International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page:www.iscientific.org/Journal.html

Evaluation of the protective effect of date palm pollen (*Phoenix dactilyfera*) against the reprotoxicity of diethyl phthalate in rabbits (*Oryctolagus cuniculus*)

Y. Bentayeb[a], Y. Moumen[a], H. Hafid[b], S. Boulahbel[a], R. Hadjeres and N. Gherraf[b]

[a]Laboratoire de Biomolécules et Amélioration Des plantes Université Oum Elbouaghi, 04000, Algeria and [b]Laboratoire des Ressources Naturelles et Aménagement des Milieux Sensibles, Université Oum Elbouaghi, 04000, Algeria

Abstract

The aim of this work is to assess the protective effect of date palm pollen (DPP) grains (*Phoenix dactylifera* L.) against the toxicity of phthalate (DEP) (it has a toxic effect on living organisms). The study was performed on adult rabbits for 30 days. The animals were divided into four groups: Group I received distilled water and was taken as a control, while group II, III and IV daily received 240 mg/kg/day of DPP; 330 ml/kg/day of DEP and a combination of both DPP and DEP .After the treatment, the rabbits were sacrificed and the testes were removed and weighed to calculate their relative weight. Blood was collected in heparinized tubes to assess the level of testosterone. The sperm was taken to estimate the reproductive parameters (speed, concentration and mobility of the sperm). The results obtained indicate that the treatment with (DEP) caused a decrease in the weight of the testes, thus a decrease in the testosterone rates and reproductive parameters (speed, concentration and mobility of sperm), compared to the control group. The administration of palm pollen seeds (DPP) showed a positive improvement in all the parameters studied. Our results confirm that DEP can effect changes in reproductive function; however, treatment with DPP can reduce the deleterious effects of DEP.

Keywords: *Phoenix dactylifera*, diethyl phthalate, testicle, testosterone, spermatozoon

Full length article **Corresponding Author*, e-mail:yasmina.bentayeb@yahoo.com

1. Introduction

Phthalates are a group of chemicals widely used in the plastics and solvent industries to make them flexible and soluble [1]. They are environmental pollutants and may generate health risks [2].The dangers of phthalates include ingestion of foods containing phthalates, inhalation of indoor and outdoor air and exposure through the skin by using personal care products containing phthalates [3].Experimental data have shown that certain phthalates can disrupt endocrine function and induce reproductive and developmental toxicity [4]. Diethyl phthalate is one of the most frequently used phthalates in many industrial products [5] and considered to belong to the low molecular weight phthalate groups [6].

It is used as a plasticizer for cellulose acetate and as a solvent and fixative for cosmetic products [7]. It is found in 71% of personal care products, 57% of perfumes and 25% of deodorants [8] and also in different medical devices with different concentrations [9]. It is therefore reasonable to assume that the antioxidants of medicinal plants and their health benefits can prevent or at least reduce the toxicity of DEP, by herbal medicines, among which the date palm pollen grains, which have a long history of use in traditional medicine [10]. Date palm pollen (DPP) is a herbal mixture that is widely used as a folk remedy to cure male infertility in traditional medicine [11]. Almost 1000 tons of DPP are reproduced each year by millions of palm trees grown in Arab regions [12]. They were used by the ancient Egyptians and Chinese as a rejuvenating medicinal agent and was called "fountain of youth" [13]. They are also used worldwide as food supplements, to increase the libido [14]. Moreover, they are regarded as a good nutritional source since they contain flavonoids, carotenoids, alkaloids, steroids, saponins, glycosides, tannins, proteins, amino acids, vitamins, dietary fibers, fatty acids, enzymes, hormones, and minerals [12].

The objective of our work is to evaluate the relative weight of the testes, the level of testosterone and the parameters of reproduction: the speed, the concentration and the mobility of the spermatozoa, after the exposure of the male rabbits to the DEP, and hence to demonstrate the therapeutic role of DPP on these parameters.

2. Materials and methods

2.1. Chemicals and reagents

- Grains of date palm pollen were obtained in the region of Biskra (Southest of Algeria).
- The diethyle phthalate ($C_{12}H_{14}O_4$), called also (ortho-phthalate of diethyl).

2.2. Animals

For this study, we used 16 pubescent male rabbits of the breed (*Oryctolagus cuniculus*).

2.3. Experimental Protocol

The rabbits were randomly divided into four experimental groups of four individuals each. The experiment consisted in administering to rabbits orally doses of diethyl phthalates and grains of palm pollen.

1. Group 1: used as a control.
2. Group 2: treated with 240 mg/kg/day of DPP.
3. Group 3: treated with 330 ml/kg/day of Diethyle phthalate.
4. Group 4: treated with a combination of 330 ml/kg/day of DEP with 240 ml/kg/day of DPP.

After 30 days of treatment the animals were sacrificed and dissected, the testes were removed to estimate their relative weight (organ weight/body weight).

At the time of sacrifice, blood samples were immediately collected in heparinized tubes, and then centrifuged at 4000 rpm/15min, subsequently, the plasma was obtained and stored at -20˚C. The testosterone concentration was estimated in the plasma according to the Enzyme-Linked Immunosorbent Assay (ELISA) method.

2.4. Le spermogramme

The spermogram was carried out according to the WHO 1993 method [15]. By making a small incision at the level of the epididymis in order to obtain a drop of sperm, which has been added to 1 ml of physiological solution (0.9% NaCl) to assess the reproduction parameters (speed, concentration and mobility of sperm). The sample was kept at a temperature of 37˚C for the microscopic analysis of sperm.

2.5. Statistical study

Data were analyzed using software (mintable 16). Statistical analysis was performed by test "t-student" to determine the significance between two means, multiple comparison of means was calculated using analysis of variance a classification criterion (ANOVA),

a) ($P < 0.05$) significant difference.
b) ($P<0.01$) highly significant difference.
c) ($P<0.001$)very highly significant difference

3. Results and discussions

The results showed a slight increase in the weight of the testes in the DPP group compared to the control group T.However, a significant decrease ($p <0.05$) was recorded in the DEP group compared to the control T. DPPDEP group showed an improvement in the weight of the testes compared to the DEP group(fiigure1).

The testosterone concentration showed a significant increase ($p <0.05$) in the DPP group compared to the control group T. On the other hand, a highly significant decrease ($P <0.01$) was recorded in the DEP group compared to the control T, the DPPDEP group showed an improvement in comparison with the DEP group.

Multiple comparison by the test (ANOVA) showed a highly significant difference ($P <0.001$) between the four groups (figure2).

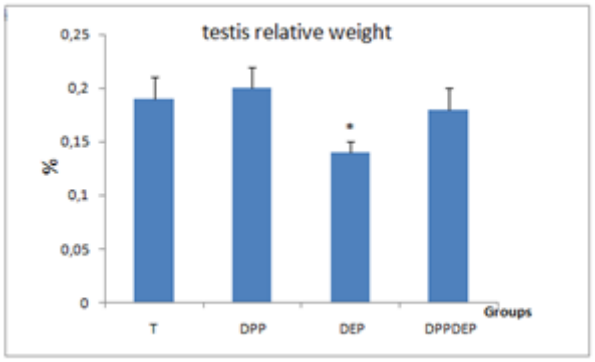

Figure 1: Estimated mean testis relative weight (X ± SD) of male rabbits (*O. cuniculus*) after four weeks of treatment

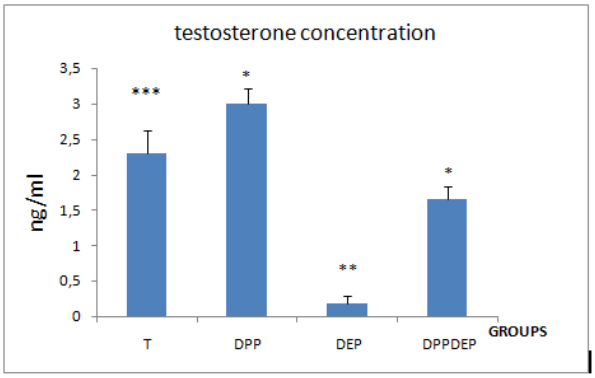

Figure 2: Estimated mean testosterone concentration (mg/ml) (X±SD) of male rabbit (*O. cuniculus*) after four weeks of treatment

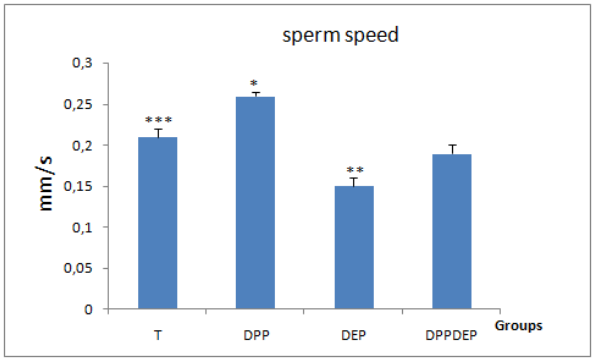

Figure 3: Estimation of the average speed (mm/s) (X±SD) of sperm in the epididymis of the male rabbit *O. cuniculus* after treatment for four weeks

The results showed a significant increase ($p<0.05$) in sperm speed in the DPP group compared to the control group T. however, a highly significant decrease ($P<0.01$) was recorded in the group DEP in comparison with the control T. The DPPDEP group showed an improvement in the speed of the spermatozoa compared to the DEP group. The multiple comparison by the test (ANOVA) showed a very highly significant difference ($P<0.001$) between the four groups (figure3).

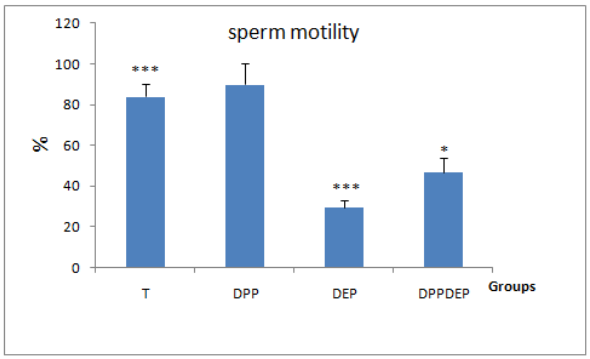

Figure 4: Estimated mean motility (%) (X±SD) of sperm in the epididymis of male rabbit *O. cuniculus* after four weeks of treatment

A slight increase was observed in the DPP group compared to the control group T. However, a very highly significant decrease ($p<0.001$) in sperm mobility was recorded in the DEP group compared to the control T, whereas, the decrease in sperm mobility in the DPPDEP group is moderate compared to the DEP group. The multiple comparison reveals a very highly significant difference ($p<0.001$) (figure4).

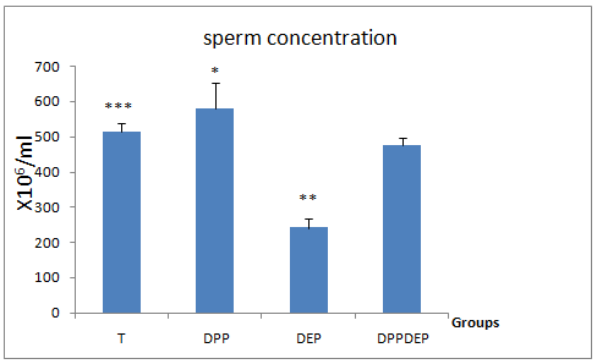

Figure 5: Estimation of the average concentration (X±SD) of spermatozoa (x106/ml) in the epididymis of the male rabbit *O. cuniculus* after four weeks of treatment

The sperm concentration is significantly increased ($p<0.05$) in the DPP group compared to the control group T. While the DEP group reveals a highly significant decrease ($p<0.001$) compared to the control group T. However, an improvement in sperm concentration was recorded in the (DPP+DEP) group compared to the DEP group. The comparison between the four groups reveals a very highly significant difference ($p<0.001$) (figure5).

Our study shows that the oral administration of 330mg/kg/day of DEP, 240mg/kg/day of DPP and the combination of 330mg/kg/day of DEP and 240mg/kg/day of DPP, for 30 days, respectively, to 3 groups of adult rabbits gives effects on the weight evolution, the change in the relative weight of the organs and the parameters of reproduction compared to the control group.

Our results revealed that the treatment with DEP induced a significant reduction ($p<0.05$) in the weight of the testes compared to the control. This reduction could be due to the degenerative effect of DEP on the testicular cells. Our results comparable to the previous study [16], which indicated that administration of (DEP) to rats caused a significant reduction in the weight of the testis. Several studies have reported that phthalates can affect the structure and functions of Sertoli cells [17] and their damage can lead to degeneration of germ cells [18].Other studies have found that (DEP) could cause oxidative stress causing a significant increase in lipid peroxidation (LPO), which leads to high production of reactive oxygen species (ROS) by testicular macrophages. It can affect Leydig cells which are particularly sensitive to ROS due to their proximity to these cells [19], [20]. This shows that oxidative stress can cause the decrease of Leydig cells [21].

We also recorded an enhancement in the relative weight of the testes in the group treated by the association of DPP and DEP compared to the group treated only with DEP. This therapeutic action of DPP in the regulation of the weight of the testes could be due on the one hand to the presence of estrogenic compounds which may be involved in the regulation and renewal of spermatogonia stem cells

[22], and on the other hand, of the increased secretion of fluids by Sertoli cells and the normal production of spermatozoa in the seminiferous tubes [23].We noted a significant increase (P <0.05) in the level of testosterone in the group treated with DPP, which agree with the results of other studies [24]. This increase is due on the one hand to the presence of zinc in (DPP), which is a necessary element for the biosynthesis of this hormone [25] and on the other hand, to the content of DPP in saponins, flavonoids and steroids. These constituents are known as stimulators for leydig cells in order to increase the secretion of testosterone and improving the levels of LH [26].

We also noticed a highly significant reduction (P<0.01) in the testosterone concentration in the group treated with DEP, which could be due to the alteration of the leydig cells. Our results are in agreement with Pereira et al [16], it was reported that DEP causes focal dilation of the smooth endoplasmic reticulum REL and mitochondrial swelling of leydig cells after administration of 2g/kg/day of DEP orally for 2 days [27].This deterioration in the functioning of leydig cells can lead to a decrease in testosterone.

Our results also demonstrate that the supplementation of 240mg/kg/d of DPP to the group treated with 330mg/kg/d of DEP causes an improvement in the secretion of testosterone. This can be explained by the presence of several factors, including mainly the steroid components and gonadotropin-like substances in DPP, which act as stimulators of the gonads by improving fertility and maintaining normal testosterone levels. In addition, the vitamin C present in DPP can also regenerate testosterone molecules up to 58% in-vitro, and is capable of increasing the level of testosterone in Leydig cells, by protecting them against oxidative stress.It acts as an electron donor [28].

In humans and laboratory animals, the evaluation of sperm parameters is considered as a useful indicator to better specify the toxic effects of environmental pollutants on testicular function. Our results prove a significant increase in the characteristics of sperm (mobility, concentration, and speed) in the group treated with DPP compared to the control group. These findings are in accord with those obtained by Al-Sanafi et al 2006 [29] who described an increase in the mobility and speed of sperm in animals treated with DPP. The findings are also similar to those of Batool H. Faleh et al (2006) [30] who found that the treatment of rabbits by DPP cause an increase in sperm concentration. The increase in sperm parameters could be due to the activation of the process of spermatogenesis which depends on the hormones of the hypothalamic-pituitary axis. Another previous study A study by rafah Razooq and al 2017 showed an increase in LH and FSH in rabbit serum after administration by DPP which confirms that the latter acts positively on this axis and therefore on the synthesis and secretion of these hormones [31].

In the present study, the group treated with DEP shows a highly significant reduction (P<0.001) in the mobility of spermatozoa, which is in agreement with a study indicating that the treatment of 234 healthy men with DEP caused decreased mobility of their sperm [32]. A significant decrease in sperm concentration was also seen in the group treated with DEP comparable to what is stated previously in rats exposed to DBP [17]. Likewise, our results show a decrease in the speed of sperm. Moreover, a positive correlation has been shown between MEP which is the metabolite of DEP and the linear speed of movement of sperm [33].

The reduction in sperm parameters could be due to a severe alteration in spermatogenesis caused by DEP which can lead to sterility or oligospermia in rabbits. It can be said that the reduction in the levels of androgens could lead to a severe alteration of spermatogenesis. Similar results stated that the secretion of testosterone is responsible for the activation and maintenance of spermatogenesis [34]. According to (contzen peireira), an increase in lipid peroxidation in the testes of rats is due to the toxic effects of DEP. This increase is accompanied by the increase in ROS and a significant reduction in the activity of antioxidant enzymes in the testes. Superoxide dismutase (SOD), glutathione peroxydas (GPX), glutathione reductase (GR), and antioxidant enzymes are part of the cellular defense against the oxygen species (ROS) [16, 35].However, an increase in the formation of free radicals leads to a deficiency of the antioxidant defense system and then a functional inactivation can occur. Spermatogenesis being a highly replicative process which occurs in the seminiferous tubes of the testes, is therefore more sensitive to oxidative stress [36]. In addition, it has been revealed that LPO is a process of degradation of polyunsaturated fatty acids PUFA and its presence in cell membranes leads to the alteration of the biological membrane [37]. Sperms are considered to be very sensitive to damage induced by oxidative stress due to their high levels of polyunsaturated fatty acids. The increase in LPO has been shown to impede the mobility of sperm so may cause a decrease in their fertilizing potential [38].On the other hand, it was shown that testosterone helps the maturation of elongated round spermatids, which suggests that a decrease in testosterone levels could cause a blockage in the spermatid phase, which can result in a decrease in the number of mature sperm [39]. Contrariwise, the reduction in sperm speed may be due to the decrease in mobility of the sperm which may be the cause of the increased time it takes for the sperm to reach the epididymis.

A prominent improvement in sperm parameters was recorded in the DPP DEP group, which could be due to the positive effect of DPP on the spermatogenesis process by promoting normal sperm production. Similar results were revealed in a study on the protective effect of DPP against testicular toxicity induced by cisplatin [40].These effects

could be attributed to the antioxidant effect of DPP which may be due to its rutin content as well as the tannins which play a very important role in the defense system against oxidative damage caused by free radicals by trapping [41].Alternatively, the flavonoids present in DPP can prevent oxidative damage due to their ability to trap reactive oxygen species such as the superoxide anion, and the hydroxyl radical. The presence of quercetin in the DPP which is a powerful antioxidant is also considered as a free radical scavenger therefore it can protect sperm from damage caused by ROS by inhibiting lipid peroxidation and changing the antioxidant defense pathway in vivo and in vitro [42].In addition, the vitamin C present in the DPP plays a beneficial role on the quality of the sperm, by the increase in the number of sperm, the improvement of the mobility and the morphology of the sperm [43], as it can neutralize the free radicals ROS due to oxidative stress. Several studies reported that vitamin (E) found in DPP which is a powerful antioxidant, helps maintain the stability of cell membranes and scavenge free radicals (ROE)] [44]. In addition, polyphenols which are powerful antioxidants present in DPP can protect cells against free radicals due to oxidative stress, because of having an anti-radical and anti-inflammatory effect [45]. Selenium is a trace element present in DPP which plays an important role in the physiology of the male reproductive system. It is present in high concentrations in the testis [46], and is an essential component of the enzyme (glutathione peroxidase) [47]. In addition, the primordial role of estrogens, especially 17B estradiol, synthesized by Sertoli, Leydig and germ cells in the process of spermatogenesis has been shown recently. The presence of phytoestrogens in the DPP, as steroid components can positively influence the parameters of the sperm by their action on the reabsorption of luminal fluid in the head of the epididymis [48].This function avoids the entry of the sperm in the epididymis in diluted form, suggesting an increase in sperm concentration (the total number of sperm) [49].

4. Conclusions

In conclusion, our results show that oral treatment for one month with a dose of 330 ml/kg/day of diethyl phthalate (DEP) can lead to toxic effects on the male reproductive system, and that the addition of DPP at240 mg/kg/day for the same period can effectively reduce the deleterious effects of (DEP). This confirms the therapeutic action of (DPP).

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