Protective Effect of Date Palm Extracts on Cadmium-Induced Infertility in Male Rats

El-Sayed Mohamed El-Habibi; Magda Mahmoud El-Komy and Heba Osama Saad

Physiology Division, Zoology Department, Faculty of Science, Mansoura University, Mansoura, Egypt

Corresponding author: Heba Osama Saad, heba_osama2014@hotmail.com

ABSTRACT

Background: Infertility is a problem which affects one in six couples. However, male factor considers solely responsible in about 20% of infertile couples and contributory in another 30–40%. Objective: The aim of this study was to elucidate the effect of date palm pollen (DPP) and date palm seed extract (DPS) on cadmium-induced infertility in male rats. Materials and Methods: Thirty six male albino rats were divided into six groups (n=6) and received their treatment orally for 30 days: group 1, control; group 2, (DPP): (240mg/kg) daily, group 3, (DPS): (100mg/kg) daily, group 4, (CdCl₂): (5mg/kg) every other day, group 5, (CdCl₂ + DPP): CdCl₂ as group 4, followed by DPP as group 2 (each dose given 2 hours after CdCl₂); group 6, (CdCl₂ + DPS): CdCl₂ as group 4, followed by DPS as group 3 (each dose was given 2 hours after CdCl₂). Results: The current data exhibited significant decrease in the sperm quality, T, E2, FSH, LH, aromatase enzyme, TAC, GSH, SOD and CAT, with marked increase in MDA& XO and severe destruction in testis histoarchitecture in CdCl₂ treated rats. However, there was a significant improvement in all these parameters with DPP& DPS administration. Conclusion: consumption of DPP or DPS might be considered as a functional treatment for retarding risks of infertility associated with cadmium exposure. Keywords: Male infertility; Cadmium; Date palm pollen; Date palm seed; Antioxidants; Testis.

INTRODUCTION

Infertility is commonly defined as the failure of conception after at least 12 months of unprotected intercourse. It is a major health problem which affects approximately 15% of all couples, however, male factor considers responsible in about 20% of infertile couples and contributory in another 30–40%. Factors like diabetes, bronchiectasis, high grade fever, long term medication, urinary tract infection, sexually transmitted diseases, epididymitis, testicular injury, un-descended testis, mumps, orchitis, excessive alcohol, smoking, exposure to heat and certain chemicals affect spermatogenesis.

Cadmium (Cd) and other heavy metals and estrogenic-based compounds (e.g., bisphenols) may account for the recent declining fertility in men by reducing sperm count and testis function. Cadmium can affect human health through several sources such as drinking water, food, manufacturing of batteries and pigments that utilize Cd, cigarette smoke, pesticides, rubber processing, electroplating, mining, alloy preparation and plastic stabilizers manufacturing.

Cadmium acts as an endocrine disruptor and oxidative stress inducer in humans and rodents. It has long been known to damage the hepatic, respiratory and reproductive systems including the ovary and testes, red blood cells, bone diseases and the skeletal muscles of rats. Renal tubular damage is probably the most common adverse effect.

Herbal medicines are gaining importance and nowadays are being studied to find the scientific basis of their therapeutic actions. Date palm (Phoenix dactylifera L., Palmae) is native to the Middle East region over centuries ago. In traditional medicine, a suspension of date palm pollen (DPP) is widely used as a folk remedy for curing male infertility. DPP mainly contains cholesterol, rutin, carotenoids, and estrone which are known to exhibit gonadotrophin activity in the rat. Its extracts contain oestrogenic compounds, oestrones, gonad stimulating compounds that can improve male infertility and elicit gonadotrophin activity in rat models. The antioxidative effect of DPP is mainly due to phenolic components, such as flavonoids, phenolic acids, and phenolic diterpenes, anthocyanins, procyanidins and coumaric acid.

Studies have been increasingly showing that date seeds possess significant nutritional value, especially in terms of their fiber and antioxidant content. Date seed contain different chemical compounds such as saturated fatty acids as stearic and palmitic acid, unsaturated fatty acids
such as linoleic, oleic acids which could inhibit the 5-α reductases enzyme, Zinc (Zn), Calcium (Ca), potassium (K)28, protein, fat, ash dietary fiber. Also, seeds contain high levels of phenolics, antioxidants,26 p-coumaric, ferulic and sinapic acids, flavonoids and procyanidins25,29 and lipids, which is either in wax, fat or oil form.30

According to these findings, the present study was undertook to evaluate the positive effect of date palm pollen (DPP) and date palm seed extracts (DPS) in treatment of male infertility induced by cadmium in rats.

MATERIALS AND METHODS

Chemicals
Cadmium Chloride (CdCl₂) was purchased from Sigma-Aldrich (Saint Louis, Missouri, USA). Ethanol was purchased from (Al-Gomohria Company for chemicals, Abou-Zabal, Egypt). All other chemicals were of analytical grade.

Preparation of DPP and DPS extracts
2 plant products (DPP and DPS) were purchased from local market in Mansoura, Egypt.

DPP suspension was freshly prepared daily by adding 5 ml distilled water to 1.2 gram of powdered pollen with stirring for 10 minutes till complete dispersion.22

The (DPS) ethanol extract was prepared by adding 120 ml of ethanol to 63gm of DPS powder and left for 48 hours. Then the mixture was filtrated and the precipitate was left to dry. The dried powder was weighted to give (2.1gm) DPS extract which was used to prepare the required dose by adding 2.5 gm of the extract to 50ml of water.

Experimental animals
Adult male Albino rats weighing 185±10g obtained from the Egyptian Organization for Biological products and vaccines, Cairo, Egypt, were used in this study. They were allocated in stainless steel cages in an automatically illuminated and thermally controlled room (22-25°C and 12 hrs light / dark cycle) at the Animal House, Faculty of Science, Mansoura University, Mansoura, Egypt. They were fed on standard chewing diet according to the National Research Council, committee on Animal Nutrition (1995), purchased from a local market at El Mansoura city, Egypt. Animals received human care and the present study conformed to the instruction’s guidelines. The local committee approved the design of the experiments, and the protocol complied with the guidelines of the National Institutes of Health (NIH).

Study design
After acclimation period of one week, rats were divided into six equal groups (rats/group). Rats were fed on standard diet and received their respective treatment orally via stomach tube for 30 days as follow: group 1; normal control (NC) group fed on standard diet without any supplementation; group 2; (DPP): rats received DPP suspension at a dose of 240 mg/kg daily, group 3; (DPS): rats received DPS ethanol extract at a dose of 100 mg/kg daily, group 4; (CdCl₂): rats received CdCl₂ solution at a dose of 5 mg/kg every other day, group 5; (CdCl₂+DPP): rats received the same dose of CdCl₂ solution every other day as group 4, followed by an identical dose of DPS suspension daily as group 2 (each dose given 2 hours after CdCl₂) and group 6; (CdCl₂+DPS): rats received the same dose of CdCl₂ solution every other day as group 4, followed by an identical dose of DPS extract daily as group 2 (each dose given 2 hours after CdCl₂).

Sample collection and tissue preparation
At the end of the experimental period, rats were fasted for 12 hrs, weighed and then sacrificed under ether anesthesia. Blood samples were collected in clean dry non heparinized centrifuge tubes. Sera were separated by centrifugation at 860 Xg for 20 min at 4°C, and frozen at -20°C for future biochemical analysis.

Rats were dissected, and the two testes and epididymis from each rat were removed. Cauda epididymis were cut into pieces in 5 ml normal saline solution and used immediately for sperm analysis.

The right testis was homogenized by tephlon homogenizer in a 10 fold volume of ice-cold saline solution, centrifuged at 860 Xg for 20 min at 4°C and the resultant supernatants were frozen at -20°C for biochemical analysis, while the left testis was kept in 10% neutral formalin fixative for histopathological examination.

METHODS
Sperm count was estimated using the haemocytometer following the method of Majumder and Biswas.32 Percent of sperm abnormalities and viability were estimated using Casa Device.
Serum testosterone was estimated by using ELISA Kit, Diagnostic Biochem Canada Inc. according to Winters et al. Serum estradiol (E2) was estimated by Enzyme linked Fluorescent Assay (ELFA) technique using kits of Biomerieux according to the methods of Dupont et al. Serum FSH & LH were measured by IMMULITE 1000 analyzers using IMMULITE FSH & LH Kit purchased from Siemens Health Care Diagnostics Products Ltd, USA according to Babson.

The quantitative measurement of aromatase activity was performed by a solid phase enzyme-linked immunosorbent assay (ELISA) (obtained from Beckman Coulter, Brea, Calif., USA), based on the sandwich principle, as described by Roselli. Malondialdehyde (MDA) (the end product of lipid peroxidation) level was measured according to Rat Malondialdehyde, MDA ELISA Kit, Catalog No. MBS268427. Superoxide dismutase (SOD) was measured by Rat Superoxide Dimutase (SOD) ELISA Kit, Catalog No. CSB-E08555r.

Reduced glutathione (GSH) content was determined according to Rat glutathione, GSH ELISA Kit, Catalog No: E0294r. Catalase activity (CAT) was determined by Rat Catalase (CAT) ELISA Kit, Cat No. MBS2600683. The total antioxidant capacity (TAC) was determined according to Rat Total Antioxidant Capacity (TAC) ELISA kit, Catalog Number: MBS733414_48T. The Xanthine oxidase enzyme activity was measured by Rat XDH / Xanthine Oxidase ELISA Kit (Sandwich ELISA) Catalog No. LS-F12985. For histopathology, the fixed testis tissues, in neutral formalin, were dehydrated through the ascending series of ethyl alcohol, cleared in xylene, infiltrated and embedded in paraffin wax. Transverse sections of testis were cut at thickness of 5 µm and stained with Mayer's hematoxylin and eosin stains according to Weesner for further examination.

The study was approved by the Ethics Board of Mansoura University.

Statistical analysis
The results obtained were evaluated by One Way ANOVA (analysis of variance) test and post comparison was carried out with Duncan test. The data were expressed as mean ± standard error (mean±SE), where \( p \leq 0.05 \) is considered statistically significant.

RESULTS
As shown in tables 1, 2 and 3, the recorded data showed some significant increases between the normal control (NC) rats group and normal rats administrated with DPP or DPS groups, indicating their safety and nontoxic effect. However, obtained data recorded a significant decrease in sperm count and viability with significant elevation in sperm abnormalities of CdCl2 rats group comparing to NC rats. While, CdCl2-DPP or CdCl2-DPS groups showed a significant amelioration in all the above mentioned changes comparing to CdCl2 rats group (Table 1). Additionally, a significant decrease in the level of sex hormones (T, E2, FSH& LH) as well as serum and testicular aromatase enzyme activity were recorded in CdCl2 rats compared to NC group. Meanwhile, administration of DPP or DPS after CdCl2 succeeded to induce a significant improvement in these biochemical parameters (Table 2).

Table 3 recorded a significant increase in testicular MDA level and XO activity associated with significant decrease in TAC, GSH level, SOD and CAT activities in CdCl2 rats compared to NC rats group. While the co-administration of DPP or DPS with CdCl2 caused a significant elevation of these parameters.

The histopathological observation of testis in control, DPP and DPS groups showed a normal testis histoarchitecture. Testis sections of DPP and DPS treated groups showed histological appearance of seminiferous tubules (ST), with arrangement of different stages of spermatogenic cells, interstitial cells (lc), sertoli cells (Sc) and many sperms (S) were observed. However the examination of testis sections of CdCl2 group showed distorted and necrotic seminiferous tubules (ST) and thickened oedematous interstitial cells (thick arrow) with dilated and congested blood vessels (CBV). There was also wide lumen (W) with no sperms, multinucleated cells (arrow head) disorganized germinal epithelium with marked vaculation (V) and wide space from plasma membrane and spermatogenic cells (curved arrow). Also degenerated germ cells with pyknotic nuclei (star) and fragmented sertoli cells were seen. On the other hand, testis sections of CdCl2+DPP and CdCl2+DPS treated animals showed an improvement in the testicular histology compared with the CdCl2 treated group, however the co-administration of DPP showed more improvement than DPS (Fig 1).
Table 1: Sperm quality in control and different treated rat groups

<table>
<thead>
<tr>
<th>NO week</th>
<th>Animal groups</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>DPP</td>
</tr>
<tr>
<td>No of sperms (x 10⁷/g epididymal tissue)</td>
<td>8.31 ±0.11</td>
<td>9.39 ±0.16</td>
</tr>
<tr>
<td>Sperm Abnormalities %</td>
<td>9.6 ±0.58</td>
<td>7.4 ±0.60</td>
</tr>
<tr>
<td>sperm viability %</td>
<td>73.0 ±4.15</td>
<td>86.0 ±2.08</td>
</tr>
</tbody>
</table>

Values are means ± SE (n=6)
a- significant compared to control.
b- significant compared to CdCl₂.
c- significant of CdCl₂+DPP compared to CdCl₂+DPS.

Table 2: Biochemical parameters in control and different treated rat groups

<table>
<thead>
<tr>
<th>NO week</th>
<th>Animal groups</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>DPP</td>
</tr>
<tr>
<td>T (ng/dl)</td>
<td>752.6 ±17.6</td>
<td>778.1 ±7.48</td>
</tr>
<tr>
<td>E2 (pg/ml)</td>
<td>43.5 ±4.66</td>
<td>52.6 ±2.56</td>
</tr>
<tr>
<td>FSH (mlU/mL)</td>
<td>0.52 ±0.03</td>
<td>0.59 ±0.04</td>
</tr>
<tr>
<td>LH (mlU/mL)</td>
<td>0.90 ±0.10</td>
<td>1.50 ±0.09</td>
</tr>
<tr>
<td>Serum Aromatase (U/ml)</td>
<td>56.9 ±5.83</td>
<td>60.0 ±6.98</td>
</tr>
<tr>
<td>Testicular Aromatase (U/mg)</td>
<td>57.5 ±8.22</td>
<td>63.2 ±6.17</td>
</tr>
</tbody>
</table>

Values are means ± SE (n=6)
a- significance compared to control.
b- significance compared to CdCl₂.
c- significance of CdCl₂+DPP compared to CdCl₂+DPS.
### Table 3: Oxidative stress and antioxidant biomarkers in control and different treated rat groups

<table>
<thead>
<tr>
<th>NO week</th>
<th>Animal groups</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>DPP</td>
</tr>
<tr>
<td>MDA (nmol/g)</td>
<td>0.18 ±0.01</td>
<td>0.14 ±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GSH (mg/g)</td>
<td>0.33 ±0.01</td>
<td>0.36 ±0.008</td>
</tr>
<tr>
<td>SOD (U/g)</td>
<td>0.24 ±0.03</td>
<td>0.30 ±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CAT (µmol/sec/g)</td>
<td>0.31 ±0.01</td>
<td>0.36 ±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TAC (mMol/g)</td>
<td>0.29 ±0.01</td>
<td>0.32 ±0.007&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>XO (ng/g)</td>
<td>0.16 ±0.01</td>
<td>0.13 ±0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE (n=6)

a- significance compared to control, b- significance compared to CdCl₂, c- significance of CdCl₂+DPP compared to CdCl₂+DPS.

**Fig. 1.** Photomicrograph of a 5µm testis section stained with H&E representative of (A) control group, (B) DPP group, (C) DPS group, (D) CdCl₂ group, (E) CdCl₂+DPP group (F) CdCl₂+DPS group. Ic:Interstitial cells, ST:Seminiferous tubule, SG:Spermatogonia, PS:Primary spermatocytes, SP:Spermatids, S:Spermatoza, SC: Sertoli cells, CBV:Congested blood vessels, Arrow:Interstitial spaces, Arrowhead:Multinucleated cells, Thickarrow:Thickened oedematous interstitial cells, Curved arrow:Wide space from plasma membrane and spermatogenic cells, Star:Germ cells with pyknotic nuclei, W:Wide lumen, V:Vaculation, GE:Germinal epithelium, L:Lyding cells.
DISCUSSION

Infertility is a major health problem that affects one in six couples; however, male infertility causes was found to be about 50% of infertile couples.

Exposure of the reproductive system to heavy metals has been reported to be a major risk factor for infertility and there has been an increasing interest in the contribution of occupational and environmental exposures to toxic metals in declining sperm concentration and human male fertility.

Cadmium is one of the most toxic industrial and environmental heavy metals. It acts as an endocrine disruptor and oxidative stress inducer in humans and rodents.

In the present study, the obtained results indicated that CdCl₂ treated rats showed a significant drop in sperm count with a significant decrease in sperm viability, while the sperm abnormalities were elevated significantly. These results run parallel to Eleawa et al., who revealed that the main cause of these results is the reduced spermatogenesis due to increased oxidative stress and the apoptotic mechanism observed in the testes of the treated rats. This reduction in sperm quality may be also due either to impairment of the H₂O₂ removal system, which leads to inhibition of steroidogenesis in the Leydig cells due to an accumulation of H₂O₂ or to membrane damage or macromolecular degradation incurred by ROS.

Furthermore, the administration of CdCl₂ caused a significant reduction of sex hormones (T, E₂, FSH, LH), and this is in accordance with Yang et al. who suggested that CdCl₂ stimulated apoptosis of the anterior pituitary. Additionally, the loss of testosterone feedback can result in pituitary cell hypertrophy, hyperplasia and eventually pituitary neoplasia. Thus the disruption of the testes-pituitary axis may contribute to the causation of both testicular and pituitary destructions. Alternatively, this reduction could result from decreased viability of Leydig cells as a consequence of the necrotic effects of toxicants such as Cd.

Additionally, the data revealed a significant reduction in both serum and testicular aromatase in Cd treated rats, while the administration of either DPP or DPS caused a significant elevation in this parameter. The ability of the testis to convert irreversibly androgens into estrogens is related to the presence of a microsomal enzymatic complex named aromatase, which is composed of a specific glycoprotein, the cytochrome P450 aromatase (P450arom). In the rat testis the P450arom has been immunolocalized not only in Leydig cells but also in germ cells and especially in elongated spermatids producing a significant amount of the estradiol in the testes. It can be concluded that a complex balance of testosterone, estradiol and aromatase in the testes confirms a highly regulated hormonal interaction in the male.

As evident from the present data there is a significant decrease in testicular TAC and GSH levels as well as SOD and CAT activities accompanied with significant increase in the lipid peroxidation product (MDA) level and XO activity in CdCl₂ treated group confirming that Cd caused testicular oxidative stress. Ikediobi et al. demonstrated that the toxic effect of CdCl₂ on the testes is known to deplete glutathione and protein-bound sulfhydryl groups, which caused enhanced production of reactive oxygen species (ROS) such as superoxide ion, hydroxyl radicals and hydrogen peroxide. El-Neweshy et al. observed a significant increase in testicular oxidative stress reflected by the significant elevation of MDA and a significant depletion of GSH, which caused irreversible testicular cell damage. The decreased level of GSH in the testis, as recorded by Imafidon et al., can be attributed to its excessive use by the testicular tissue to scavenge the free radicals that were generated following exposure to Cd toxicity and or reduced GSH production by the tissue; a consequence evidently enhanced by the increased use of SOD in the oxidative process which resulted in its reduced testicular level.

The administration of CdCl₂ also resulted in histopathological gonadal lesions, such as testicular damage in seminiferous tubules (STs), Leydig cells and Sertoli cells in addition to decreases in the spermatogonial population and number of spermatozoa. These results run parallel with the previous studies of El-Neweshy et al. and Eleawa et al. who revealed that the testicular injury was confirmed by marked alterations in the histological structure of the Cd-treated rats testes where it exhibited degeneration, necrosis and atrophy of almost all of the STs with incomplete to complete spermatogenic arrest. Cd enters the seminiferous tubules through a breach of the blood-testis barrier and causes focal testicular necrosis and dystrophy with consequent reduction in germ cell numbers, leading to infertility.
On the other hand, the supplementation of DPP or DPS extracts significantly improved the sperm quality, which may be due to DPP estradiol and flavonoid components or to its scavenging properties that is said to be the main important effects on the sperm parameters. Furthermore, phenol and tocopherol profiles of DPS were found to be a rich source of natural phenolic compounds, which was one of the main reasons for its better oxidative stability.

Moreover, DPP and DPS co-administration showed a significant increase in sex hormones, which is in accordance with Abedi et al. This effect may be due to the presence of flavonoids, steroids, saponins and estradiol compounds in DPP that affect the hypothalamus pituitary axis and have thus increased concentrations of these hormones through raising the level of luteinizing hormones (LH) leading to stimulate estradiol levels and endogenous testosterone levels.

Shagauo and Davidson showed that DPP is capable of releasing LH hormone by affecting hypothalamus axis which increases secretion of gonadotropin releasing hormone (GnRH). Concerning DPS extract, Ammar et al. reported the presence of different sterols such as; campasterol, stigmasterol, b-amyrin and b-sitosterol which exhibited a remarkable antioxidant and estrogen like activity which can lead to increase the mentioned hormones.

Additionally, there was a significant increase in the levels of TAC, GSH, as well as SOD and CAT activities associated with significant decrease in MDA and XO activities in the testis of rats co-administrated with either DPP or DPS. These results are in harmony with who reported that the antioxidative effect of DPP is mainly due to its phenolic components, such anthocyanins, procyanidins and coumaric acid. The antioxidant activity of phenolic compounds is a result of their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.

Additionally, the decreased MDA level associated with elevated other antioxidants in DPS rat group may be attributed also to the wide range of phenolic compounds in DPS including p-coumaric, ferulic and sinapic acids, flavonoids and procyanidins.

The administration of DPP or DPS caused significant suppress of Cd induced histopathological damage in testicular tissues. It is reported that free radicals induce oxidative damage that is resulted in destructive testicular architecture causing male infertility, so the DPP administration showed an antioxidant effect, as evidenced by improved GSH and restored LPO in the Cd-treated rats’ testes. Thus, DPP can ameliorate Cd-induced oxidative stress in the testicular tissues, as evidenced by the renewal of spermatogenesis in the seminiferous tubules and normalisation of the testicular histoarchitecture. Investigations have also reported that DPP extracts have the capacity to improve this ulceration is attributed to its content of estrogenic materials that are considered as gonad-stimulating compounds where estrogen is involved in regulating the renewal of spermatogonial stem cells. Vayalil and Bashir et al. also attributed the positive effect of DPS on the testicular architecture to its antioxidant effect that was reported to possess both androgenic and antioxidant properties.

CONCLUSION
In this study, the data showed that Cd can induce significant spermatological damage, oxidative stress and histopathological alterations in the testicular tissue of male rats 30 days after exposure, ultimately resulting in infertility. Remarkably, administration of DPP and DPS once daily for 30 days effectively prevented the deleterious effects of Cd. The pro-fertility properties of DPP and DPS are mainly achieved through its endocrine-mediated effects, consistent with their vital role in the antioxidant systems that protect against Cd damage, and possibly due to their prevention of oxidative damage to testicular tissues. Also it can be concluded that DPP is more effective than DPS in ameliorating the above mentioned infertility features.

Taken together, our findings support the hypothesis that the testis is very sensitive to Cd, which can induce testicular damage and infertility that can be blocked by the therapeutic administration with DPP or DPS.

REFERENCES
Protective Effect of Date Palm Extracts…


58-Fatma BA, Nozha CF, Ines D, Hamadi A, Basma H and Leila AK(2009): Sperm quality improvement after date seed oil in vitro supplementation in


