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Semen traits, testicular morphometry and histopathology of cadmium-exposed rabbit bucks administered methanolic extract of *Phoenix dactylifera* fruit

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ABSTRACT. This study evaluated the effect of Cd on some male reproductive parameters; and explored the therapeutic potentials of methanolic extract of *Phoenix dactylifera* (MEPD) fruit in averting such reproductive damages. 45 rabbit bucks aged 24-28 weeks and weighing 1.41-1.43 kg was used. The rabbits were assigned to 5 treatment groups (control, Cd-only, Cd + 300 mg MEPD, Cd + 600 mg MEPD and Cd + 900 mg MEPD) in a completely randomized design. The rabbits were dosed with 3 mg $CdCl_2$ kg^{-1}feed for 7 days followed by MEPD for 56 days after every 72 hours. Result of semen evaluation indicates that semen volume, motility, libido, concentration, total ejaculate, viability and morphology were significantly reduced by Cd compared to the normal control group. Testis density and epididymis length of the control rabbits were significantly different from the Cd-only exposed rabbits. Histopathological examination revealed severe testicular damage due to Cd. Indeed, treatment with MEPD significantly reversed the deleterious reproductive effects caused by Cd. In conclusion, cadmium drastically affected the testis of rabbits and treatment with MEPD alleviated some deleterious effects.

Keywords: testis, sperm, heavy metal, methanol, plant extract.

Características do sêmen, morfometria testicular e histopatologia de cadáveres de coelho expostos, administrado extrato metanólico de frutos de *Phoenix dactylifera*

RESUMO. Este estudo avaliou o efeito da Cd em alguns parâmetros reprodutivos masculinos e explorou os potenciais terapêuticos de extrato metanólico de *Phoenix dactylifera* (MEPD), fruto na prevenção de tais danos reprodutivos. Foram utilizados 45 machos de coelho com idade entre 24-28 semanas e pesando 1,41-1,43 kg. Os coelhos foram designados a cinco grupos de tratamento (controle, Cd-apenas, Cd+300 mg MEPD, Cd+600 mg MEPD e Cd+900 mg MEPD) num delineamento inteiramente casualizado. Os coelhos foram doseados com 3 mg de CdCl2 kg^{-1} de alimento durante sete dias seguido por MEPD durante 56 dias após cada 72h. O resultado da avaliação do sêmen indica que o volume de sêmen, a motilidade, a libido, a concentração, a ejaculação total, a viabilidade e a morfologia foram significativamente reduzidos pelo Cd em comparação com o grupo controle normal. A densidade do testículo e o comprimento do epidídimo dos coelhos de controle foram significativamente diferentes dos coelhos expostos. O exame histopatológico revelou dano testicular grave devido ao Cd. Na verdade, o tratamento com MEPD reverteu significativamente os efeitos deletérios reprodutivos causados por Cd. Em conclusão, o cádmio afetou muito os testículos de coelhos e o tratamento com MEPD aliviou alguns efeitos deletérios.

Palavras-chave: testículo, esperma, metal pesado, metanol, extrato vegetal.

Introduction

Environmental contamination has been reported to threaten animal health and limits productivity (Galadima, Garba, Leke, Almustapha, & Adam, 2011). Potentially toxic metal pollution in the environment has given rise to growing concerns from scientists, and a considerable amount of researches have been done all over the world (Shi et al., 2007; Amara et al., 2008; Suruchi & Pankaj, 2011). The impact of toxic metals contamination on animals result in serious economic losses, thus, there is an increasing concern about environmental pollutants emanating into the livestock production systems (Patra, Rautray, & Swarup, 2011). Cadmium (Cd) is one of these known toxic metals that is most significant in public health (Orisakwe, 2014). Cd is used in industrial processes and where regulations are lax in the disposal of Cd in used industrial products, Cd finds its way into the soil. Cd has high rates of soil to plant transference compared with other non-essential elements, and plants accumulate large amounts of cadmium from

low cadmium content soils more avidly than they do other heavy metals such as lead and mercury (Satarag et al., 2003). Through plant uptake of Cd, this toxic metal of increasing environmental concern enters the food chain of livestock in significant amounts. Farm animals such as rabbits, goats, sheep and cattle feed on these plants and plants products which have absorbed and accumulated these toxic elements from the soil over time.

Sequel to physiological imbalances as a result of Cd contamination, there has been increasing demand for the use of plant products in enhancing male fertility and management of some physiological disorders (Neelesh, Sanjay, Bihari, & Savita, 2011). This is probably due to low cost, easy availability and lesser side effects of organic medicinal products compared to their allopathic counterpart. Hence, plants are continuously scrutinized and explored for their beneficial effects. A larger number of these tropical plants and their extract have shown beneficial therapeutic effects including fertility enhancing compounds, anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial and aphrodisiac (Petrovska, 2012). In view of the concerns on male animal infertility in our livestock industry, the scope of the biologic actions of this commonly used botanical remedy needs to be assessed. Among the promising medicinal plants, *Phoenix dactylifera* (Date palm) is a tropical and subtropical tree belonging to the Palmae family. Date fruit pulp is reported to be rich in phytochemicals like phenolics, sterols, carotenoids, anthocyanins, procyanidins, and flavonoids (Al-Daihan & Bhat, 2012). Given these inherent phyto-constituents, date fruit is claimed to have potential role to protect against cellular damage (Saafi et al., 2011). In the light of the foregoing, this study was designed to evaluate the potentials of *Phoenix dactylifera* fruit extracts in protecting against the toxic effect of Cd on the reproductive system of rabbit bucks.

Material and methods

Location of study: The experiment was carried out at the Rabbitary Unit of the Teaching and Research Farm of the University of Benin, Benin City. The University of Benin is in Ugbowo and Ugbowo is situated in Ovia-North, Edo State, Nigeria.

Acquisition of Phoenix dactylifera fruits: *Phoenix dactylifera* fruits were sourced from Nigerian Institute for Oil Palm Research (Nifor) Benin City, Edo State, Nigeria. The acquired fruits was authenticated by a botanist, split, air-dried, finely ground with the aid of an electric blender and stored in an air-tight container.

Preparation of fruit extracts: The ground *Phoenix dactylifera* fruits was weighed and extraction was carried out with 99% methanol in a soxhlet apparatus. The extract obtained was concentrated by recovery of methanol. The solvent was recovered using rotary vacuum evaporator and the concentrated extract was preserved in an air-tight bottle.

Experimental materials and management: A total of forty-five (45) composite rabbit bucks aged 24-28 weeks and weighing between 1.41-1.43 kg was used for this study. The rabbits were managed intensively in a hutch. They were quarantined for 2 weeks during which they were treated with Ivomec® injection for the control of haemoparasite, internal and external parasites. The rabbits were allowed *ad libitum* access to water and feed (commercial growers' diet).

Experimental design: The treatment protocols consisted of 5 groups: group 1 (control), group 2 (3 mg of $CdCl_2$ kg^{-1} feed day^{-1} for 7 days), group 3 ($CdCl_2$ kg^{-1} feed day^{-1} for 7 days + 300 mg kg^{-1} body weight of methanolic extract of *Phoenix dactylifera* (MEPD) fruits for 56 days), group 4 ($CdCl_2$ kg^{-1} feed day^{-1} for 7 days + 600 mg kg^{-1} body weight of MEPD fruits for 56 days) and group 5 ($CdCl_2$ kg^{-1} feed day^{-1} for 7 days + 900 mg kg^{-1} body weight of MEPD fruits for 56 days). Each treatment group consisted of three replications with three bucks per replicate in a completely randomized design.

Data collection and evaluation

Semen collection: Two weeks prior to semen collection, the rabbit bucks were trained to serve an Artificial vagina (AV) using a teaser rabbit doe. On the 57^{th} days following the administration of the experimental diets, the bucks under study were placed on a semen collection schedule of twice per week. One ejaculate was collected from each rabbit buck once between 08:00 to 13:00 hours (local time) on Mondays and Thursdays for 3 consecutive weeks.

Semen characteristics evaluation: Semen evaluation involved the estimation of both microscopic and macroscopic indices. Ejaculate volume was read-off directly in milliliters from a calibrated glass collection tube attached to the AV. Sperm motility percentage score was subjectively assessed in a drop of fresh semen on a warm glass slide covered with a warm cover slip and examined using a microscope at x40 magnification. Sperm cell concentration ($\times 10^6$ mm^{-3}) was determined using a haemocytometer at a dilution of 1 in 100 in a

solution of 45 mL normal saline and 5 mL formalin (Lanes, Okamoto, Bianchini, Marins, & Sampaio, 2010). Total sperm ($\times 10^6$ mm^{-3} per ejaculate) was determined by multiplying the semen ejaculate volume by the sperm cell concentration. Morphological examination of the semen was done by performing different counts of the morphologically normal and abnormal sperm cell types on eosin-nigrosin stained preparations. The viability (live:dead ratio %) was calculated by counting the number of live cells (without colour) and dead cells (pink) using optical microscopy (400X), after combining one drop of semen with one drop of eosin-nigrosin. Libido was estimated by observing the reaction time (s) which elapsed between exposure of a buck to a doe and the first copulation (serving the AV).

Evaluation of testicular morphometry: At the end of the experiment, 3 bucks from each treatment groups were randomly selected from the experimental bucks for morphometric analysis. The bucks were euthanized and the liver, kidney and testis were harvested and measured. Testis, epididymis and vas deferens were carefully separated and freed of tunica albuginea and all adhering connective tissues. The length of each testis was measured using vernier caliper. Testis circumference, length of epididymis and vas deferens were determined with a measuring tape. The testes and epididymis weights were measured on an electronic scale. Testis volume was determined volumetrically using the Archimedes principle of water displacement in a measuring cylinder. The testes density was derived thus Equation 1:

$$\text{Testes density} = \frac{\text{Testes weight (g)}}{\text{Testes volume (cm}^3\text{)}} \quad (1)$$

The weights of the liver and kidney were also measured using the electronic scale.

Histopathological studies: Histopathological examination was carried out at the College of Veterinary Medicine Laboratory, Michael Okpara University of Agriculture, Umudike, Nigeria. The testes recovered from testicular morphometry were fixed in Bouin's fluid for 24 hours. The tissues were washed in ascending grades of ethanol (50, 75 and 100%) and cleared with xylene. They were embedded in paraffin wax and then sectioned using microtome at 4-5 µ thickness. Dewaxed sections were stained with Haemotoxylin and Eosin (H&E). The slides were covered with DPX (Distyrene, Plasticizer, and Xylene) mountant to increase refractive index of the stained preparation and then covered with slides to prevent scratches. All sections were examined under light microscope using × 400 magnification. Photomicrographs of the testicular tissues were taken with Olympus photomicroscope for observation and documentation of histopathology.

Statistical analysis: The data generated was subjected to statistical analysis of variance (ANOVA) procedure of GenStat 12th edition at 5% probability level. Occurrence of significant means was separated using Duncan Multiple Range Test (DMRT) of the same statistical software (Duncan, 1955).

Results and discussion

The mean of semen characteristics of Cd exposed rabbit bucks administered methonolic extract of *Phoenix dactylifera* (MEPD) fruit is presented in Table 1.

The result on semen evaluation in this study indicates that semen volume, semen motility, libido, semen concentration, total ejaculate, viability and morphology were significantly ($p < 0.05$) reduced by Cd compared to the normal control group. Following treatment with MEPD for 56 days, the mean values of semen motility, concentration, libido, viability, and sperm morphology were significantly ($p < 0.05$) different from Cd-only treated rabbit bucks. Mean value of semen concentration at 900 mg kg^{-1} was statistically ($p > 0.05$) similar with the normal control rabbit bucks. However, MEPD treatment did not significantly ($p > 0.05$) affect the mean values of semen volume and total ejaculate concentration. Also, the mean semen concentration value at 300 and 600 mg kg^{-1} did not significantly ($p > 0.05$) differ from the Cd exposed rabbit bucks.

The result of testicular morphometry for rabbit bucks in this study is presented in Table 2.

In all the parameters measured for testicular morphometry (Table 2), testis density and epididymis length of the control rabbits were significantly ($p < 0.05$) different from the Cd-only treated rabbits. Testis length, testis circumference, testis weight, testis volume, epididymis weight and vas deferens length of the control rabbits were not significantly ($p > 0.05$) different but showed higher mean values compared to the control rabbits. Treatment with MEPD significantly ($p < 0.05$) improved testis volume and testis density. Although, the mean values obtained for testis length, testis circumference, testis weight and epididymis weight in the extract treated groups did not significantly differ from the Cd-only treated rabbit bucks, they showed marked improvement in these measured parameters.

Table 1. Mean of semen characteristics of cadmium-exposed rabbits administered *Phoenix dactylifera* extract.

Parameters	Control	Cd	Cd + 300 MEPD	Cd + 600 MEPD	Cd + 900 MEPD	SEM
Volume (mL)	1.17[a]	0.40[b]	0.51[b]	0.63[b]	0.49[b]	0.17
Motility (%)	74.48[a]	20.25[b]	79.00[a]	66.97[a]	80.46[a]	9.26
Libido (sec)	13.84[b]	23.19[a]	13.07[b]	11.69[b]	10.90[b]	3.10
Concentration ($\times 10^6$ mm^{-3})	251.70[a]	22.50[c]	111.70[bc]	119.20[bc]	201.20[ab]	29.60
Total ejaculate ($\times 10^6$ mm^{-3})	294.40[a]	9.50[c]	58.60[b]	81.40[b]	96.80[b]	44.00
Viability (%)	72.00[a]	55.00[b]	72.00[a]	73.00[a]	75.33[a]	3.93
Morphology (%)	70.33[a]	64.67[b]	71.00[a]	70.00[a]	73.33[a]	1.48

[ab]Means bearing different letters of superscript within the same row differ significantly ($p < 0.05$).

Table 2. Mean testicular morphometry of cadmium-exposed rabbits administered *Phoenix dactylifera* extract.

Parameters	Control	Cd	Cd + 300 MEPD	Cd + 600 MEPD	Cd + 900 MEPD	SEM
Paired testis length (cm)	5.53	5.00	5.63	5.63	5.70	0.27
Paired testis circumference (cm)	6.03	5.23	6.13	6.03	5.80	0.37
Paired testis weight (g)	2.57	2.10	2.90	2.67	2.83	0.33
Paired testis volume (cm^3)	2.63[ab]	1.57[b]	3.00[a]	2.80[a]	2.77[a]	0.36
Paired testis density (g cm^{-3})	0.99[b]	1.35[a]	0.98[b]	1.00[b]	1.03[b]	0.06
Paired epididymis length (cm)	16.73[a]	14.17[b]	16.23[a]	15.93[ab]	16.07[ab]	0.60
Paired epididymis weight (g)	1.100	0.833	0.867	1.067	0.867	0.08
Paired vas deferens length (cm)	17.07	16.03	15.60	18.30	16.47	1.34

[ab]Means bearing different letters of superscript within the same row differ significantly ($p < 0.05$).

Histopathologically, the microscopic observations of the testis in the control group showed normal appearance of seminiferous tubules (STs), spermatogenic cells and interstitial cells (Figure 1a). The rabbits treated with Cd (Figure 1b) showed many histopathological changes. All the rabbits in this group developed severe testicular lesions, haemorrhage, widening of the central tubules lumen and germ cell necrosis. Multiple vacuoles were seen within the tubules. Furthermore, Cd inhibited spermatogenesis which was visible in the absence of the advance stages of sperm production. However, a photomicrograph of a section of testis of 300 mg kg^{-1} MEPD group (Figure 1c) showed the appearance of some shrunken tubules, separated from each other with widened interstitial space and had different shapes with irregular outlines. Nevertheless, some lumen contained sperm and Sertoli cells are capped by tufts of late spermatids attached by their heads in the lumen. In 600 (Figure 1d) and 900 mg kg^{-1} (Figure 1e) groups, the histological features were almost similar to those in the normal control group. Testicular sections showed STs with near normal germ cell population layer thickness with a normal orderly arranged pattern up to mature spermatids. There were adequate Sertoli cell populations.

In this study, the results of semen evaluation indicates that the dose of 3 mg kg^{-1} day^{-1} of cadmium chloride for 7 days in feed caused alterations in macroscopic and microscopic characteristics of ejaculates in rabbit bucks. This observation was reached based on the examination of certain indices of the ejaculate which constitutes the commonest criteria used for semen quality evaluation.

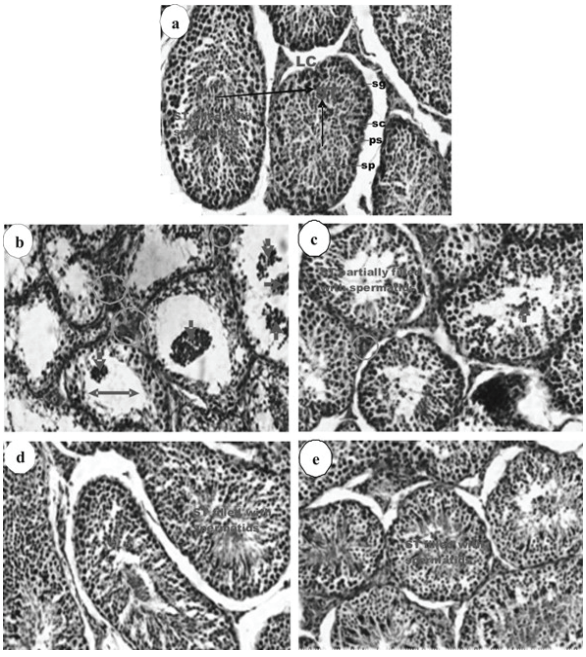

Figure 1. A photomicrograph of the testis of cadmium exposed rabbits administered MEPD fruit stained with H&E, 100 X. (a) The control rabbit showing normal appearance of spermatogenic cells; spermatogonia (sg), primary spermatocyte (ps), spermatid (sp), and Sertoli cells (sc). Leydig cells in interstitial tissue are noticed (LC). (b) Cross section of Cd-only treated rabbit showing: degeneration of spermatogenic cells, aggregation of necrotic spermatozoa at the seminiferous tubules (STs) lumen (thick arrow), interstitial haemorrhage (encircled), wide ST lumen (c) Section of rabbit treated with Cd+ 300 mg kg^{-1} MEPD showing; aggregation of few necrotic spermatozoa at the STs, mild interstitial haemorrhage (encircled), STs partially filled with spermatozoa. (d-e) sections of rabbits treated with Cd+600 and Cd+900 mg kg^{-1} MEPD showing; mild vacuolation, STs containing sperms and Sertoli cells are capped by tufts of late spermatids attached by their heads in the lumen.

These characteristics were volume of ejaculate, sperm motility, sperm concentration, sperm

viability, presence of morphologically abnormal spermatozoa and libido. Compared with the control group, a significant ($p < 0.05$) decrease was observed in all measured semen traits in Cd-only treated rabbit bucks. However, these semen traits were improved following treatment with MEPD, although, not to the control level in some characteristics.

The testis is extremely sensitive to Cd exposure and one of the possible explanations for this sensitivity is its unique morphological layout of the blood testis barrier (BTB). It is known that Cd treatment induces metallothioneins (MTs) expression in several tissues. Thus, MTs protects tissues against Cd toxicity in this way. Yet, the steady level of MTs does not appear to be induced in sufficient levels in the testis (Siu, Mruk, Porto, & Cheng, 2009). In a related study, Dalton, Fu, Enders, Palmiter, and Andrews (1996) documented that the toxic effects of Cd in the testis could not be reduced in MTs transgenic mice even at an adequate expression of MTs. This raised the question why the testis displays a relatively high basal level of MTs compared to other organs, yet, it fails to detoxify Cd.

In the present study, the significant reduction in semen volume in Cd-only and Cd-extract treated groups compared to the control group could probably be attributed to the impairment of accessory sex glands by Cd. Visual observations in this study revealed a higher volume of seminal plasma in the ejaculate of the control group compared to the other groups which received 3 mg kg^{-1} of cadmium chloride in feed for 7 days. The higher volume of seminal plasma from the supposed intact accessory organs in the control group could have contributed to the observed higher semen volume (1.17 mL) recorded in this study. This assumption is supported by Rehm et al. (2008), Saeed (2013) and Zakaria and Al-Busadah (2015) who independently reported a reduction in accessory sex gland weights and secretions after Cd administration. Predes, Diamante, and Dolder (2010) and Elgawish and Ghanem (2014) specifically reported a reduction in weight of seminal vesicle and prostate gland of cadmium exposed rats. In this study, after the treatment of cadmium exposed rabbits with MEPD, semen volume was only numerically higher but not significantly different ($p > 0.05$) from the Cd-only treated rabbits. Similarly, Ahmed, Hasona, and Selemain (2008) reported that weight of testis and epididymis was increased while there was little positive effect on prostate and seminal vesicle following *Phoenix dactylifera* pollen administration to Sprague-Dawley rats. Therefore, it was not puzzling to observe the slight and not significant ($p > 0.05$) improvement in semen volume of Cd-extract treated rabbits in the present findings. However, the lowest mean semen volume obtained in this study was not below the 0.4 mL low limit reported by Herbert and Acha (1995) and Brackett (2004) for rabbit bucks.

Cadmium contamination also drastically impaired the sperm motility (20.25%) of rabbit bucks below the minimum score documented for quality ejaculates. Brackett (2004) reported sperm motility of 70% and above for good quality fresh sperm. Pineda (2003) advocated a low score of 30% as a minimally acceptable spermatozoa motility levels for fresh ejaculates for breeding organisations. However, the significant difference ($p < 0.05$) in sperm motility observed in the Cd-extract group compared with Cd-only treated group may undoubtedly be due to the ameliorating effect of the phytochemical component(s) present in *Phoenix dactylifera* fruits. For instance, saponin found in *Phoenix dactylifera* is believed to have contributed to the observed increase in sperm motility in this study. Supporting this assertion, Oyeyemi, Soetan, and Akinpelu (2015) reported that increase in sperm motility was observed with increasing dose of saponins extract with the highest percentage in the group that received the highest dose of 400 mg kg^{-1} body weight. This could have been achieved through the inhibition of the cyclic Adenosine Monophosphate (cAMP) phosphodiesterase by saponins in MEPD, thus increasing intracellular cAMP which is reported to be susceptible to Cd toxicity (Gunnarsson, Nordberg, & Selstam, 2007). cAMP has a role in sperm kinematics. So, treatments that increase intracellular cAMP concentration cause an increase in kinematics and sperm motility (Henkel & Schill, 2003). Secondly, the antioxidant potentials of phenol is well established and this could have provided an oxidative stress-free testicular environment for sperm production and maturation which favoured the Cd-extract treated groups in this study.

The result obtained for libido as measured by reaction time has validated the use of dates as important aphrodisiacs and tonic confections in the various traditional medicines. Methanolic extract of date have been shown to restore loss of sex drive in rabbits caused by cadmium intoxication (El-Neweshy, El-Maddawy, & El-Sayed, 2013). Incidentally, the libido stimulating property of date palm extract affected the experimental rabbits in a dose-dependent manner (13:07, 11:69 and 10:90 s for 300, 600 and 900 mg kg^{-1} MEPD respectively).

Concerning the result of semen concentration, it was observed that the mean value of 22.50 × 10^6 mm^{-3} obtained for the Cd-only group was below the range of 50 to 350 ×10^6 mm^{-3} reported by Brackett (2004) for rabbit bucks. Although the observed significant ($p < 0.05$) improvement in Cd-extract group was lower than the control group, the dose-dependent significant increase suggests the therapeutic effect of MEPD in alleviating cadmium induced reproductive dysfunction in rabbits. Laskey and Phelps (1991) showed that cadmium impairs testosterone production in Leydig cells without affecting their viability. This demonstrates that steroidogenic disruption in Leydig cells is likely to be the initial target of cadmium toxicity as an endocrine modulator. However, there are reports that show that the administration of *P. dactylifera* resulted both in an increase in sperm density and testosterone level, indirectly promoting spermatogenesis (El-Neweshy et al., 2013; Saeed, Tahir, & Lone, 2015). The result of semen evaluation herein confirmed that the administration of MEPD to cadmium exposed rabbit bucks increased the number of spermatogonia which resulted in the significant ($p < 0.05$) increase in sperm concentration. This may have been due to the stimulation of androgen biosynthesis, possibly by regulating progesterone synthesis and metabolism which may have been altered through direct interaction of cadmium with deoxyribonucleic acid (DNA) and competitive inhibition of essential enzymes (Yang, Kim, Weon, & Seo, 2015). Also by the initiation of FSH synthesis (Mehraban et al., 2014) which perhaps increased the proportion of cells passing through meiosis and spermatogenesis thus increasing semen concentration and total sperm per ejaculate.

Contrary to the result of semen traits obtained in this study, Doyle, Pfander, Grebing, and Pierce (1974) found that when cadmium chloride was administered to 4 months-old ram at a dose of 60 mg kg^{-1} feed for 191 days, sperm concentration, was unaffected. Also, Lymberopoulos, Kotsaki-Kovatsi, Taylor, Papaioannou, and Brikas (2000) found that sperm viability, grade motility and the percentage of live $dead^{-1}$, and the number of morphologically abnormal spermatozoa was not affected by 3 mg kg^{-1} body weight of cadmium chloride orally for 7 months in Chios ram-lambs.

In this study, the post treatment effects of Cd on testicular morphometric indices as well as the therapeutic possibilities of MEPD against Cd-induced toxicity was also examined. The results indicated that Cd significantly ($p < 0.05$) reduced the volume of testis, carrying capacity and the epididymal length. These effects may be associated with a reduction in serum testosterone levels as earlier postulated by El-Neweshy, El-Maddawy, and El-Sayed (2013). This findings which are consistent with previous reports (Qadori & Al-Shaikh, 2011; El-Neweshy et al., 2013; Saeed, 2013; Zakaria & Al-Busadah, 2015) confirm the toxic effect of Cd on the testis. Cd has been reported to induce necrotic degenerative changes in the testis (Predes et al., 2010) and destruction of testicular germ cells due to membrane damage or macromolecular degeneration (Qadori & Al-Shaikh, 2011). This may have contributed to the reduced testicular weight and consequent decrease in sperm characteristics in this study.

Concerning the significant ($p < 0.05$) reduction in testis volume and density, previous authors have described a marked reduction of seminiferous tubular diameter along with conspicuous decrease of testicular volume density after Cd administration (França & Russell, 1998; Qadori & Al-Shaikh, 2011). Taking into account the fact that the weight of the testis was reduced, it can be deduced that the seminiferous tubule diminished as a consequence of Cd exposure, thus reducing the carrying capacity of the testis.

Interestingly, the therapeutic intervention in Cd-intoxicated rabbits with MEPD effectively attenuated the deleterious reproductive defects of Cd, restoring the epididymis length, testis density and volume of the testis with concomitant improvements in sperm motility, sperm concentration, viability and morphology as shown in this study. The observed therapeutic potency of date extract might be due to several contributing factors primarily including the hormone mediated effects elicited through its content of gonadotropin-like substances or steroidal components (Bajpayee, 1997). This acts like gonad stimulating compounds, improving male fertility and maintaining normal serum levels of testosterone (El-Neweshy et al., 2013). Ahmed et al. (2008), El-Neweshy et al. (2013) and Wafaa, El-Kashlan, and Ehssan (2012) independently reported increase in testis weight and epididymis weight of Cd induced testicular dysfunction in rats treated with *Phoenix dactylifera* pollen extract.

The histopathological examination of the testicular tissue revealed the true position of the reproductive potentials of Cd exposed rabbits. In agreement with the results of Saygi, Deniz, Kutsal, and Vural (1991), Lymberopoulos et al. (2000), El-Shahat, Gabr, Meki, and Mehana (2009) and El-Refaiy and Eissa (2013), the current study showed

marked generalized disorganisation of the architectural structure of the seminiferous tubules (STs), a decrease in thickness of germ cell layer, widening of the central STs lumen, prominent germ cell population necrosis and leakage of blood into the interstitial spaces causing haemorrhage and oedema. Sertoli cells were abnormal in number and shape when compared to the control group. In the lumen of the STs, some tubules contained a large number of intraluminal collection of degenerated, necrotic, desquamated spematogenic cells and a very small number of spermatozoa were observed. Other tubules showed complete absence of sperm in the lumen, multiple vacuoles and sloughing of all layers of STs. The appearance of the testis resemble that of the immature testis and there were hallmark of degeneration and could indicate an end of reproduction.

Indeed, the results of these histopathological changes are harmonious with the decreased semen and testicular morphometric parameters recorded in this study. Thus it appears that Cd damaged the STs, probably by causing a cessation of spermatogenesis; interfered with the testicular vasculature and blood flow; and probably gave rise to an increase in the absorption of dead spermatozoa by the epididymis, resulting in their disappearance from the reproductive tract. Blanco et al. (2007) reported that even low doses of $CdCl_2$ (1 mg kg^{-1} for one month) induced lack of spermatogenesis and severe necrosis of the testes of rats. Moreover, Santos et al. (2004) reported that endothelial damage of the small blood vessels, oedema and haemorrhage of the rat testes can be demonstrated by using just a single parenteral dose of Cd chloride at 2-4 mg kg^{-1}. Several studies focusing on Cd-related changes in testicular histopathology have implicated testicular blood vessel damage, followed by the degeneration of spermatopoietic epithelial, as the main cause of Cd toxicity (Thompson & Bannigan, 2008; Messaoudi, Banni, Said, Said, & Kerkeni, 2010).

The present study demonstrated that treatment with *Phoenix dactylifera* fruit extract has a protective effect on histopathological damage induced by Cd in the tissues of testis by maintaining membrane integrity of the reproductive organ, thus, reversed the induced impairment of spermatogenesis. The seminiferous tubules nearly retained their normal architecture which was generalized. The normal spermatogenic columns are almost recognizable. Sertoli cells are capped by tufts of late spermatids attached by their heads in the lumen. Though the interstitial spaces are widen and the spermatogonia resting on a thin basement membrane.

Phoenix dactylifera fruit extract may have achieved this therapeutic effect by inducing the synthesis of Metallothionein by the liver, a cadmium binding protein, which is widely implicated in the sequestration of this metal, and as a result it may prevent the toxic effects of Cd. It is also possible that the phytochemicals contained in MEPD may have had a direct therapeutic effect on the testis. El-Kott, Sayed, El-Sayad and Abdoulrahman (2014) reported that dates contain estradiol and flavonoids that increase sperm health thus improving male reproductive activity. More so, report by Bahmanpour et al. (2006) confirmed that *Phoenix dactylifera* have gonadotrophin-like effects which could be due to its steroidal components. The date phytochemicals genistein (Roberts, Veeramachaneni, Schlaff, & Awoniyi, 2000; Eustache et al., 2009), vitamin A (Bartlett, Weinbauer, & Nieschlag, 1989) and selenium (Jana et al., 2008) have all been reported to protect testicular functions against various stress and possess gonadotropic activity, and the presence of these compounds may have contributed to the observed ameliorating effects recorded here in.

Conclusion

From the results of this finding, it has been observed that cadmium is toxic to testis and suggest that the methanolic extract of *P. dactylifera* fruits can ameliorate the toxic effect of cadmium to the testis by restoring some semen characteristics.

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