Egyptian Date Palm Pollen Ameliorates Testicular Dysfunction Induced by Cadmium Chloride in Adult Male Rats

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Abstract: Pollen of the date palm (DPP) has been used for long time as a traditional Egyptian herbal medicine for improving male and female fertility. The purpose of this study was to investigate the protective effect and the possible mechanisms of DPP against cadmium-induced testicular dysfunction in adult male rats. Testicular dysfunction was induced by an oral administration of cadmium chloride ($CdCl_2$, 5mg/kg body wt, every other day for 30 days). Oral administration of DPP (240mg/kg body wt, daily for 30 days) dramatically increased estradiol level of normal rats. Co administration of DPP with $CdCl_2$ significantly restored the reduction in sex organs weight and the decline in sperm counts and their motility as well as the decrease in testosterone level induced by $CdCl_2$ challenge. Treatment with DPP counteracted the increases in antioxidant systems in rat testis as assessed by restoration of reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT).

Co administration of DPP with $CdCl_2$ inhibited the abnormalities in testicular morphology, but partially attenuated the elevation in the percentage of testicular DNA damage induced by $CdCl_2$. This study provides evidence that DPP exert a significant protective effect against testicular dysfunction induced by $CdCl_2$ through increases in estradiol level as well as normalization of testosterone levels and sperm parameters. This report also shows some evidence that DPP protects rat's testicular tissue via suppressing testicular histological abnormalities and modulating its DNA damage.

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1. Introduction

Cadmium (Cd) is an industrial and environmental pollutant, primarily from battery, electroplating, pigment, plastic and fertilizer industries and cigarette smoke (**Stohs & Bagchi, 1995**). The general population is exposed to Cd via contaminants found in drinking water and food (**Atsdr, 2008**). Acute and chronic Cd toxicity is associated with severe damage in various organs, particularly the testes in both humans and animals (**Liu *et al.,* 2009).** More important, Cd and other toxicants, such as heavy metals (e.g. lead, mercury) and estrogenic-based compounds (e.g., bisphenols) may account for the recent declining fertility in men among developed countries by reducing sperm count and testis function (**Siu *et al*., 2009**).

The mechanism responsible for Cd testicular toxicity is not yet completely clear. Findings from recent studies suggest disruption of the blood testis barrier causing hemorrhage and edema (**Siu *et al*., 2009**). In addition, Cd affects Leydig cells, thereby promoting a reduction in steroidogenesis (**Gunnarsson *et al*., 2007**), induces cell necrosis by production of reactive oxygen species (**Tremellen, 2008**) and may disrupt the zinc and calcium mediated cellular events (**Martin *et al*., 2007**). Based on this concept, a variety of anti-oxidant agents have been employed to counteract Cd-induced testicular damage (**Acharya *et al*., 2008** and **Ognjanovic *et al*., 2010**).

Nevertheless, so far there is still no single agent proven effective enough to prevent or reverse Cd testicular toxic effects.

Date palm (*Phoenix dactylifera* L., Palmae) is native to the Middle East region over centuries ago (**Copley *et al*., 2001**). In Folkloric practice, date represents an essential meal in some Arab area (**Miller *et al*., 2003**). Pollen of the date palm has been used for thousands of years as a traditional Egyptian herbal medicine for improving male and female fertility (**Soliman & Soliman, 1957** and **Amin *et al*., 1969**). Extracts of fruits, pits and edible kernels showed improvement of vital activities and increased the hormonal concentration in rats (**El-Mougy *et al*., 1991; Ali *et al*., 1999; Bauza, 2002; and Vayalil, 2002).** Phytochemical studies of DPP showed the presence of estrone, α-amirin, triterpenoidal saponins, flavonoids and a crude gonadotrophic substance (**Mahran *et al*., 1976; Mahran *et al*., 1985 and Bennet *et al*., 1996**). Recently, **Abbas & Ateya (2011)** also revealed the presence of estrone, estradiol and estriol, besides five flavonoids compounds. Only few studies have been conducted to prove its fertility-improving effects in males. One study showed its functional health benefits on sperm parameters and reproductive system of adult male rats (**Bahmanpour *et al*., 2006**) and the other shed light on its protective effect on cisplatin-induced male infertility in rats (**Al-Kharage, 1982**). Taken together, these studies could

imply DPP to be a promising agent against a range of exogenous testicular toxic stimuli.

To determine whether DPP could also attenuate Cd-induced testicular damage, this study was designed to examine the antagonistic actions of DPP on several aspects of testicular dysfunctions, biochemical and pathological abnormalities in male rat testes induced by Cd, as well as on testicular DNA damage to access the genotoxic and genoprotective effect of DPP using comet assay.

2. Material and Methods:
Chemicals

Cadmium chloride, thiobarbituric acid, reduced glutathione, 5,5-dithiobis (2-nitrobenzoic acid), Folin's reagent, pyrogallol, superoxide dismutase, sulfanilamide, N-(1-naphthyl)ethyl-enediamine, vanadium chloride were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other used chemicals were of the highest analytical grades commercially available.

Date palm pollen and preparation

Fresh pollen of date palm was collected in March 2010 from Zagazig city, Egypt. Its botanical identification and authentication were confirmed at the Department of Phytochemistry, University of Zagazig, Egypt. The pollens were separated from the kernels with a fine gauze sieve and kept refrigerated (4^oC). The suspension was freshly prepared daily by adding 5 ml of distilled water to 1.2 gram of powdered pollen (concentration: 240 mg/kg), with stirring for 10 minutes on a magnetic stirrer till complete dispersion (**Bahmanpour et al., 2006).**

Animals

Adult male albino rats weighed 210 to 230 g. They were obtained from the animal house of the National Organization for Drug Control and Research (NODCAR). They housed under controlled conditioning ($25 \pm 1°C$ constant temperature, 55% relative humidity, 12 h lighting cycle), kept under laboratory conditions 2 weeks prior to experiment for acclimatization and received standard diet and water *ad libitum* during the study period. All animal procedures and the experimental protocols were approved by the Institutional Ethics Committee at NODCAR and were carried out in accordance with the *Guide for the Care and Use of Laboratory Animals*.

Induction of testicular dysfunction

Testicular dysfunction was induced by an oral administration of CdCl2 (5mg/kg body wt, every other day) for 30 days (**El-Demerdash et al., 2004**). Testicular injury was identified by the reduction in sex organs weight, sperm counts and their motility as well

as abnormalities in histological and DNA testicular tissue.

Experimental groups

Forty-eight rats were randomly assigned to four groups according to the pharmacological treatment they received. The control group (Cont); rats received only distilled water orally. The $CdCl_2$ group (Cd); rats were given cadmium chloride solution (5 mg kg^{-1}; orally.) every other day for 30 days. The DPP group (DPP); rats received DPP suspension (240 mg kg^{-1}; orally) daily for 30 days. The $CdCl_2$ + DPP group (Cd-DPP); rats were given the same dose of $CdCl_2$ solution as the Cd group, followed by an identical dose of DPP suspension as the DPP group (each dose given 2 hours after $CdCl_2$).

Collection of serum and testicular samples

At the end of the experimental period, the final body weight of the animals was determined. Blood samples were collected from the retro-orbital plexus in plastic centrifuge tubes, left to clot at $4°C$ for 30 minutes and serum was obtained by centrifugation at 3000 rpm for 20 minutes. Then, rats were sacrificed under ether anesthesia. Testes, cauda (full of secretion), prostate gland and epididymes were immediately removed, cleared of adhering tissues, rinsed in ice-cold saline and weighed. The relative weight of organs (%) was calculated as g/100 g body weight. The testicular tissues were either fixed in 10% formalin for histopathologic examination or stored at -80 $°C$ in association with the serum samples till later analysis.

Epididymal sperm counts and motility

Epididymal sperm concentration and sperm progressive motility were evaluated by the method of **Bearden & Fuquay (1997)**. Sperm cells count was determined using the Improved New Pauer haemocytometer and haemocytometer pipette of erythrocytes for counting of sperm cells. The whole epididymus was thoroughly macerated so as to bring about most if not all the epididymal content and then the content was diluted with 100 μL eosin aqueous solution (1%). The diluted stained content was withdrawn up to the mark 0.5 on the erythrocyte pipette; the pipette was then filled up to mark 101 by 1% eosin aqueous solution. The contents in the pipette bulb were mixed and the sperm heads were counted in five large squares (80 small squares).

Progressive motility was assessed using a phase-contrast microscope ($\times$ 40 magnification), with a warm stage maintained at 37 °C. A wet mount was made using a 10μl drop of semen placed directly on a

microscope slide and covered by a cover slip. Sperm-motility estimations were performed in three microscopic fields for each semen sample. The mean of the three successive estimations was recorded as the final motility score.

Hormone assays in serum

Serum testosterone and estradiol were measured using commercial ELISA kits from NovaTec Immunodiagnostica (Dietzenbach, Germany) and BioCheck (Foster city, U.S.A.) respectively.

Tissue preparation

Frozen testicular samples were homogenized in ice cold medium as 10% w/v homogenate according to the parameter measured (1.15% kcl for all except GSH, where 0.01M sodium potassium phosphate buffer, PH=7.4 was used for its homogenization).The homogenates were centrifuged at 10000 x g at 4°C. Aliquots were prepared and used for determination of different oxidant / antioxidant parameters.

Prooxidant and antioxidant status in testicular tissues

Malondialdehyde (MDA) is the most abundant individual aldehyde resulting from lipid peroxidation (LP) breakdown in biological system. It's used as an indirect index of LP. The determination of MDA in biological materials was carried out with the spectrophotometric method described by **Simon et al. (1994),** which detected the reaction of the sample with thiobarbituric acid to quantitate the resultant lipid peroxidation as a surrogate of MDA level. Nitric oxide (NO) content was quantified as nitrite concentration according to the method of **Miranda et al. (2001),** which depended on reduction of nitrate to nitrite by vanadium chloride, then the released nitrite was colorimetrically detected by Griess' reagent. The reduced glutathion (GSH) content in the testis was determined using the method of **Van Dooran et al. (1978)**. The basis of the GSH determination method is the reaction of Ellman's reagent (5,5′ dithiobis - (2-nitrobenzoic acid) with thiol groups of GSH at pH 8.0 to produce the yellow 5-thiol-2-nitrobenzoate anion. Superoxide dismutase (SOD) activity in testis homogenate was determined according to the method described by **Nandi & Chatterjee (1988)**. This method is based on the ability of SOD to inhibit the auto-oxidation of pyrogallol at an alkaline pH. One unit of SOD is described as the amount of enzyme required to cause 50% inhibition of pyrogallol auto-oxidation. The Catalase (CAT) activity was determined by measuring the exponential disappearance of H_2O_2 at 240nm and

expressed in units per mg protein as described by **Aebi (1984)**. The total protein contents were determined according to the **Lowry** method as modified by **Peterson (1977)**. Absorbances were recorded using a Shimadzu recording spectrophotometer (UV-160) in all measurements.

Histopathologic examination

Testicular tissues for histopathological examination were fixed in 10% buffered formalin overnight and then embedded with paraffin. When analyzed, all paraffin-embedded tissue was sectioned at 4 μm, deparaffinized in xylene, dehydrated by ethyl alcohol in decreasing concentrations (100%, 95% and 70%), and stained with haematoxylin (Merck KGaA, Darmstadt, Germany) and eosin (Sigma, St. Louis, MO, USA). These specimens were examined under bright-field optical microscopy using a light microscope and × 40 magnification power. Corresponding digital images were captured for later analysis (**Bancroft & Stevens, 1996**).

Testicular DNA damage

Testicular DNA damage was measured using a single-cell gel electrophoresis (comet) assay **(Singh et al., 1988),** 1 g of crushed samples were transferred to 1 ml ice-cold PBS. This suspension was stirred for 5 min and filtered. Cell suspension (100 μl) was mixed with 600 μl of low-melting agarose (0.8% in PBS). 100 μl of this mixture was spread on precoated slides. The coated slides were immersed in lyses buffer (0.045 M TBE, pH 8.4, containing 2.5 % SDS) for 15 min. The slides were placed in electrophoresis chamber containing the same TBE buffer, but devoid of SDS. The electrophoresis conditions were 2 V/cm for 2 min and 100 mA. Staining with Ethidium bromide 20 μg/ml at 4 °C. the observation was with the samples still humid, the DNA fragment migration patterns of 100 cells for each dose level were evaluated with a fluorescence microscope (with excitation filter 420 - 490 nm [issue 510 nm]). The comets tails lengths were measured from the middle of the nucleus to the end of the tail with 40x increase for the count and measure the size of the comet. For visualization of DNA damage, observations are made of EtBr-strained DNA using a 40x objective on a fluorescent microscope.

Although any image analysis system may be suitable for the quantitation of SCGE data, we use a komet 5 image analysis software developed by kinetic imaging, Ltd. (Liverpool, UK) linked to a CCD camera to assess the quantitative and

qualitative extent of DNA damage in the cells by measuring the length of DNA migration and the percentage of migrated DNA. Finally, the program calculates tail moment. Generally, 50 to 100 randomly selected cells are analyzed per sample.

Statistical analysis

The quantitative data of continuous variables were expressed as mean ± S.D. Significance of mean values of different parameters between the groups were analyzed using One Way Analysis of Variance (ANOVA) after ascertaining the leven test for homogeneity of variance between the treatments. Pair wise comparisons were done using (Tukey HSD or Gsmes-Howell) test. In case of non-homogeneity, a Welch and Brown Forsythe adjustment for the ANOVA. All analyses were performed using SPSS 12 for Windows (SPSS Inc., Chicago) and differences were considered statistically significant at probability level less than 0.05.

3. Results
Effect of Cd, DPP and Cd-DPP on body weight, sex organs weight and spermatogenic structures.

During the entire study period Cd-administered animal gained the same weight as the controls (Table 1). The same happened with animals administered DPP alone or Cd + DPP. With regards to testicular weight, Cd-administered animals gained less testicular weight than the controls (p <0.05; Table 1). However, those co-administered DPP were largely spared from this weight reducing effect caused by Cd (p <0.01; Table 1), and their testicular weight were maintained at the level of the controls. A similar antagonism of Cd and normalization by DPP co-administration (Cd-DPP), on the weight of prostate gland and seminal vesicles could be deduced from the significance between group differences in their weights respectively. The epididymus weight was reduced in Cd-administered animals when compared to control (p <0.01; Table 1). However, animals co-administered DPP were spared from this Cd reducing effects (p <0.05), though their epididymal weight was still less than the control weight (p <0.05; Table 1). The counts of epididymal sperm and their motility were notably decreased by Cd while DPP co-administration largely counteracted these unfavorable action of

Cd, and preserved the integrity of spermatogenic structures. These results suggested that DPP could antagonize the negative effect of Cd on testicular and accessory sex organ weights as well as on sperm parameters.

Effect of Cd, DPP and Cd-DPP on serum testosterone and estradiol concentration.

Cd-administered animals had lower serum testosterone concentration than the controls (p <0.05; Figure 1). In contrast, those co-administered DPP were largely spared from this testosterone reducing effect caused by Cd and kept testosterone concentration at the level of the corresponding control. A different outcome was happened regarding estradiol serum level. Cd-administered animals had the same estradiol concentration as controls. However, those administered DPP alone or Cd-DPP showed a drastic increase in serum estradiol level (p <0.01; Figure 1).

Effect of Cd, DPP and Cd-DPP on prooxidant/antioxidant status.

From Figure (2) Cd-treated testis contained the same amount of MDA as control and higher level (p <0.01) of NO and GSH as well as higher activity (p <0.01) of SOD and CAT. DPP co-administration effectively neutralized these abnormalities in GSH level (p <0.01) and antioxidant enzyme activities (SOD and CAT, p <0.01). However, DPP didn't modulate NO level.

Effect of Cd, DPP and Cd-DPP on testicular morphology.

Microscopically, Cd disrupted rat spermatogenic structures, causing hypertrophy of seminiferous tubules and depletion of spermatids (Figure 3, Cd), whereas DPP co-administration significantly alleviated these abnormalities (Figure 3, Cd-DPP).

Effect of Cd, DPP and Cd-DPP on testicular DNA damage using gell electrophoresis (comet assay):

Single cell gell electrophoresis analysis on Cd-treated rats revealed the highest DNA damage with a mean DNA migration tail length of 7.6μm (6.77%) of damaged DNA concentration in the comet tail. Co-administration of DPP with Cd partially reduced the tail length of DNA migration (5.26) and the intensity of damaged DNA (5.02%) in testicular tissue (Table 1 and Figure 4)

Table 1: Effect of Cd, DPP and Cd-DPP on body and sex organs' weights and sperm characteristics in adult male rats. Values are expressed in terms of Mean ± S.D. (n=10)

	Cont.	Cd	DPP	Cd-DPP
Body Weight (g)				
Baseline	243 ± 8.7	239 ± 3.02	249 ± 10.02	242 ± 10.48
Final	298 ± 3.7	303.5 ± 2.8	298 ± 8.9	298 ± 11.08
Sex organ weights (g/100 g body weight)				
left testis	0.54 ± 0.014	0.47 ± 0.019 [a]	0.53 ± 0.012[b]	0.55 ±0.029[b+]
Right testis	0.53 ± 0.011	0.46 ± 0.0171[a]	0.52 ± 0.030[b]	0.54 ±0.023[b+]
Epididimus	0.067 ±0.002	0.052 ±0.003[a+]	0.066 ±0.003[b+]	0.059 ±0.004[ab]
Prostate gland	0.144 ± 0.010	0.086 ±0.007[a+]	0.146 ±0.011[b+]	0.124 ±0.011[b+]
Seminal vesicle	0.226 ± 0.013	0.163 ± 0.004[a+]	0.226 ± 0.014[b+]	0.208 ± 0.015[b]
Sperm characters				
Sperm count ($\times 10^6$/ml)	78.33 ± 1.19	57.21± 2.03[a+]	87.13 ± 1.83[a+b+]	76.02 ± 2.03[b+c+]
Sperm motility (%)	86.25 ± 1.70	63.65 ± 3.50[a+]	93.75 ± 25.00[a+b+]	80.31±3.11[b+c+]
DNA damage				
Comet tail length (µm)	1.95 ± 0.05	7.61 ± 0.46	1.82 ± 0.098	5.26 ± 0.23
Tail DNA %	1.89	6.77	1.69	5.02

a: $p < 0.05$ and a+: $p < 0.01$ versus Cont group, b: $p < 0.05$ and b+: $p < 0.01$ versus Cd group and c+: $p < 0.01$ versus DPP group.
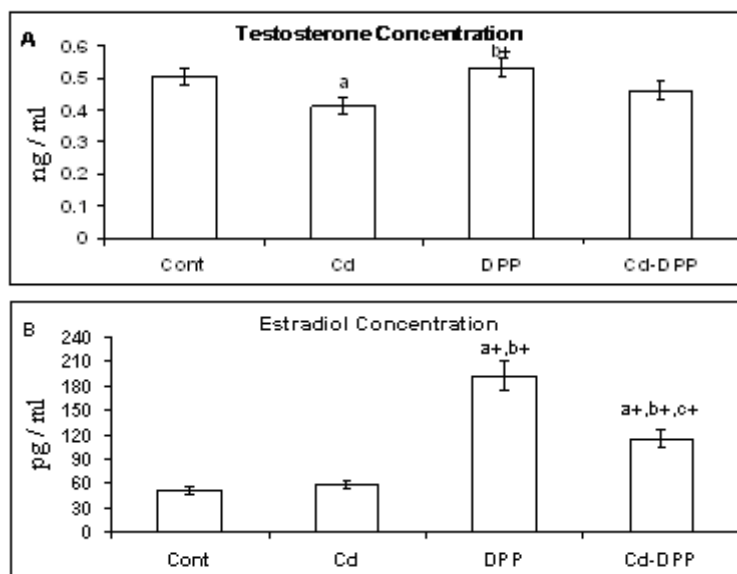

Figure (1): Effect of Cd, DPP and Cd-DPP on serum testosterone and estradiol level in adult male rats. a: $p < 0.05$ and a+: $p < 0.01$ versus Cont group, b+: $p < 0.01$ versus Cd group, c+: $p < 0.01$ versus DPP group.
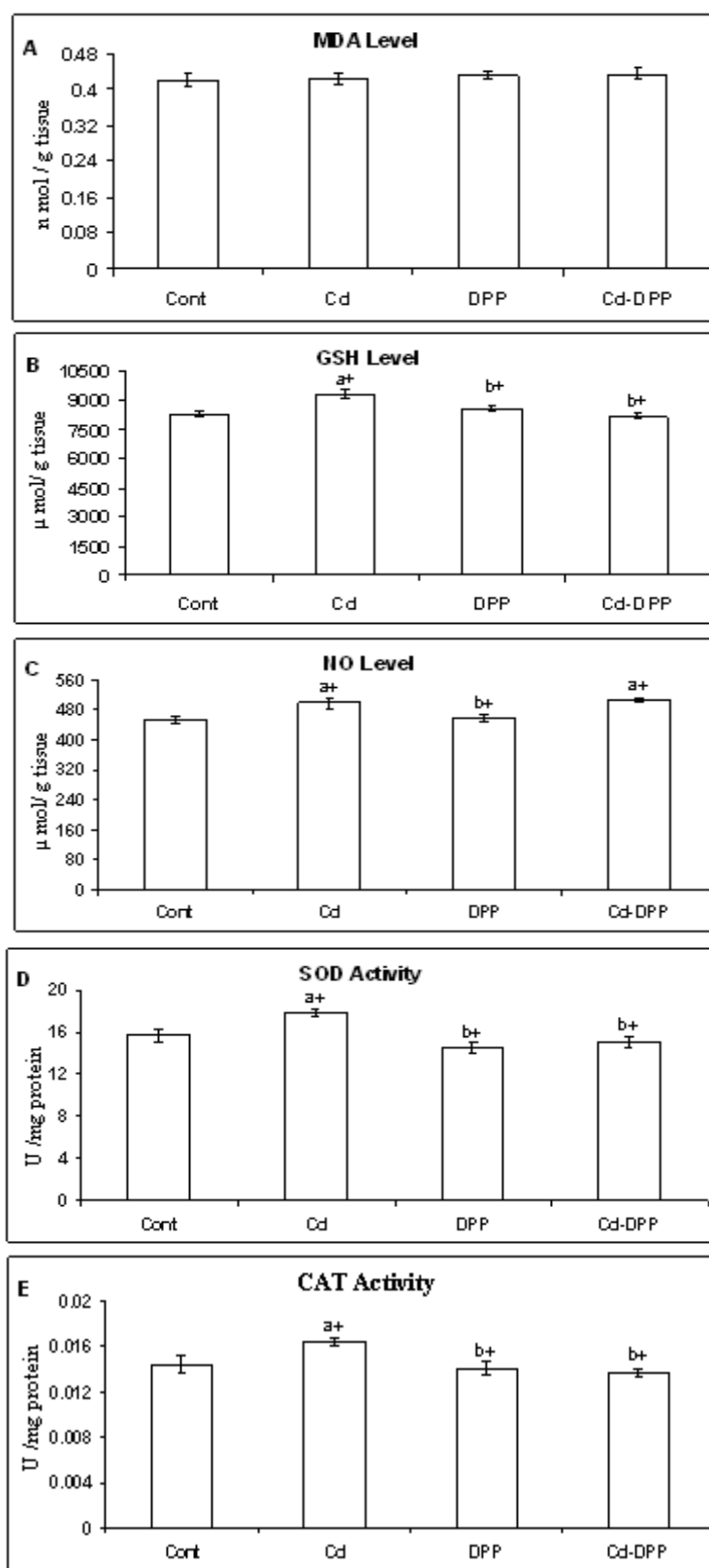
Figure (2): Effect of Cd, DPP and Cd-DPP on prooxidant parameters (MDA and NO) and antioxidant parameters (GSH, CAT and SOD) in adult male tats. a+: $p < 0.01$ versus Cont. and b+: $p < 0.01$ versus Cd group.
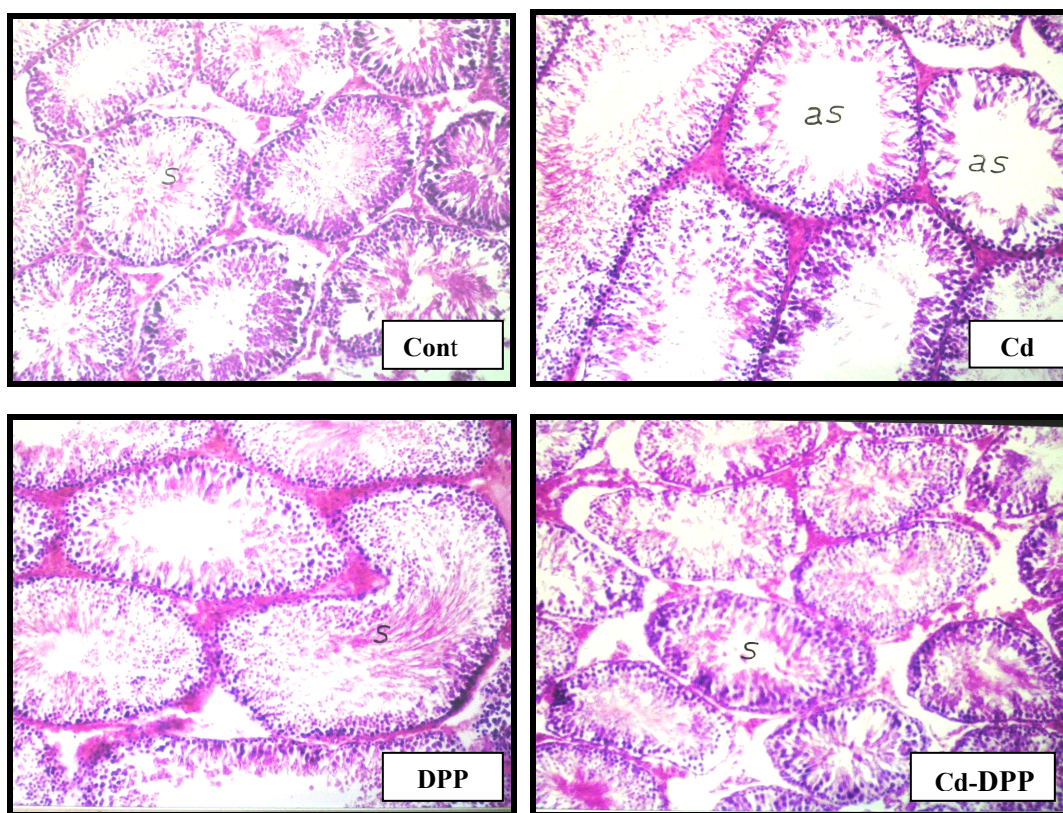
Figure (3): Representative illustrations of histological morphology of rat testes. Cross sections of testes were stained with hematoxylin and eosin (H and E). Testes from control (Cont) and date palm pollen extract (DPP) groups of rats exhibit typical features of seminiferous epithelium, while those from cadmium (Cd) group animals display impaired spermatogenesis, thickening and hypertrophy in the interstitial of leydig cells in the stroma. In DPP co-administered rat (Cd-DPP), testes could be protected from these Cd-induced abnormalities and show nearly normal Histological morphology in testicular sections.
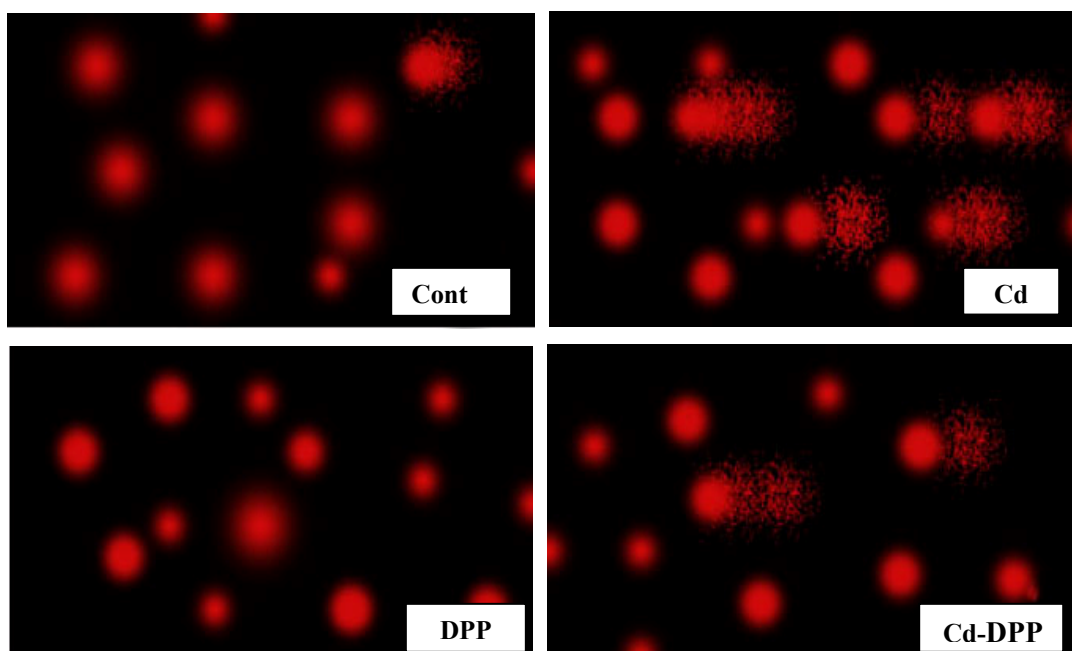

Figure (4): Effect of Cd, DPP and Cd -DPP on testicular DNA damage in adult male rats (40X).

4. Discussion

Cadmium has been shown to have gonadotoxic and spermiotoxic potentials **(El-Demerdash *et al*., 2004 and Yang *et al*., 2006)**. It exerts adverse effects on reproductive structures and functions directly at the testicular level or by altering post-testicular events such as sperm progress motility and/or function (viability), all of which may culminate in hypogonadism and infertility **(Akinloye *et al*., 2006)**. In the present study, administration of Cd caused a significant weight loss of the sex organs (testes, epididimus, seminal vesicle and prostate gland), reduction in epididymal sperm count, suppression of sperm progress motility and decline in testosterone concentration. Histological alterations in the testis were also noticed as evident by the decreases in spermatogenesis and stromal interstitial Leydig cell hypertrophy. Also testicular DNA damage was observed as represented by the increase in tail length of DNA migration in the comet assay.

Cadmium has been reported to induce necrotic degeneration of testicular tissues **(Shen & Sangiah, 1995),** which may contribute to weight loss of testis. Moreover, the weight loss of the testis is largely dependent on the mass of undifferentiated spermatogenic cells. Thus the weight loss of the testis may be due, in part to the adverse effect of Cd on the number of germ cells and elongated spermatids **(Yang *et al*., 2006)**. Some reports suggest that toxic exposure to Cd decreases the testicular sperm count **(Oteiza *et al*., 1999)** perhaps due to the low production of sperm in testes and that could be related to the low levels of gonadotrophins and/or plasma testosterone level **(Steinberger, 1971 & 1975)** and may be due to testicular necrosis **(Manna *et al*,. 2008). Santos *et al.* (2006) and Thompson & Bannigan (2008)** showed significant decline in testosterone concentration following Cd challenge. These finding run parallel with the results of the present study which indicated that Cd-administration lowered sperm counts and relative testicular weight associated with the reduction in serum testosterone level. The present results also attributed the reduction in the weights of sex organ and the decline in the number of sperm following Cd treatment to the abnormalities in testicular morphology as represented by thickening and hypertrophy in the interstitial of Leydig cells in the stroma and disruption of spermatogenic structures and also attributed to the testicular DNA damage.

Regarding the sperm motility, the present data demonstrated that Cd-challenge rats exhibited a significant inhibition in sperm motility. This result is in agreement with the results of **Oliveira *et al*. (2006)** who noticed that exposure of mice to $CdCl_2$ for 35 days caused a drastic reduction in sperm motility. This reduction may be due to Cd competes with calcium for calmodulin binding which was found to be important for sperm motility. Calmodulin inhibitions lead to decrease in sperm motility **(Schlingmann *et al*., 2007)**.

In the present study treatment with Cd caused non significant change in MDA level and significant increases in NO level and antioxidant system (GSH, SOD and CAT) of rat testis. Several studies reported absence or slightly difference in testicular MDA in response to Cd- challenge **(EL-Demerdash *et al*., 2004; Thijssen *et al.,* 2007; and Djukić-Cosić *et al.,* 2008)**. It is hypothesized that during Cd exposure, adaptation mechanism are induced to offset Cd-induced ROS and oxidative stress **(Liu *et al*., 2009)**. On the other hand several studies showed that Cd toxicity is associated with oxidative stress **(Manna *et al,.* 2008 and Liu *et al .,* 2009)** and that may be one of the causes of testicular steroidogenic disorders. Such discrepancies could be attributed to differences in experimental design and / or Cd dosing or rout of administration.

A number of cellular defense mechanisms are known to be activated in response to Cd exposures; among them are induction of Metallothionein, increase in cellular glutathione, activation of antioxidant transcription factor and other antioxidant components **(Waisberg *et al.,* 2003 and Prozialeck *et al*., 2007)**. Metallothionein is a cycteine –rich, Cd-binding protein playing an important role in protecting Cd toxicity and in competing Cd-induced ROS through Cd sequestration or through its free radical scavenging activity **(Prozialeck *et al*., 2007 and Thijssen *et al*., 2007)**. It is conceivable that GSH is though to be the first line of defense against Cd toxicity. In contrast to sharply depletion of GSH during acute Cd exposure **(Dudley & Klaassen, 1984),** chronic Cd exposure often result in elevations of tissue GSH level **(Waisberg *et al.,* 2003)**, which in turn will diminish oxidative damage from the metal. These mechanisms are not mutually exclusive, but may function together in an integrated way to confer resistance to Cd-induced oxidative stress. This notion is supported by our observation that chronic Cd administration leads to increase antioxidant status, GSH, SOD and CAT along with nonsignificant change in MDA level.

The current study also shows that administration of DPP alone caused a prominent increase in estradiol concentration associated with an elevation in epididymal sperm counts and their motility. DPP has been used for long time in traditional medicine to improve fertility in males and females. However, few studies are available to justify this use and to offer mechanism for its fertility improvement effect. **Mahran *et al.* (1976)** attributed DPP fertility improvement effect to its gonadotrphic activity. Previously, Egyptian scientists have reported the gonad stimulating potency of DPP **(Soliman & Soliman, 1957)**. **Miura *et al. (*2003) and Nayernia *et al.* (2004)** suggested that the improving effect of DPP on the

quality of sperm parameters in male rats might be due to the presence of gonadotrophins like substance or steroidal compound present in DPP, a result which is consistent with the data of the present work. Investigation have revealed that DPP grain extract contain estrogenic material as gonad-stimulating compound that improve male fertility **(Zargari, 1999)**. Recently, **Abbas & Ateya (2011),** reported that column chromatography of the Egyptian DPP resulted in the isolation of estradiol, estrone and estriol compounds. In accordance with the above studies it may be suggested that the improving effect of DPP on the quality of fertility as represented by increase serum concentration of estradiol and sperm characteristic may be due to the presence of estrogenic compound in DPP.

Another aim of this study was to reveal weather DPP can improve fertility when administered to infertile rats. For this purpose, DPP was given to rats challenged with CdCl$_2$. Our results showed the ability of DPP to reverse the testicular dysfunction caused by Cd; as represented by normalization of epididymal sperm counts and testosterone level and improvement of sperm motility along with restoration of spermatogenesis in seminiferous tubules as shown via the histopathological testicular examination as well as the reduction in the intensity of DNA damage caused by Cd. An increasing number of scientific evidence has revealed that male fertility is greatly influenced by estrogen **(Hess et al., 2001 and Sierens et al., 2005).** Investigations have reported that palm kernels and date pollen grain extracts contain estrogenic materials as gonad-stimulating compounds that improve male infertility **(Zargari, 1999)**. **Kostyuk et al. (2004)** showed that estrogen is synthesized in male reproductive system by at least three different cell type, Sertoil, Leydig and germ cells. It has been shown that estrogen may be involved in the regulating the renewal of spermatogonial stem cell **(Miura et al., 2003)**, and male reproductive tissue with estrogen receptor (**Amin et al., 1969)**. **Eddy et al. (1996)** demonstrated that disruption of the estrogen receptor gene in male mice causes alteration of spermatogenesis and infertility. In Knockout mice (with functionally inactive estrogen receptors) showed number of abnormalities, including dilated rete testis and seminiferous tubules lumen, a thin epithelial layer in seminiferous tubule, and reduced rete testis epithelial height **(Lee et al., 2008, 2009).** These results in dilution of cauda epididymal sperm, disruption of sperm morphology, inhibition of sodium transport and subsequent water reabsorption, increased secretion of chloride ion and eventual decreased fertility **(Hess, 2003)**. Estrogen regulates the reabsorption of luminal fluid in the head of epididymus. Disruption of this essential function causes the sperm to enter the cauda epididymus in

diluted form and reduce the sperm cell count **(Hess et al., 2001).**

Reports are indicated that DPP contain flavonoid components **(Mahran et al., 1985 and Abbas & Ateya, 2011)** that have positive effect on the sperm quality **(Vayalil, 2002 and Kostyuk et al., 2004).** The scavenging properties of DPP is said to be the main important effects on the sperm parameters. Several studies have shown their functional health benefits on sperm parameters and reproductive system of adult rats **(Bahmanpour et al., 2006)** and their protective effect on cisplatin induced male infertility in rats **(AL-Kharage, 1982)**. An investigation by **Morales et al. (2006)** showed that querecetin, a bioactive flavonoid component of DPP exerted a synergistic action with Cd in increasing the expression of stress protein, metallothionein, which offer protection against Cd toxicity **(Kara et al., 2005 and Morales et al., 2006)**. This may plausibly explain the protective effect of DPP against Cd-induced testicular damage.

Conclusion

The results of the present study suggest that unrelated to ROS production, other effects of Cd are involved in testicular dysfunction as represented by decreases the relative weight of sex organs, testosterone hormone, epididymal sperm counts and their motility as well as alteration in testicular morphology and its DNA damage, consequently impaired fertility. Administration of DPP suspension separately increased estradiol hormone, sperm counts and their motility in normal rats and consequently improves fertility. Co-administration of DPP suspension with Cd show protective effect against testicular dysfunction induced by Cd. DPP modulating effects on Cd-induced testicular dysfunction are through normalization of serum testosterone level and increases serum estradiol level and sperm characteristic. DPP also protects rats' testicular tissue via suppressing testicular histopathological abnormalities and modulating its DNA damage. The result of our work suggest that, the benefit effect of DPP suspension is not only for its antioxidant characteristic but also due to estrogenic properties, so the DPP may be useful to solve the infertility problems in males results from low concentration of estradiol or elevated level of ROS. On the other hand, it may be used as replacement therapy for menopausal women as source of estrogen. These results support the folkloric use of DPP such as vaginal pesseris used for infertility and menopausal problems by women in ruler area in the middle east.

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