
<td>Sig</td>
<td>Sig</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>902±4</td>
<td>13.0±1.0</td>
<td>38.0±2.9</td>
<td>16±2.3</td>
<td>31±2.0</td>
<td>98.0±12.2</td>
<td>9.8</td>
</tr>
<tr>
<td>Control+</td>
<td>902±4</td>
<td>14.2±3.1</td>
<td>35.0±2.8</td>
<td>15±5.3</td>
<td>32±3.0</td>
<td>96.2±18.2</td>
<td>9.62</td>
</tr>
<tr>
<td>CMN</td>
<td>933±12</td>
<td>16.2±4.0</td>
<td>18.4±4.5</td>
<td>18±2.7</td>
<td>14±2.0</td>
<td>66.6±25.2</td>
<td>6.66</td>
</tr>
<tr>
<td>Cd</td>
<td>878±9</td>
<td>17.4±2.0</td>
<td>46.8±4.1</td>
<td>18±2.6</td>
<td>45±2.0</td>
<td>127.2±19.7</td>
<td>12.7</td>
</tr>
<tr>
<td>Cd CMN</td>
<td>894±11</td>
<td>13.4±1.0</td>
<td>22.6±5.5</td>
<td>17±2.2</td>
<td>23±6.0</td>
<td>76.0±14.7</td>
<td>7.60</td>
</tr>
<tr>
<td>CMN Cd</td>
<td>845±3</td>
<td>8.0±1.0</td>
<td>53.6±4.5</td>
<td>39.6±4.0</td>
<td>58±3.0</td>
<td>159.2±12.5</td>
<td>15.9</td>
</tr>
</tbody>
</table>

a; significant with control, b; significant with control +, c; CMN, d; significant with Cd, e; significant with Cd CMN, f; significant with CMN Cd. Significant means P<0.05.
Figure (1): Metaphase spread from mouse bone marrow cells showing (a) normal chromosomes spread, (b) deletion (D), (c) fragment (F), (d) centric fusion (C.F), (e) centromeric attenuation (C.A), (F) end to end (E.to end), (g) gap, (h) ring (R), (i) polyploidy, (j) stickiness.
Figure (2): It shows (a) Normal sperm, (b) Hummer sperm, (c) Without hook, (d) Banana shape.

DISCUSSION

Cd burden has been correlated with chromosomal aberrations (IARC, 1993). It was observed that, chromosomal fragments, centric fusion, gaps, stickiness and aneuploidy produced by Cd injection, were also in *O. mossambica* (Chandra and Khuda-Bukhsh, 2004). Singh et al. (2007) showed that low dose of Cd (1mg/kg/day) for 30 days resulted in chromosomal aneuploidy, breaks, gaps and centromeric fusion. However, dose of 25mg and 200mg Cd /kg/day for 20 and 5 days resulted in severe damage of chromosome. Singh and Sankhla (2010) have assured that Cd increased the number of chromosomal aberrations and declined the mitotic index. Shaikh et al. (1999) referred that damage of chromosome to stimulation of free radical which in turn declined mitotic index. The present study significant decrease in sperm count associated with significant increase in sperm abnormalities in Cd-treated mice. The abnormalities included hummer, without hook, amorphous and banana. The Cd-induced ROS may affect the specific gene loci of germ cell chromosomes that may dysfunction the maintenance of normal sperm structure. The same result was explained by Acharya et al. (2003) ; Oldereid et al. (1994) and Salama and EL-Baher (2007) in Cd-treated mice due to either membranous or macromolecular damage. As well as, the decreased sperm count and alterations in motility have been associated with cigarette (Kulikauskas et al., 1985). Authors found that cadmium induced spermatogenic damage, decreased sperm count, reduced testosterone level due to generation of free radicals. Zemjanis (1970), Chandra and Khuda-Bukhsh (2004) and Kini et al. (2012), reported that spermatozoa
abnormalities such as headless tails and bent mid-piece are considered to reflect disturbances to spermatogenesis. Whereas secondary abnormalities such as bent tails and abnormal acrosome are believed to arise after spermatogenesis is completed due to epididymal dysfunction. Thus, the increase in abnormal sperms in the cadmium-treated rats may be due to both testicular dysfunction and impairment of epididymal function. Oliveira et al. (2009) also demonstrated that Cd induced increase in the percentage of cells with abnormal head and tail morphology. Bekheet (2010) pointed out that Cd-induced loss of spermatogenic cells has appeared by apoptosis and structural ultra-changes in the Sertoli and spermatogenic cells cytoplasm. The intake of low and oral doses of cadmium over a long period of time induces quantitative changes in apoptosis of the seminiferous epithelium of the rats (Herranz et al., 2010). Even a low level of cadmium accumulation in semen might contribute to male infertility by reducing sperm quality (Wu et al., 2008).

Currently, decreased chromosome abnormalities and mitotic index were observed in CMN-treated group and cadmium then curcumin as treatment trial (Cd CMN) in comparison to Cd group. In contrast, curcumin then cadmium as protection trial (CMNCd) group has the same values of Cd in chromosome abnormalities. The present experiment confirms the protective role of curcumin in bone marrow cells as shown by (Singh and Sankhla, 2010). Curcumin attenuated the cadmium induced malondialdehyde (MDA) formation and the decreased testicular reduced glutathione (GSH) (both discussed below), possibly due to its intrinsic antioxidant properties. Curcumin may thus prevent per oxidative changes in the sperm and the testicular membrane, thus enhancing sperm motility and decreasing spermatozoa abnormalities. CMN administration after Cd couldn't ameliorate, the effect of Cd. No reduction in cadmium tissue concentration was observed in cadmium exposed animals and cadmium treated with curcumin suggesting that curcumin is devoid of any cadmium removing activity. This may be due to the fact that, the elimination half-life of cadmium is 10-30 years. (Jarup et al., 1998). Hence, may suggest that curcumin may act by mechanisms different from chelation therapeutic approaches. Curcumin as antioxidant may be important in the treatment of cadmium intoxication. Curcumin represents a class of anti-inflammatory and antioxidants reported to be a potent inhibitor of ROS formation (Venkatesan et al., 2000 and Biswas et al., 2005). Thus, it is believed that antioxidant should be one of the important components of an effective therapy of Cd poisoning. Treatment with curcumin was effective in decreasing oxidative damage induced by Cd which resulted in markedly lower MDA concentration. Also, in the present study CMN lead to increase in sperm count associated with significant decrease in sperm head abnormalities. In contrast, curcumin then cadmium as trail protect (CMNCd) group has the
same values of Cd in both sperm abnormalities. Curcumin may protect from the damaged effect of Cd on mice testis. Curcumin as a stronger antioxidant may relieve the effect of free radicals induced by Cd exposure. The administration of curcumin to cadmium-treated rats prevents the cadmium induced chromosomal aberration, spermatogenic damage and decreased sperm count.

REFERENCES


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تقييم دور الكركم ضد السمية الخلوية لكميريد cadmione في الفئران

محمد زويل، خالد شرف الدين، أسامة شحور

قسم علم الحيوان- كلية العلوم- جامعه بنها – مصر

تهدف الدراسة إلى تقييم دور الكركم (CMN) ضد السمية الخلوية لكميريد cadmione. حيث تم تقسم ثلاثين من الذكور الفئران البالغين إلى ست مجموعات. تم حقنها في التجويف البروتيوني بخمسين ملجم كركم / كجم و 75, 105, كجم كلود cadmione / كجم لمدة 96 ساعة، على حدة أو بتناوب لمدة 8 أيام. أظهرت النتائج أن حقن الفئران بالكادميوم أدى إلى زيادة معدل التشوهات الكروموسومية مثل الشظايا، الالتحامات التجويفي، كرومسومين ملتقيين، الوجه، النزوجة وعدم توازن الصبغيات متماثلة في الثلاث الصبغاء. كما يسبب كلود cadmione الخيوانات المنوية شكل المطرقة، بدون خطاف وشكل موزه وتشوهات غير منتظمة. وأظهرت أيضًا أن الكركم له تأثير في تحسين وتيره التشوهات الصبغية والخيوانات المنوية الناتجة عن كلود cadmione وهذا يدل على أن الكركم بعد كلود cadmione يحمي الجسم من السمية الخلوية الناتجة عن كلود cadmione وايضا تحسن قيم الحيوانات المنوية المسجلة. تتناول الكركم قبل الحقن بـ كلود cadmione يقلل تأثير كلود cadmione.

وعلية يمكننا استنتاج أن الجرعه المستخدمة من الكركم يمكن استخدامها لحماية الجسم من السمية الخلوية الناتجة عن كلود cadmione.