Curcumin rendered protection against cadmium chloride induced testicular damage in Swiss albino mice

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Abstract
Fertility interference, regulation and control have become a matter of global concern in order to maintain adequate, sustainable and healthy population. Cadmium is known to interfere with reproductive physiology and adversely affects the process of spermatogenesis, whereas curcumin is known to be a potent, protective herbal derivative, which renders protection at the physiological, metabolic and cellular levels against numerous toxicants. In the present research, four groups of Swiss albino mice, each group consisting of six mice, were treated with cadmium chloride and curcumin. Group 1 was the control group, where mice were administered only vehicle. In the second group, mice were administered only curcumin. In the third group mice were administered single oral dose of CdCl₂, 50mg/kg/animal/day, for a day and were left for 15 days. The fourth group was pre-treated with curcumin (10mg/animal/day) for 15 days and on the 16th day they were administered with single oral dose of CdCl₂ (50mg/kg/animal). In animals administered only cadmium, significant perturbations were observed in the process of spermatogenesis. In the seminiferous tubules there is loss of cellular contact and associations, as manifested by loss of germ cells. In organisms pre-treated with curcumin, marked decline in histopathological damage was observed, where the loss of germ cells was not so pronounced. Hence, the present research categorically elucidates the protective effect of curcumin against a single high dose of cadmium chloride induced perturbation in the process of spermatogenesis.

Keywords: Curcumin, cadmium chloride, testicular damage, spermatogenesis, reproduction

Özet
Zerdeçal Swiss albino farelerde kadmiyum klorür ile indüklenmiş testiküler hasara karşı koruma sağlar

Fertilitenin engellenmesi, düzenlenmesi ve kontrollü, syeterli, devamlı ve sağlıklı bir popülasyonun sağlanması için dünyanın ilgilendirdiği bir sorun haline gelmektedir. Kadmiyumun üreme fizyolojisini engellediği ve spermatogenesis sürecini ters olarak etkilediği bilinirken, fizyolojik, metabolik ve hücresel, seviyelerde pek çok toksik maddede karşı koruyucu olan zerdeçalin güçlü, koruyucu bir bitki türevi olduğu bilinmektedir. Bu araştırma, her bir grupta altı fare bulunan dört grup Swiss albino fareye kadmiyum klorür ve zerdeçal uygulanmıştır. Sadece distile su verilen Grup1, kontrol grubunu oluşturmaktaidır. İkinci grupta farelere sadece zerdeçal verilirken üçüncü gruptaki farelere bir gün için 50mg/kg/hayvan/gün CdCl₂ tek doz oral olarak uygulanmış ve 15 gün boyunca beklenmiştir. Dördüncü grubu önceden 15 gün boyunca (10mg/hayvan/gün) zerdeçal uygulanmış ve 16. günde farelere tek doz oral olarak 50mg/kg/hayvan CdCl₂ uygulanmıştır. Sadece kadmiyum uygulanan hayvanlarda spermatogenesis sürecinde belirgin bir düzensizlik gözlenmiş ve germ hücre hasar haline getirilmiş gibi seminifer tübülerde hücresel ileşim ve birliktelik kaybı gözlenmiştir. Önceden zerdeçal uygulanan organizmalarla germ hücre hasarının bildirildiği yerlerde histopatolojik hasarlarla belirgin bir düşüş görülmüştür. Bunun sonucu olarak bu araştırma çalışma yüksek tek doz kadmiyum klorürün spermatogenesis sürecindeki düzensizliği indüklemesine karşı, zerdeçalin koruyucu etkisini kategorik olarak açığa kavuşturmaktadır.

Anahtar Kelimeler: Zerdeçal, kadmiyum klorür, testiküler hasar, spermatogenesis, üreme
Cadmium is highly toxic to various biological systems, e.g. kidney (Friberg et al., 1974; Buchet et al., 1980; Goyer, 1991), male and female reproductive systems (Ferm and Carpenter, 1967; Massanyi et al., 2007; Wu et al., 2008; Monsefi et al., 2010), brain (Beton et al., 1966; Taylor et al., 1984; Bernard and Lauwerys, 1986), gastrointestinal tract (Sugawara and Sugawara, 1974), liver, circulatory system (Stowe et al., 1972) and skeletal system (Kawamura et al., 1978; Blumenthal et al., 1995; Staessen et al., 1999). Due to the rapid industrialization and overgrowing urbanization, the toxic effects of cadmium on male reproduction is to be assessed and monitored and an effort has to be made to check, counter balance or nullify its toxicity.

India has a rich history of using plants for medicinal purposes. Turmeric derived from Curcuma as a medicine is used as home remedy for various diseases (Ammon and Wahl, 1991; Eigner and Scholz, 1999). Curcumin has been considered as a potent healing herbal formulation, a strong antioxidant, which has been considered to be more than three hundred times more potent than vitamin E (Rao et al., 1982; Dikshit et al., 1995). Curcumin is also reported to have anti-bacterial, anti-amoebic, antifungal, anti-viral and anti-HIV activities (Ammon et al., 1992; Azuine and Bhide, 1992; Ruby and Kutan, 1995 and Mortellini et al., 2000). Hence, in the present study an effort has been made to assess and monitor cadmium-induced reproductive toxicity as after one single chance exposure and to observe whether curcumin, derived from Curcuma longa has the potential to protect and prevent testes from such toxicity.

Materials and methods

32-50 days old adult Swiss albino mice, weighing around 30-40 g, were maintained in plastic cages under controlled lighting conditions (12 h light/12 h dark regime), relative humidity (50 ± 5%) and temperature (37 ± 2°C). The animals were fed on standard mice feed. Food and water were given ad libitum. Each batch comprised of 6 mice and their dose protocol was as follows:

Group 1 - Mice were administered the vehicle (distilled water) for 16 days.
Group 2- Mice were administered curcumin 10mg/animal for 16 days.
Group 3- Mice were administered single oral dose of CdCl₂ 50mg/kg/animal/day for a day and left for 15 days.
Group 4- Mice were pretreated with curcumin (10mg/animal/day) for 15 days and on the 16th day mice were administered with single oral dose of CdCl₂ (50mg/kg/animal).

Twenty-four hours after administration of the last dose, control and experimental animals were sacrificed. Testes were excised and subsequently fixed in Bouins solutions. After fixation testes were processed, wax blocks were made. Wax sections were cut and slides were prepared.
then stained in haematoxylin and eosin for histopathological studies (Kiernan, 2008). For statistical evaluation of significance, 100 seminiferous tubules (‘a’ and ‘c’), per group were assessed and $X^2$ (Chi Square) test was conducted using the formula as per the two by two table.

\[
\begin{array}{ccc}
  & a & b \\
  c & d & c+d \\
 a+c & b+d & N \\
\end{array}
\]

where ‘b’ the Observed Frequency for category ‘a’
‘d’ is the Expected Frequency in the corresponding category ‘c’

This experimental study was done after taking approval from the Institutional Animal Ethics Committee (No.Cs/Res/07/759).

\[
X^2 = \frac{N(ad-bc)^2}{(a+c)(b+d)(a+b)(c+d)}
\]

Results

In the present study the testes of cadmium and curcumin treated animals were assessed using the following parameters: Changes in morphology, testicular pathology, and cytostatic and cytotoxic changes in the germ and Leydig cells.

The testes of control group mice administered only vehicle showed normal pathology with distinct seminiferous tubules undergoing different stages of spermatogenesis. The interstitium was compact with distinct Leydig cells (Fig.1). Spermatogonial mother cells, primary and secondary spermatocytes, maturing spermatids and spermatoozoons embedded in Sertoli cells were clearly visible (Fig.2). Histopathological evaluation of only curcumin treated mice testes showed normal structures similar to control group. Testes of the cadmium treated mice testes showed normal structures in comparison with normal structure of seminiferous tubules in control mice. The seminiferous tubules of experimental group animals pre-treated with curcumin for 15 days and cadmium chloride on 16th day showed very slight histopathological damage in the peripheral tubules and the inner tubules appeared to be normal showing all stages of spermatogenesis viz. spermatogonial mother cells, primary and secondary spermatocytes, maturing spermatids and spermatoozoons embedded in Sertoli cells (Fig.7 and 8).
Figure 1. 1) Photomicrograph of testis of control group mice administered only vehicle. Normal seminiferous tubules (ST) and Leydig cell (LC) are clearly visible (20X). 2) Testis of control group mice administered only vehicle. Seminiferous tubule with distinct spermatogonial mother cells (SMC), primary spermatocyte (PS), secondary spermatocytes (SS), maturing spermatids (MS) and spermatozoa (S) are seen (40X). 3) Testis of cadmium chloride (50mg/kg) treated group mice. Degenerate seminiferous tubules(ST) are clearly visible (20x). 4) Testis of cadmium chloride(50mg/kg) treated group mice. Degenerated germ cells in lumen of seminiferous tubules are clearly evident (40x). 5) Testis of cadmium chloride(50mg/kg) treated mice showing exfoliated germ cells(GC) in tubular lumen (100x). 6) Testis of cadmium chloride (50mg/kg) treated mice. Seminiferous tubules are left with only vacuolised spermatogonial mother cells (SMC) and Sertoli cell (SC) (100x). 7) Testis of group pre-treated with curcumin(10mg/kg for 15 days) and then administered cadmium chloride(50mg/kg). Seminiferous tubules (ST) showing stages of spermatogenesis and pyknotic nuclei in Leydig cells (LC) are clearly evident (40x). 8) Testis of group pre-treated with curcumin (10mg/kg for 15 days) and then administered cadmium chloride(50mg/kg). Maturing spermatids (MS) and adhered germ cells are visible (100x)
Discussion

The management of infertility problems is the need of time. The importance of drugs from plant origin, as fertility regulating agents for the males has long been recognized. Medicinal plants present a repertoire capable of providing varied constituents which could be helpful in infertility management. Curcumin, a potent antioxidant compound derived from turmeric, has been used for centuries as a natural dye, seasoning and medicine (Huang et al., 1988). In Ayurveda, a 5000 year old system of medicine originating in India, curcumin in turmeric has been used to treat dozens of common conditions. Hence, in the present experiment an effort has been made to observe ameliorative effect of curcumin on testicular damage induced by cadmium chloride. The evidence of the past twenty years have shown a disturbing trend in male reproductive health hazards due to careless use of certain chemicals cadmium being one of them, which causes detrimental effects on different organs. Broad-spectrum irreversible toxic actions of cadmium at the cellular and molecular levels have been observed mainly on the reproductive system of humans and experimental animals, by a number of researchers (Batra et al., 2001; Chowdhury, 2004; Massanyi et al., 2007; Burukog and Bayc, 2008; Almansour, 2009; Obianime and Roberts, 2009). Cadmium has also been reported to cause testicular damage in Leydig cells and seminiferous tubules (Massanyi et al., 2007; Burukog and Bayc, 2008; Almansour, 2009; Obianime and Roberts, 2009; De Souza Predes et al., 2009 and Al attar 2011). These observations are similar to the results of the present study, which has revealed that cadmium chloride induced severe alterations in histopathological profile of testes as manifested by disarrangement of morphology of Leydig cells and 100% seminiferous tubular damage within which spermatogonia, spermatocytes and differentiating spermatids were severely affected and were lost in the luminal space of the tubules culminating in total suppression of spermatogenesis. There was induction of azoospermia. The results of present experiment also correlate well with other reports where cadmium has been shown to induce testicular damage in rat and mice (Gunn et al., 1970., Herranz et al., 2010; and Mathur et al., 2010). Our observations are also similar to the observations of Monsefi et al., 2008; Chowdhury, 2009 and Adamkovicova et al., 2010 where, similar to our results cadmium chloride has shown to cause rapid testicular edema, haemorrhage, necrosis and degeneration of testicular membrane tissue. Adaikpoh and Obi (2009) have reported that cadmium increased total cholesterol levels in the testes and prostate of rats, which affects Leydig cell function negatively. In the present experimental design administration of curcumin protected testis of mice exposed to cadmium as evidenced by appearance of about 75% normal structures of seminiferous tubule showing the ongoing process of spermatogenesis as evidenced by the presence of spermatids and spermatocytes, and lack of exfoliated cells in the luminal space. Additionally, the present study indicated that the exposure to heavy metals produce testicular damage, which leads to spermatogenic arrest which is rectified and prevented by curcumin intake.

Table 1. Alterations in the seminiferous germ cells of Swiss albino mice challenged with cadmium chloride and curcumin

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Experimental Protocol</th>
<th>Types Of Germ Cells Present (P) And Exfoliated (E)</th>
<th>Total number of germ cell layers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SMC</td>
<td>SC</td>
</tr>
<tr>
<td>1.</td>
<td>Control (distilled water as a vehicle for 16 days)</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>2.</td>
<td>Curcumin (10 mg/animal/day for 16 days)</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>3.</td>
<td>Cadmium Chloride(50 mg/kg/animal/day for 15 days)</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>4.</td>
<td>Curcumin (10 mg/animal/day for 16 days) + Cadmium Chloride(50 mg/kg/animal/day for 15 days)</td>
<td>P</td>
<td>P</td>
</tr>
</tbody>
</table>

SMC – Spermatogonial Mother Cells; SC – Spermatogonial cells ; PSp- Primary Spermatocytes; SSp- Secondary Spermatocytes; S- Spermatids; SZ- Spermatozoa; SeC- Sertoli Cells *$X^2$ significant at P ≤0.05
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