

Effect of Date Palm Pollen (DPP) on Serum Testosterone Levels in Prepubertal Albino Rats

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ABSTRACT

Date Palm Pollen (DPP) is suggested to increase the concentration of testosterone, follicle stimulating hormone, and luteinizing hormone in rats. We, therefore, planned this study to examine its effect on Serum Testosterone and body weight using prepubertal rats. 4-day old rats were divided into four groups of 12 pups each (Control I, Experimental I, Control II, and Experimental II). Experimental groups I and II were given (Date Pal Pollen (DPP) suspension, in a single oral dose of 120mg/kg daily for 18 and 35 days respectively. At the end of the experiment blood from each animal was drawn by cardiac puncture for serum testosterone levels. Pups were weighed and blood was collected through cardiac puncture on day 22nd and 39th post natally. 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 serum testosterone levels as compared to Control group. Their mean body weight also significantly increased from 89.25±2.00g; in the Control group II to 98.91±6.28g in the Experimental group II. Date Palm Pollen suspension given orally at a dose of 120mg/kg for 35 days resulted in an increase serum testosterone levels with concurrent increase in body weight.

Key word: Date Palm pollen, Testosterone, Body weight, Testosterone Replacement Therapy.

INTRODUCTION

Recent years have witnessed a resurgence of interest in exploring the anabolic applications of androgens, particularly to reverse the sarcopenia associated with the human immunodeficiency virus, cancer, aging, and other chronic illnesses. Replacement doses of testosterone increase body weight, primarily by increasing fat-free mass.¹ Moreover, many of the symptoms of hypogonadism can be improved by injectable testosterone esters, which have formed the cornerstone of treatment since the 1950s. Male hypogonadism can cause significant morbidity and substantial reduction in quality of life².

Even in the females androgens are essentially prohormones for other steroids including estrogens. Androgens influence sexual desire, bone density, muscle strength and mass, mood, energy and psychological well-being³. Testosterone replacement therapy improves mood in hypogonadal men⁴.

Hyperinsulinaemia and insulin resistance are antecedents to clinically established type 2 diabetes. Insulin resistance is also an essential component of the metabolic syndrome, which is defined as the presence of three or more of the following factors: central obesity, hypertriglyceridaemia, low high-density lipoprotein (HDL), hypertension and a raised fasting blood glucose⁵. Studies in healthy men have shown an inverse relationship between total testosterone levels and insulin concentrations.⁶ Low testosterone levels in men have been found to predict insulin resistance and the future development of type 2 diabetes.⁷ Testosterone therapy is mainly indicated in Adult hypogonadism, delayed puberty sarcopenia, female-to-male transexualism, osteoporosis and cardiometabolic disorders.⁸ Testosterone is much more than a sex hormone. There are testosterone receptor sites in cells throughout the body, most notably in the brain and heart. Youthful protein synthesis for maintaining muscle mass and bone formation requires testosterone. Testosterone improves oxygen uptake throughout the body, helps control blood sugar, regulate cholesterol and maintain immune surveillance. The body requires testosterone to maintain youthful cardiac output and neurological function⁹.

The beneficial health and nutrition values of *Phoenix dactylifera L.* for human and animal consumption have been claimed for centuries.¹⁰ Experimentally, date extracts have been shown to increase sperm count in guinea pigs and to enhance

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spermatogenesis and increase the concentration of testosterone, follicle stimulating hormone (FSH), and luteinizing hormone (LH) in rats¹¹.

The effect of DPP is studied in adult male rats; the present study was therefore designed to see the effect of Date Palm Pollen on the testosterone levels in prepubertal rats to validate an easier and alternative source for increasing the testosterone levels in the prepubertal subjects where replacement of testosterone is the necessary mode of treatment.

MATERIAL AND METHODS

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

Preparation of the herbal cocktails: Date Palm Pollen was obtained from Dera Ghazi Khan District of Punjab, through University of Health Science, Pakistan and was grounded into powdered form in the Pharmacology laboratory, UHS. It was mixed in distilled water to form a suspension. The dose was adjusted daily according to the body weight of the pups during the course of treatment and administered orally.

Sample Size: A total of 48 prepubertal male albino rats weighing 5 g were divided into four groups, each group consisting of 12 rats. Rats were kept in the experimental research laboratory of UHS.

Parameters Studied

Body Weight: It is suggested that DPP has some androgenic activity and testosterone is known to increase the lean body mass and weight, thus body weight of the experimental and control animals were monitored.

Serum Testosterone Levels: Due to proposed androgenic activity of DPP, serum testosterone levels were measured.

Experimental Procedure: Twelve female and four male adult albino rats were procured from National Institute of Health, Islamabad and were kept for two weeks in Experimental research laboratory of University of Health Sciences to acclimatize them. 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.¹² Rats were monitored at 8-hour interval to observe the time they deliver.¹³ 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,¹⁴ as a single oral dose daily for 18 days starting at 4 days of age¹⁵.

Experimental II: Received 120mg/kg body weight of DPP suspension¹⁴, as a single oral dose daily for 35 days starting at 4 days of age¹⁵. Each group was kept at controlled room temperature (22±2°C) and humidity of 55±10%.¹² 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.

The pups of Control I and Experimental I groups were weighed and their blood was drawn on day 22nd 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 their blood was drawn on day 39th post-natally after 35 days of treatment.¹⁴

Weight of Animals and Collection of Blood

Samples: At the end of the Experimental periods, each animal was weighed and anaesthetized by putting it in plastic container with chloroform soaked cotton wool¹⁶ 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. 2 ml blood was drawn in 5 ml disposable syringe by cardiac puncture¹⁷. It was allowed to stand for one hour before centrifuging it at a speed of 3000r/pm for 10 minutes. The clear serum was collected with the help of a clean dropper in a sterilized disposable plastic tube for testing at a later date: the tube was properly labeled before placing in a freezer set at -20°C. Serum testosterone levels were measured by using commercially available Testosterone Enzyme Immunoassay Test Kit prepared by Bio Check Company.

Statistical Analysis: The statistical analysis was carried out using computer software Statistical Package for Social Sciences (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

All animals of Experimental and Control groups at the time of taking blood sample were active and healthy; there was no morbidity or mortality among the groups; their suckling/feeding behaviour was normal and showed no sign of any ailment.

Body Weight (18 days Therapy): In the Control group I, body weight of animals varied from 20g to 30g with an average of 25.41 ± 2.57 g; in the treated group I it ranged from 22g to 29g with the average of 25.00 ± 2.55 g. Difference in the body weight of animals in Control group I and Experimental group I, was not statistically significant $p=0.695$ (Table 1).

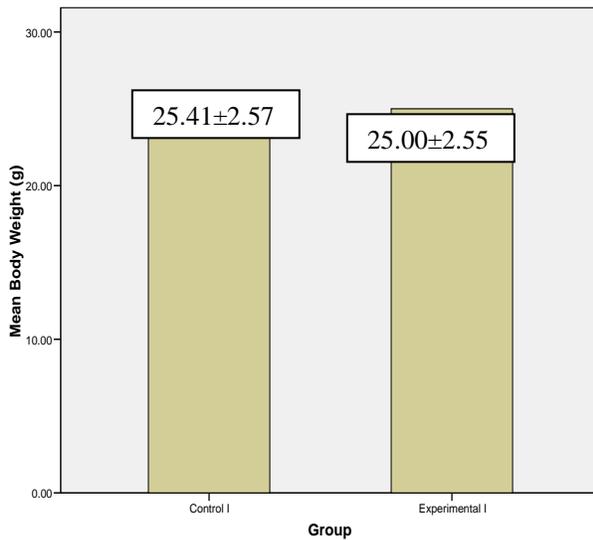


Fig. 1: Mean Body Weight (g) of Animals of Control I and Experimental I Groups after 18 days Therapy.

Table 1: Comparison of Mean Body Weight (g) of Animals among Experimental and Control Groups I after 18 days therapy (P value: 0.695)

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

Figure in parenthesis indicate total number of animals in each group.

Body Weight (35 days Therapy): In the Control group II, body weight of animals varied from 87g to 93g with an average of 89.25 ± 2.00 g; in the treated group II it ranged from 89g to 107g with the average of 98.91 ± 6.28 g. Difference in the body weight of animals in Control group II and Experimental group II was statistically significant $p < 0.001$ (Table 2).

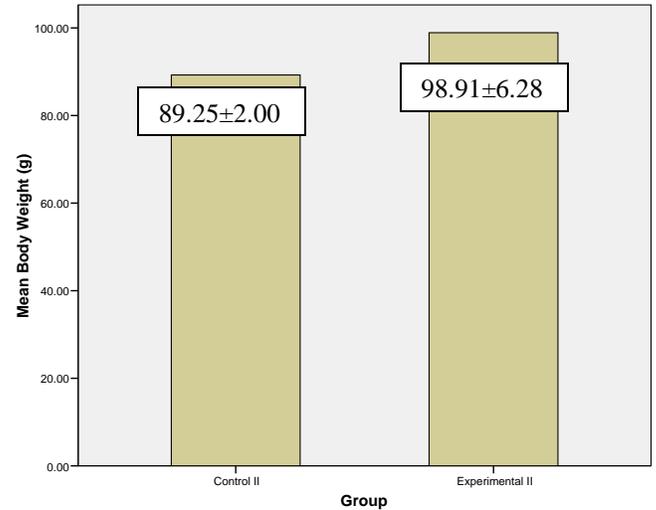


Fig. 2: Mean Body Weight (g) of Animals of Control II and Experimental II Groups after 35 days Therapy.

Table 2: Comparison of Mean Body Weight (g) of Animals among Experimental and Control Groups II after 35 days therapy (P value: < 0.001)

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

Figure in parenthesis indicate total number of animals in each group.

Serum Testosterone Levels (18 days Therapy) : In the animals of Control group I, serum testosterone levels varied from 0.27ng/ml to 0.39ng/ml with an average of 0.32 ± 0.04 ng/ml; in the treated group I it ranged from 0.26ng/ml to 0.38ng/ml with the average of 0.32 ± 0.04 ng/ml. Difference in serum testosterone levels of animals of Control and Experimental groups I was not statistically significant $p=0.916$ (Table 23).

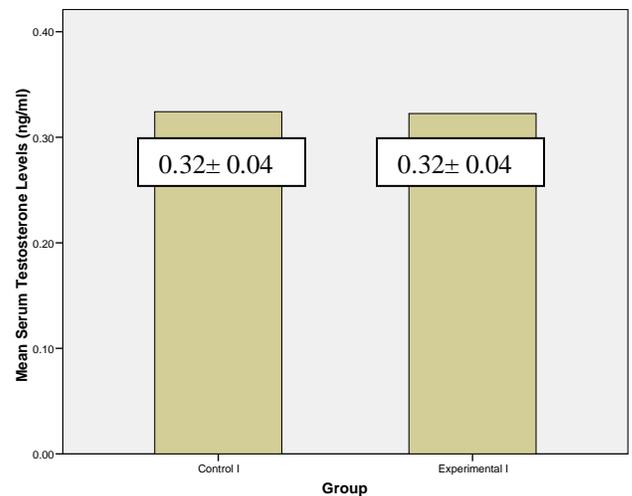


Fig.3. Mean Serum Testosterone Levels (ng/ml) of Animals of Control I and Experimental I Groups after 18 days Therapy.

Table 3. Comparison of Mean Serum Testosterone Levels (ng/ml) of Animals among Experimental and Control Groups I after 18 days Therapy (P value: 0.916)

Parameter	Control Group I (12) Mean ± SD	Experimental Group I (12) Mean ± SD
Serum Testosterone Levels	0.32± 0.04	0.32± 0.04

Figure in parenthesis indicate total number of animals in each group.

Serum Testosterone Levels (35 days Therapy): In the animals of Control group II, serum testosterone levels varied from 0.75ng/ml to 0.95g/ml with an average of 0.82± 0.06ng/ml; in the treated group II it ranged from 0.90ng/ml to 1.12ng/ml with the average of 0.99± 0.64ng/ml. Difference in serum testosterone levels of animals of Control and Experimental groups II was statistically significant p<0.001 (Table 24).

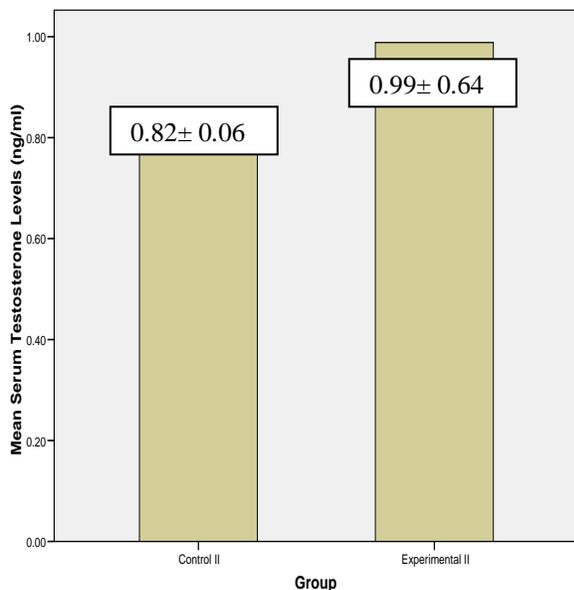


Fig.4. Mean Serum Testosterone Levels (ng/ml) of Animals of Control II and Experimental II Groups after 35 days Therapy.

Table 4. Comparison of Mean Serum Testosterone Levels (ng/ml) of Animals among Experimental and Control Groups II after 35 days Therapy (P value: <0.001)

Parameter	Control Group II (12) Mean ± SD	Experimental Group II (12) Mean ± SD
Serum Testosterone Levels	0.82± 0.06	0.99± 0.64

Figure in parenthesis indicate total number of animals in each group.

DISCUSSION

In our study the 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 I. Millsap RL and Jusko WJ in 1994 suggested that processes controlling the absorption, distribution, metabolism, excretion, and pharmacologic effects of drugs are likely to be immature or altered in neonates¹⁸. This also agrees with Schwark W.S (1992), who stated that drugs administered to neonatal animals may exhibit significantly different pharmacokinetic/disposition characteristics than they do in adult animals of the same species¹⁹. Therefore, this may be due to age-related differences in maturation pathways responsible for drug absorption, distribution, metabolism and excretion²⁰ or treatment with DPP took somewhere between 18 and 35 days to produce its effects, this agrees with a study carried out by Bahmanpour S, et al (2006)¹⁴, where they observed the effect of *Phoenix Dactylifera* pollen on sperm parameters and reproductive system of adult male rats after 35 days of treatment.

Oral administration of DPP suspension resulted in increased body weight from 89.25±2.00g in the Control group II to 98.91±6.28g in the Experimental group II, their p value being <0.001. 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 K 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 weight²¹.

It is suggested that DPP suspension has some androgenic activity as a significant increase in the serum testosterone levels was observed in the treated group II, where DPP suspension was given for 35 days. The serum testosterone levels in the Control group II were 0.82± 0.06 and in the treated group II they increased to 0.99± 0.64 with p-value <0.001. A significant increase in the serum testosterone levels in our study is in concert with Bahmanpour S et al (2006), where they observed similar increase in the testosterone levels of mature albino rats after administration of DPP suspension.¹⁴ A study was carried out by Beckk V et al. in 2005, where they stated that the benefits of plant extracts

from soy and red clover as alternatives to conventional hormone replacement therapy (HRT) have been debated in the past and they suggested phytoestrogens derived from red clover as an alternative to estrogen replacement therapy.²² This supports our suggestion to use DPP as an alternative source for the conditions where testosterone replacement is indicated. A similar study carried out by Adimoelja A in 2000 suggested that the use of *Tribulus terrestris* (puncture wine) also results in an increase of LH levels by 72%, and free testosterone levels by 41%, enhancing sperm quality and mobility, and for increasing libido and sexual performance in experimental animals and men²³. Yakubu, M. T et al., in their study using a herb "Fadogia agretis" suggested that it may be used to modify impaired sexual functions in animals, especially those arising from hypotestosteronemia. They attributed this effect to the ability of the aqueous extract of the stem of *Fadogia agretis* to increase serum testosterone concentration and suggested it to be the mechanism responsible for its aphrodisiac effect and various masculine behaviors.²⁴ Another study carried by Bawazir A.E suggested *Talbina* (barley water) increased the plasma levels of testosterone which had a positive correlation with increased active spermatogenesis and significant rise of number of mature sperms. He concluded that *Talbina* is beneficial for male reproductive activity²⁵.

The Experimental animals had normal food and water intake and were active without any sign of ill health; there was no mortality seen in either of the four groups; the Experimental animals in treated group II weighed more than those of the Control indicating that Date Palm Pollen administration did not have any negative effect on the survival of experimental animals this is consistent with Al-Qarawi AA et al (2004) and Bahmanpour S et al (2006), who did not report any mortality or morbidity in their studies on Date Palm Pollen^{14,26}.

CONCLUSION

Date Palm Pollen suspension given orally at a dose of 120mg/kg for 35 days resulted in an increase serum testosterone levels with concurrent increase in body weight. It is suggested that it may be used as an alternative or adjunctive/supplementary source of increasing the serum testosterone levels in conditions where testosterone replacement therapy is indicated in adults as well as prepubertal subjects.

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