

Effect of Date Palm Pollen (DPP) on Development of Reproductive Organs and their Relative Tissue Body Weight Indices in Prepubertal Male Rats – A Potential Remedy for Hypogonadism in males

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ABSTRACT

Background: Date Palm Pollen (DPP) is suggested to increase the concentration of testosterone, follicle stimulating hormone, and luteinizing hormone in rats.

Aim: To examine its effect on body weight and weight of testosterone dependent sex organs using prepubertal rats as an experimental model with a hope that the results of this study may pave the way for treating male hypogonadism and delayed puberty.

Methods: 4-day old rats were randomly divided into four groups (Control I, Experimental I, Control II, and Experimental II). Experimental groups I and II were given DPP suspension, in a single oral dose of 120mg/kg daily for 18 and 35 days respectively.

Results: At the end of the experiment pups were weighed and sacrificed on day 22nd and 39th post-natally; their testes, liver, ventral prostate and seminal vesicles were removed and weighed. There was no statistically significant difference in the variables of Control and Experimental groups I. Pups who received DPP for 35 days showed statistically significant increase in mean body weight, seminal vesicle, ventral prostate and paired testes weight also significantly increased from 89.25±2.00g, 0.0354±0.0039g, 0.0539±0.0035g and 0.75±0.01g in the Control group II respectively to 98.91±6.28g, 0.0417±0.0057g, 0.0611±0.0055g and 0.85±0.08g in Experimental Group II. There was no significant effect on the mean relative tissue body weight indices of both the groups except for that of RTWI (Liver) which decreased implying no change in the weight of liver in group II but increased body weight.

Conclusion: Our data regarding the effects of Date Palm Pollen on reproductive organs implied that it had a complex stimulating effect on size of the organs, producing their precocious development suggestive of its androgenic activity.

Keywords: Date palm Pollen, testes, ventral prostate, seminal vesicles, body weight

INTRODUCTION

Throughout the world 50-80 million couples suffer from infertility. Male factors are thought to be the major cause of infertility in 30% of cases and to contribute to 50% of cases over all. However, this number does not accurately represent all regions of the world (Agarwal et al., 2015, Organization, 1991). Male reproductive system is complex and involves the testes, epididymis, vas deferens, accessory sex glands, and associated hormones (Yakubu, 2012, Odusoga et al., 2014). Factors like diabetes, bronchiectasis, high grade fever, long term medication, urinary tract infection, sexually transmitted infection, epididymitis, testicular injury, un-descended testis, mumps, orchitis, excessive alcohol, smoking, exposure to heat and certain chemicals effect spermatogenesis (Pant, 2010). The idiopathic causes of infertility are oligospermia, asthenozoospermia, teratozoospermia, azospermia etc. Others are obstructive azospermia, isolated seminal abnormalities, sexual or ejaculatory dysfunction and delayed puberty (Jungwirth et al., 2017). Androgens play a

key role in the development of male reproductive system. Chemicals, like those found in majority of pesticides, alter the function of androgens resulting in disruption or delay in this development (Blystone et al., 2007) Inability of the testis to produce physiological levels of testosterone due to a perturbation occurring at one or more levels of the hypothalamic-pituitary-testicular axis leads to clinical syndrome of androgen deficiency (Watcho et al., 2017). In milder cases of delayed puberty, treatment is often not required; however, considerable evidence exists for the efficacy and safety of short courses of low-dose testosterone therapy for appropriately selected individuals, this treatment is associated with high levels of patient satisfaction (Ambler, 2009). Exogenous testosterone effects androgen-dependent tissue weights; for example, seminal vesicle and paired testes weights (Howdeshell et al., 2007). There is an undeniable role of androgens in sexuality. They play a critical role in the development of male external genitalia, secondary sexual characters and regulation of erectile response (Soliman, 2014). At early developmental stage, reduction in testosterone levels manifests as lack of virilization, incomplete sexual development, aspermia and hindrance in pubertal growth spurt. In adulthood it results in loss of libido (Arver and

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Lehtihet, 2009). Androgen-receptor antagonists, significantly delay development of androgen-sensitive organs (Kelce et al., 1995).

Date palm pollen (DPP) has long been used as a dietary supplement to increase libido and ameliorate fertility in both women and men. It is suggested that it increases the concentration of testosterone, follicle stimulating hormone, and luteinizing hormone in rats (Iftikhar et al., 2011, Abdi et al., 2017). Date pits have been included in animal feed to enhance growth, an action that has been ascribed to an increase in the plasma level of estrogens or testosterone (Jassim and Naji, 2010).

As DPP reportedly increases testosterone levels, in the present study its effects were observed on testosterone dependent reproductive organs and pubertal development was evaluated using the male prepubertal rats.

MATERIALS AND METHODS

This study was carried out at the University of Health Sciences (UHS), Lahore, Pakistan.

Preparation of DPP Powder and Method of Administration: Date Palm Pollen was grounded into powdered form. It was mixed in distilled water till it appeared homogenous. The dose was adjusted daily according to the body weight of the pups during the course of treatment and administered orally.

Parameters Studied

- Body Weight
- Weight of Reproductive Organs
- Weight of Liver
- Relative Tissue Weight Index (RTWI)

$$RTWI = \text{weight of organ (g)} \div \text{body weight (g)} \times 100$$

Experimental Procedure: Twelve female and four male adult albino rats were kept for two weeks in the animal house of University of Health Sciences to acclimatize. One male and three female rats were housed together in a single cage for mating. Pregnancy was confirmed by observing vaginal plug in the morning after keeping the female and male rats together (Song et al., 2015). Rats were monitored at 8-hour interval to observe the time they deliver (Dhungel et al., 2006). Neonates were born after 21 days; they were kept with their mothers and examined for any congenital anomaly. Each of the 48 male neonates so obtained was given identification mark and divided randomly into following four groups of 12 pups each.

Control I: Received equal amount of distilled water as experimental animals daily for 18 days starting at 4th day of age.

Control II: Received equal amount of distilled water as experimental animals daily for 35 days starting at 4th day of age.

Experimental I: Received 120mg/kg body weight of DPP suspension (Bahmanpour et al., 2006) as a single oral dose daily for 18 days starting at 4 days of age (Picó et al., 2007).

Experimental II: Received 120mg/kg body weight of DPP suspension (Bahmanpour et al., 2006), as a single oral dose daily for 35 days starting at 4 days of age (Picó et al., 2007). Each group was kept at controlled room temperature (22±2°C) and humidity of 55±10% (Sato et al., 2005). They were kept under natural light and dark cycle.

All pups were fed on mother's milk and gradually weaned to normal rat chow and water ad libitum. The mothers were, however, fed ad libitum on normal rat chow and water. 4-day old rats were selected since the experiment consumed 35 days in total and rats normally attain puberty from 40-50 days (Roy et al., 1983, Robb et al., 1978). The pups of Control I and Experimental I groups were weighed and sacrificed on day 22 post-natally after 18 days of treatment to determine if DPP affected the above mentioned parameters in the middle of the experimental period, and Control II and Experimental II were weighed and sacrificed on day 39 post-natally after 35 days of treatment (Bahmanpour et al., 2006)

Weight of Animals and dissection: At the end of the Experimental periods, each animal was weighed and anaesthetized by putting it in plastic container with chloroform soaked cotton wool (Umoh and Ekanem, 2017). The lid of the container was closed tightly till the animal was completely anaesthetized. The rat was then removed from the container and laid on its back upon a clean paper towel. A vertical midline skin incision was given from xyphoid to symphysis pubis; it was extended laterally by a transverse incision on each side of the midline. The skin was reflected laterally and abdomen was opened using a pair of sterile scissors; both skin and muscles were removed. The testes were retractable; they were pushed forward into the body cavity and removed by pulling the tails of the epididymides along with their head and body, vas deferens and spermatic blood vessels. The blood vessels and vas deferens were severed allowing removal of the testes and the epididymides. The lumpy brown glands located to the right and left of urinary bladder were the seminal vesicles. They along with the ventral prostate were removed. Whole liver was also removed by ligating all its ligaments. Weight of paired testes, epididymides, liver, seminal vesicles and ventral prostate from each animal was recorded in grams using electronic balance.

Statistical Analysis: The statistical analysis was carried out using SPSS version 18.0. The arithmetic mean of observations and standard deviation values were calculated; two independent samples t-tests were applied to observe differences in mean values. The difference was regarded statistically significant if the 'p' value was < 0.05.

RESULTS AND DISCUSSION

Table 1. Comparison of Mean Body Weight (g) of Animals among Experimental and Control Groups I after 18 days therapy.

Parameter	Control Group I (12) Mean ± SD	Experimental Group I (12) Mean ± SD
Body Weight	25.41±2.57	25.00±2.55

P value 0.695

Table 2. Comparison of Mean Body Weight (g) of Animals among Experimental and Control Groups II after 35 days therapy.

Parameter	Control Group II (12) Mean ± SD	Experimental Group II (12) Mean ± SD
Body Weight	89.25±2.00	98.91±6.28

P value <0.001

Table 3. Comparison of Mean Weight of Paired Testes (g) of Animals among Experimental and Control Groups I after 18 days Therapy.

Parameter	Control Group I (12) Mean ± SD	Experimental Group I (12) Mean ± SD
Weight of Paired Testes	0.23±0.02	0.24±0.02

P value 0.186

Table 4. Comparison of Mean Weight of Paired Testes (g) of Animals among Experimental and Control Groups II after 35 days therapy.

Parameter	Control Group II (12) Mean ± SD	Experimental Group II (12) Mean ± SD
Weight of Paired Testes	0.75±0.01	0.85±0.08

P value <0.001

Table 5. Comparison of Mean Relative Tissue Body Weight Indices (Testes) of Animals among Experimental and Control Groups I after 18 days Therapy.

Parameter	Control Group I (12) Mean ± SD	Experimental Group I (12) Mean ± SD
Relative Tissue Body Weight Index	0.92±0.08	0.98±0.10

P value 0.093

Table 6. Comparison of Mean Relative Tissue Body Weight Indices (Testes) of Animals among Experimental and Control Groups II after 35 days therapy.

Parameter	Control Group II (12) Mean ± SD	Experimental Group II (12) Mean ± SD
Relative Tissue Body Weight Index	0.85±0.02	0.86±0.03

P value 0.202

Table 7. Comparison of Mean Weight of Liver (g) of Animals among Experimental and Control Groups I after 18 days therapy.

Parameter	Control Group I (12) Mean ± SD	Experimental Group I (12) Mean ± SD
Weight of Liver	1.17± 0.05	1.21±0.04

P value 0.083

Table 8. Comparison of Mean Weight of Liver (g) of Animals among Experimental and Control Groups II after 35 days therapy.

Parameter	Control Group II (12) Mean ± SD	Experimental Group II (12) Mean ± SD
Weight of Liver	5.8± 0.30	5.8±0.36

P value 0.672

Table 9. Comparison of Mean Relative Tissue Body Weight Indices (Liver) of Animals among Experimental and Control Groups I after 18 days Therapy.

Parameter	Control Group I (12) Mean ± SD	Experimental Group I (12) Mean ± SD
Relative Tissue Body Weight Index	4.64±0.32	4.87±0.53

P value 0.216

Table 10. Comparison of Mean Relative Tissue Body Weight Indices (Liver) of Animals among Experimental and Control Groups II after 35 days Therapy.

Parameter	Control Group II (12) Mean ± SD	Experimental Group II (12) Mean ± SD
Relative Tissue Body Weight Index	6.49±0.33	5.92±0.37

P value 0.001

Table 11. Comparison of Mean Weight Seminal Vesicles (g) of Animals among Experimental and Control Groups I after 18 days of Therapy.

Parameter	Control Group I (12) Mean ± SD	Experimental Group I (12) Mean ± SD
Weight of Seminal Vesicles	0.0085±0.0003	0.0084±0.0004

P value 0.592

Table 12. Comparison of Mean Weight Seminal Vesicles (g) of Animals among Experimental and Control Groups II after 35 days Therapy.

Parameter	Control Group II (12) Mean ± SD	Experimental Group II (12) Mean ± SD
Weight of Seminal Vesicles	0.0354±0.0039	0.0417±0.0057

P value 0.005

Table 13. Comparison of Mean Relative Tissue Body Weight Indices (Seminal Vesicles) of Animals among Experimental and Control Groups I after 18 days Therapy.

Parameter	Control Group I (12) Mean ± SD	Experimental Group I (12) Mean ± SD
Relative Tissue Body Weight Index	0.0338±0.0029	0.0340±0.0023

P value 0.816

Table 14. Comparison of Mean Relative Tissue Body Weight Indices (Seminal Vesicles) of Animals among Experimental and Control Groups II after 35 days Therapy.

Parameter	Control Group II (12) Mean ± SD	Experimental Group II (12) Mean ± SD
Relative Tissue Body Weight Index	0.0398±0.0040	0.0421±0.0037

P value 0.168

Table 15. Comparison of Mean Weight Ventral Prostate (g) of Animals among Experimental and Control Groups I after 18 days Therapy.

Parameter	Control Group I (12) Mean ± SD	Experimental Group I (12) Mean ± SD
Weight of Ventral Prostate	0.0184±0.0008	0.0185±0.0007

P value 0.767.

Table 16. Comparison of Mean Weight Ventral Prostate (g) of Animals among Experimental and Control Groups II after 35 days Therapy.

Parameter	Control Group II (12) Mean ± SD	Experimental Group II (12) Mean ± SD
Weight of Ventral Prostate	0.0539±0.0035	0.0611±0.0055

P value 0.001

Table 17. Comparison of Mean Relative Tissue Body Weight Indices (Ventral Prostate) of Animals among Experimental and Control Groups I after 18 days Therapy.

Parameter	Control Group I (12) Mean \pm SD	Experimental Group I (12) Mean \pm SD
Relative Tissue Body Weight Index	0.0728 \pm 0.0054	0.0744 \pm 0.0057

P value 0.481.

Table 18. Comparison of Mean Relative Tissue Body Weight Indices (Ventral Prostate) of Animals among Experimental and Control Groups II after 35 days Therapy.

Parameter	Control Group II (12) Mean \pm SD	Experimental Group II (12) Mean \pm SD
Relative Tissue Body Weight Index	0.0604 \pm 0.0032	0.0617 \pm 0.0028

P value 0.281

In our study different parameters analyzed above were assessed to see the effect of DPP suspension (120mg/kg) given orally for 18 and 35 days in premature albino rats. Our data showed that there was no statistically significant difference in any of the observed parameters after 18 days of treatment with Date Palm Pollen suspension in Control and Experimental groups (Table 1, 3, 5, 7, 9, 11, 13, and 15). Milsap and Jusko (1994) suggested that processes controlling the absorption, distribution, metabolism, excretion, and pharmacologic effects of drugs are likely to be immature or altered in neonates. This also agrees with Schwark (1992) stating that drugs administered to neonatal animals may exhibit significantly different pharmacokinetic/disposition characteristics than they do in adult animals of the same species. Therefore, this may be due to age-related differences in maturation pathways responsible for drug absorption, distribution, metabolism and excretion (Espandiani et al., 2010, Zhao et al., 2016), or treatment with DPP took somewhere between 18 and 35 days to produce its effects, this agrees with a study carried out by Bahmanpour et al. (2006) where they observed the effect of *Phoenix Dactylifera* pollen on sperm parameters and reproductive system of adult male rats after 35 days of treatment. A significant increase was observed in the body weight, weight of the paired testes, seminal vesicles and ventral prostate of the animals in the Experimental group II, treated with same dose of DPP Suspension for 35 days. There was no significant change in the weight of liver in Control and Experimental groups I and also in Control and Experimental groups II respectively (Table 7 and 8), indirectly being an evidence that increase in the weight of the sex organs in Experimental group II might be due to the presence of gonadotropin like substances in the DPP, as it had no influence on an organ that is not dependent on sex hormones for growth (Azooz et al., 2001). Oral administration of DPP suspension resulted in increased body weight from 89.25 \pm 2.00g in the Control group II to 98.91 \pm 6.28g in the Experimental group II, their *p* value being <0.001 (Table 2). This weight gain in the treated group II may partly be attributed to the androgenic effects of testosterone as its levels increased; this is in concert with the study carried out by Gauthaman et al. (2002), they reported that androgens have a major role in the growth and differentiation of many tissues in addition to the organs

of reproduction; testosterone is the main hormone having nitrogen-retaining (anabolic) properties which increases lean body mass and body weight. The testicular weight in the treated group II was also increased significantly. The weight of the paired testis of Control group II was 0.75 \pm 0.01g, whereas in the Experimental group II it was 0.85 \pm 0.08g with a *p*< 0.001 (Table 4). Oral administration of DPP suspension resulted in increased wet weight of seminal vesicles and ventral prostate from 0.0354 \pm 0.0039g and 0.0539 \pm 0.0035g in the Control group II respectively to 0.0417 \pm 0.0057g and 0.0611 \pm 0.0055g in the treated group II, their *p* values being 0.005 and 0.001 (Table 12, Table 16). These findings are also in accordance with Bahmanpour et al. (2006) where they observed weight gains in epididymis, testes and seminal vesicles after DPP administration. The increase in weight of ventral prostate is not in keeping with their observations as they observed no change in the weight of ventral prostate. This might be due to the difference in the age and the process of development in our experimental model, as they used adult rats and we had prepubertal rats with a maximum age of 39 days when they were sacrificed. However, the increase in the weight of ventral prostate is in accordance with Azooz et al. (2001), whereby they observed that administration of testosterone revived the reduced weights of seminal vesicles and ventral prostate in rat model of colitis, again supporting the evidence of increased testosterone being responsible for this weight gain. Our results also agree with the findings of Gauthaman et al. (2002) who studied the aphrodisiac properties of *Puncture Vine* extract (Protodioscin) in normal and castrated rats; their study showed that treatment of castrated rats with their extract resulted in increased body and prostate weight; they implied that it increased testosterone levels in rats. Androgens are also responsible for the pubertal development of the testes (Bagatell and Bremner, 1996). Thus the androgenic activity of DPP could have possibly produced enhanced pubertal development of testes and an increase in the body, testicular, seminal vesicle and prostate weight. Date Palm Pollen suspension given orally at a dose of 120mg/kg for 35 days resulted in an increase in body, seminal vesicle, ventral prostate and testicular weight without significant effect on relative tissue body weight indices (Table 6, 14, 18) implying that the increase in body weight and weight of these organs was proportionate. Moreover there was no significant effect on relative tissue body weight indices of liver after 18 days therapy (Table 9) however it was significant after 35 days of therapy (Table 10) indicating that DPP had no effect on liver (a non-androgen dependent organ).

CONCLUSION

Date Palm Pollen suspension given orally at a dose of 120mg/kg for 35 days resulted in an increase in body, seminal vesicle, ventral prostate and testicular weight without significant effect on relative tissue body weight indices. Our data regarding the effects of Date Palm Pollen on reproductive organs implied that it had a complex stimulating effect on size of the organs, producing their precocious development suggestive of its androgenic activity.

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