

*Full Length Research Paper*

## Effect of date palm pollen on serum testosterone and intra-testicular environment in male albino rats

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Date palm pollen (DPP) is suggested to increase the concentration of serum testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) in rats. Intra-testicular testosterone (ITT) is thought to play a key role in the control of spermatogenesis but is rarely measured. We therefore planned this study to examine its effect on intra-testicular testosterone levels and serum testosterone in male albino rats. Adult rats were divided into two groups, control and experimental, with 12 rats in each. Blood samples were drawn for analysis from tail vein at day 0, 12, 24 and 35. The experimental group was given DPP suspension in a single oral dosage of 120 mg/kg daily, starting at day 1, for 35 days (dose was confirmed by a pilot study). At the end of the experimental period, the rats were dissected and the testes were removed and weighed. Testes were minced in phosphate buffer solution at a proportion of 1:10. The tissue homogenate obtained was cold centrifuged. The supernatant was taken for the estimation of ITT levels. There was a statistically significant increase in serum testosterone levels at day 24 which increased from (mean  $\pm$  SD)  $1.81 \pm 0.05$  to  $2.12 \pm 0.13$  and from  $1.82 \pm 0.05$  to  $3.09 \pm 0.22$  on day 35, and ITT levels were raised from  $76.48 \pm 2.4$  to  $129.90 \pm 9.66$  on day 35 in experimental groups as compared to control group. The results of the present study demonstrate that date palm pollen is involved in the increased reproductive activity and serum levels of testosterone and intra-testicular testosterone.

**Key words:** Date palm pollen, serum testosterone, intra-testicular testosterone, infertility.

### INTRODUCTION

Infertility affects 13 to 18% of married couples. Growing evidence exists from clinical and epidemiological studies, suggesting an increasing incidence in male reproductive

problems (Kolettis, 2003; Nayernia et al., 2004). Male factors are thought to play 50% role in the etiology of infertility (Amelar and Dubin, 1977). The hormonal control

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of spermatogenesis is based on the action of the pituitary gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH) on the testis. LH stimulates the leydig cells in the testes to produce testosterone. Intra-testicular testosterone (ITT) mediates its effects within the testes through the androgen receptor that is found in leydig cells, sertoli cells and peritubular cells (McLachlan et al., 2002). ITT is believed to stimulate spermatogenesis directly in rats (Zirkin et al., 1989) and men (Matsumoto and Bremner, 1985). Nevertheless, ITT is thought to play a key role in the control of spermatogenesis but is rarely measured (Coviello et al., 2004). Studies in rats have shown that the ITT concentration is much higher, approximately 30-fold, than serum testosterone (Turner et al., 1984). Additionally, exogenous testosterone administration has shown to restore spermatogenesis in rats with gonadotropin suppression (Awoniyi et al., 1992; Zirkin and Santulli, 1989).

Various parts of date palm plant have been known to enhance growth, an action that has been ascribed to increase in the plasma level of estrogen and testosterone (Ali et al., 1999). Suspension of *Phoenix dactylifera* (date palm pollen, DPP) is a herbal mixture that is widely used as a folk remedy for curing male infertility in traditional medicine (Zargari, 1990). The present study is therefore designed to see the effect of DPP on the plasma and intratesticular testosterone levels of male adult rats with the hopes to add a valuable contribution in advancement to treatment of infertility.

## MATERIALS AND METHODS

The rats were procured from National Institute of Health, Islamabad, Pakistan and were kept for two weeks in experimental research laboratory of University of veterinary and animal sciences, Lahore, Pakistan for acclimatization. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals. All experimental procedures followed the principle of laboratory animal care and were carried out according to a protocol approved by the local animal ethics committee and performed in accordance with the National Institutes of Health (NIH, USA) guidelines for the use of experimental animals. Dissections were performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering. Local anesthetic was applied before drawing blood from rat tail.

### Preparation of the herbal cocktails

The date palm pollen powder was imported from Saudia Arabia, it was mixed with distill water to form a homogenized suspension. The dose was adjusted daily according to the body weight of the rats during the course of treatment and administered by oral gavage.

### Control and experimental animals

A total of 24 adult male albino rats, weighing 156 to 167 g, were randomly divided into control and experimental groups containing

12 rats each. All animals were fed normal rat chow and water *ad libitum*. Experimental rats were provided herbal cocktails daily according to the body weight.

## 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.

### Weights of paired testes

Weights of paired testes after removal were measured to determine the effect of DPP on testes.

### Serum testosterone level

Due to proposed androgenic activity of DPP, serum testosterone level was measured.

### Intra-testicular testosterone level

It was measured to evaluate the effect of DPP on the intra-testicular environment.

## Experimental procedure

As mentioned earlier, male albino rats were randomly divided into two groups of control and experimental, each containing 12 male rats. Each group was kept at controlled room temperature ( $22 \pm 2^\circ\text{C}$ ) and humidity of  $55 \pm 10\%$ . They were kept under natural light and dark cycle. All animals were fed on normal rat chow and water *ad libitum*.

Control group: Distill water was given to control group daily for 35 days starting at day 1.

Experimental group: DPP suspension was given to the experimental group of rats by 120mg/kg body weight as a single oral dose daily for 35 days, starting at day 1 (Bahmanpour et al., 2006). The rats of control and experimental groups were weighed and their blood was drawn on day 0, 12, 24 and 35 through tail vein.

### Preparation of rats for collection of blood and testicular tissue

At the beginning of the experimental period, that is, day 0, weight of each rat from control and experimental groups was recorded. Local aesthetic cream was then applied on the surface of the tail 30 min before the procedure. A 23G needle was inserted into the blood vessel and blood was collected using a syringe. Having completed blood collection, pressure was applied to stop the bleeding (Parasuraman et al., 2010). The blood was allowed to stand for one hour and then was centrifuged at a speed of 3000 rpm (Eppendorf 5810R, Germany) for 10 min to get clear serum. Serum was separated into the sterilized eppendorf tube and was stored at  $-80^\circ\text{C}$  until further analysis. The same procedure was repeated on day 12, 24 and 35. At the end of the experiment, that is, day 35, the

rats were dissected and the testes of both control and experimental groups were removed. Their weights were measured and preserved at -80°C.

#### Intra-testicular and serum testosterone quantitation

Testes were thawed, decapsulated and were subsequently homogenized in phosphate buffer solution of pH 7.4 at a ratio of 1:10 with a polytron homogenizer (PT 2100). Samples were vortexed vigorously for 1 min. The tissue homogenate obtained was cold centrifuged at 800 g for 10 min and the supernatant was obtained in a sterilized disposable Eppendorf tube. Samples were preserved at -80°C (Ultra-freezer SANYO, Japan) until the estimation of intra-testicular testosterone levels was quantified by enzyme-linked immunosorbent assay (ELISA) (Vijay et al., 2009). No androgen was detected in blank samples processed through the extraction protocol, indicating that neither solvent residues nor buffers interfered with the ELISA. The serum and intra-testicular testosterone levels were measured by using commercially available testosterone ELISA kit (BioCheck, USA). The concentrations of testosterone were determined by semi-automated ELISA analyzer (ELISA plate reader 680, Biorad). About 50 µl of calibrators and tissue homogenate sample were added into appropriate wells of strips. About 200 µl of horseradish peroxidase–testosterone conjugate was added to each well in sequence. Mixture was incubated for 2 h at 37°C without covering the plate, after which the solution was discarded. The wells were rinsed thrice with washing solution (Tween 20) and amphotericin–B (2.5 µg/ml) in citrate-borate buffer and the residual fluid was removed. Immediately, 100 µl of chromogen substrate mixture (0.26 mg/ml of 3,3',5,5'-tetramethyl benzidine) and 0.01% (w/v) of hydrogen peroxide in citrate buffer) was added to the wells and incubated for 15 min at room temperature, avoiding exposure to sunlight. Reaction was stopped by pipetting 100 µl of the stop solution (sulfuric acid - 0.3 mol/l) into the wells. Absorption was read in ELISA at 450 nm within 1 h from the addition of stop solution, as per manufacturer's instructions.

#### Statistical analyses

Statistically significant differences were determined using t-test in Prism statistical software (GraphPad Software, San Diego, CA, USA). A  $P < 0.05$  was considered significant in all cases. Data was reported as mean  $\pm$  SD.

## RESULTS

#### Body weight

There was no difference in the body weight of both control and experimental group at day 0 and 12. However, there was a significant difference in control and experimental rats on day 24 and 35 of experiment, respectively, with  $p$ -values  $< 0.05$  (Table 1 and Figure 1).

#### Measurement of serum testosterone levels

There was no significant difference between the serum testosterone levels among the control and the experimental group at day 0 and 12. However, on day 24

and 35, a significant increase was observed in experimental group with  $p$ -values  $< 0.05$  (Table 2 and Figure 2).

#### Weight of paired testes

The paired testes were weighed at the end of experiment after 35 days of treatment of DPP suspension. Weight of paired testes, in the control group varied from 1.52 to 1.78 g, with an average of  $1.65 \pm 0.09$  g. In the treated group, it ranged from 1.78 to 2.50 g, with the average of  $2.26 \pm 0.28$  g. Difference in the weight of paired testes of animals in control group and experimental group after 35 days of therapy was statistically significant  $p < 0.01$  (Table 3 and Figure 3).

#### Intra-testicular testosterone levels

In the control group, intra-testicular testosterone levels of albino male rats varied from 72.80 to 80.41 ng/ml, with an average of  $76.48 \pm 2.4$  ng/ml. In the treated group, it ranged from 116.20 to 145.29 ng/ml, with the average of  $129.90 \pm 9.66$  ng/ml. Difference in the intra-testicular testosterone levels of albino male rats in control group and experimental group was statistically significant  $p < 0.01$  (Table 3), as shown in Figure 4.

The scatter diagram for (Figure 5) suggests, there is a positive linear relationship between these two variables. These are serum and intra-testicular testosterone, the logarithmically increased level after 35 days of DPP treatment.

## DISCUSSION

The current study has shown that rats exposed to DPP suspension at levels comparable to control exhibit significant changes in developmental profiles in terms of increased body weight and also increase in serum testosterone levels after 24 and 35 days of treatment. There was a significant increase in the weight of testes at day 35 along with an increase in intra-testicular testosterone. These findings support those of previous studies and suggest that 120 mg/kg of DPP suspension can influence the endocrine regulation of male reproductive function in rats.

In this study, different parameters analyzed were assessed to see the effect of DPP suspension (120 mg/kg) given orally for 35 days in adult male albino rats. The data collected showed that up to 24 days, treatment with date palm pollen suspension did not have statistically significant effect on serum testosterone levels and body weight. Treatment with DPP took somewhere between 24 and 35 days to produce its effects. This agrees with the study carried out by (Bahmanpour and Talaei, 2006),

**Table 1.** Body weight of control and experimental rats.

Body weight	Control group	Experimental group	p-value
	Mean $\pm$ SD		
Day 0	160.33 $\pm$ 7.03	161.17 $\pm$ 7.12	0.776
Day 12	160.67 $\pm$ 6.44	161.58 $\pm$ 6.65	0.735
Day 24	163.42 $\pm$ 6.14	172.58 $\pm$ 5.85	0.001
Day 35	165.83 $\pm$ 5.65	191.83 $\pm$ 7.54	0.001

Each value represents the mean  $\pm$  SD for 12 mice with p-value ( $P < 0.05$ ).

**Table 2.** Effect of DPP on serum testosterone level (ng/ml) in control and experimental rats.

Serum testosterone level	Control group	Experimental group	p-value
	Mean $\pm$ SD		
Day 0	1.81 $\pm$ 0.05	1.82 $\pm$ 0.06	0.717
Day 12	1.81 $\pm$ 0.06	1.84 $\pm$ 0.06	0.277
Day 24	1.82 $\pm$ 0.05	2.12 $\pm$ 0.13	0.001
Day 35	1.82 $\pm$ 0.05	3.09 $\pm$ 0.22	0.001

Each value represents the mean  $\pm$  SD for 12 mice with p-value ( $P < 0.05$ ).

**Table 3.** Comparison of mean weight of paired testes and mean intra-testicular testosterone levels (ng/ml) of albino male rats among experimental and control groups.

Parameter	Control group	Experimental group	p-value
	Mean $\pm$ SD		
Weight of paired testes	1.65 $\pm$ 0.09	2.26 $\pm$ 0.28	0.001
Intra-testicular testosterone levels	76.48 $\pm$ 2.4	129.90 $\pm$ 9.66	0.001

Each value represents the mean  $\pm$  SD for 12 mice with p-value ( $P < 0.05$ ).

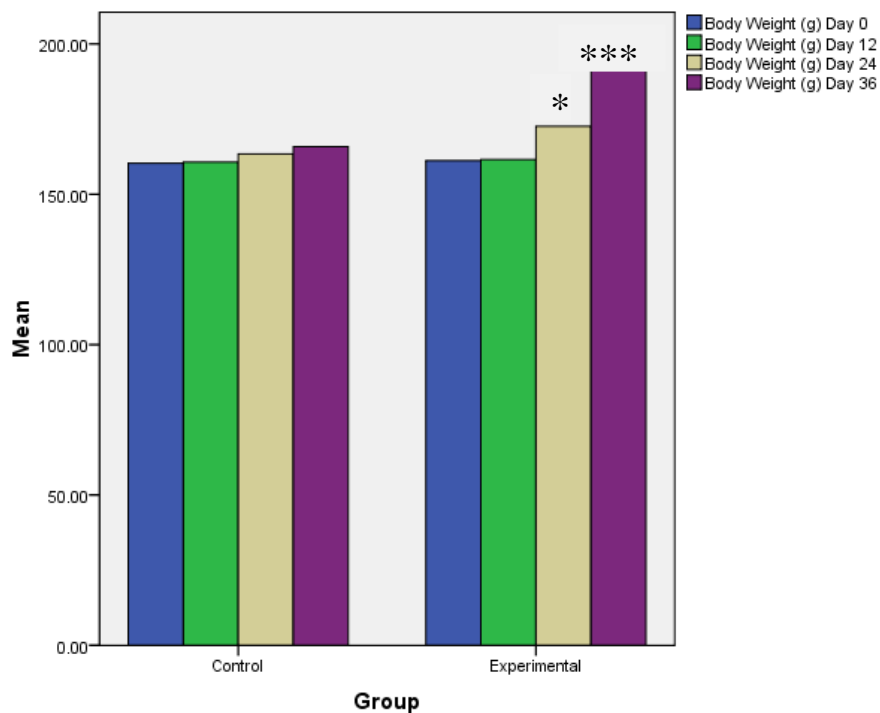
where they observed the effect of DPP on sperm parameters and reproductive system of adult male rat after 35 days of treatment. Significant increase observed in the body weight and serum testosterone, served an evidence that increase in the body weight, weight of paired testes, serum testosterone levels and intra testicular testosterone levels in experimental group might be due to the presence of gonadotropin like substances in the DPP (Azooz et al., 2001).

A comparison of body weight between control and experimental animals was made. The weight gain in the experimental group may partly be attributed to the androgenic effects of increased testosterone levels. This is in concert with the study carried out by Gauthaman where they maintained 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

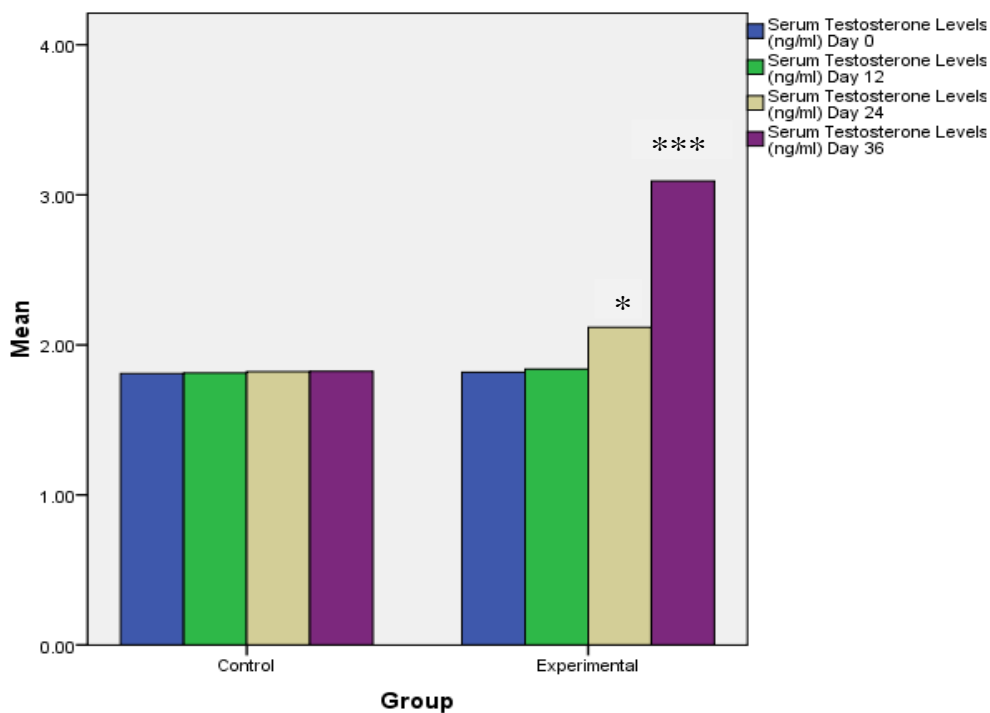
(Gauthaman et al., 2002).

Similarly, when a comparison of testicular weight and intra-testicular testosterone levels was made, an increase of 1.4 times in the weight of testes and 1.7 times in the intra-testicular testosterone was observed. It is suggested that this improvement in both parameters is attributed to the ability of DPP to increase the testosterone levels. These results agree with the findings of Gauthaman who studied aphrodisiac properties of the puncture vine extract (protodioscin) in normal and castrated rats. They concluded that treatment of castrated rats with their extract resulted in increased body weight and weight of testes with increase in the testosterone levels in rats due to protodioscin content (Gauthaman and Adaikan, 2002).

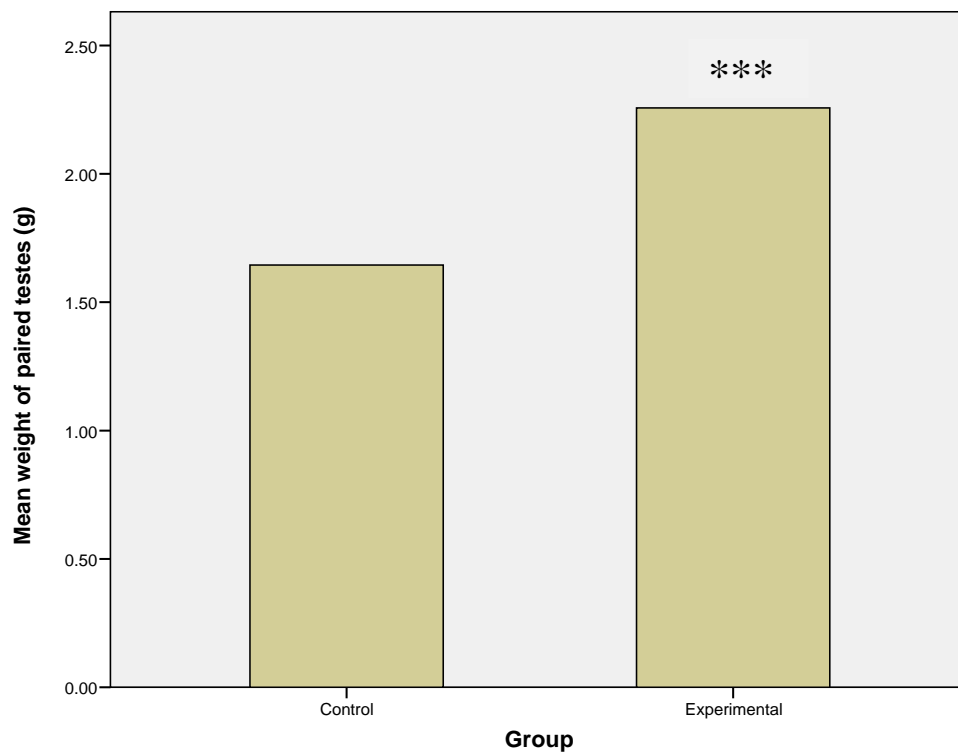
The scatter plot between serum and intra-testicular testosterone showed that there is positive correlation between these two parameters when treated with DPP for 35 days. It is suggested that DPP, through its complex mechanism, may increase both serum and intra-testicular



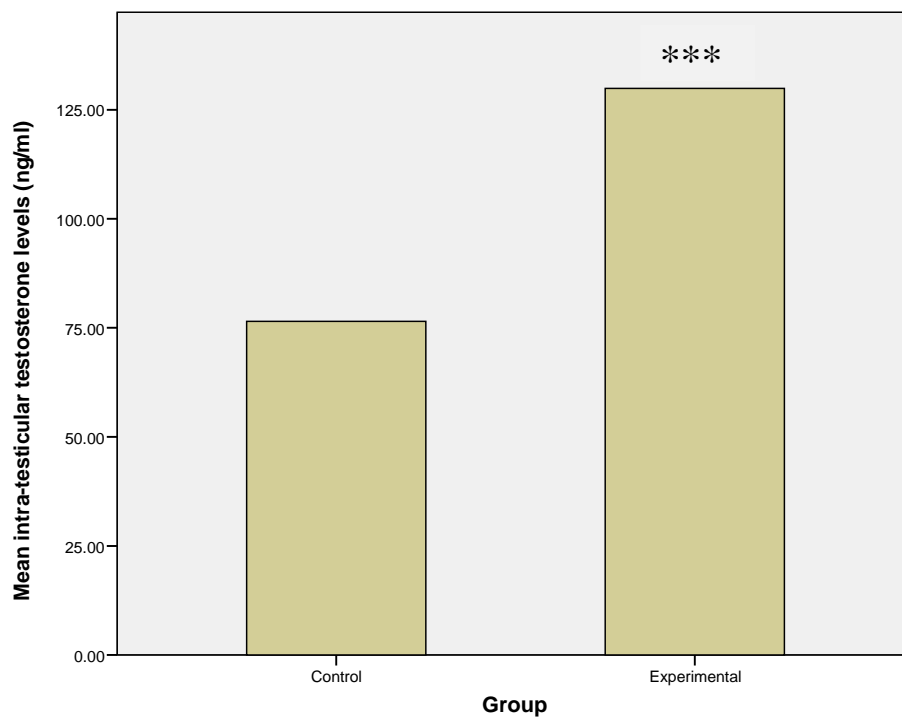
**Figure 1.** Body weight comparison of control and experimental rats. Values represent the mean ± SD of 12 rats in each group. Each value represents the mean ± SD for 12 mice with p-value ( $P < 0.05$ ).



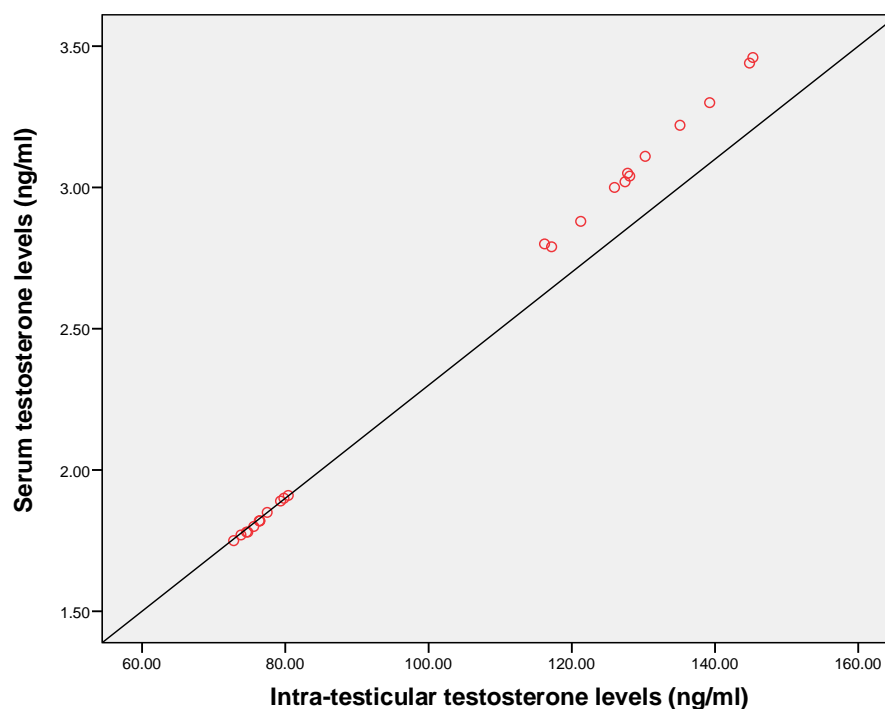
**Figure 2.** Serum testosterone levels (ng/ml) in control and experimental rats (treated with DPP suspension). Testosterone levels were noted on day 0, 12, 24 and at 35 days with DPP treatment. Each value represents the mean ± SD for 12 mice with p-value ( $P < 0.05$ ).



**Figure 3.** Weight of paired testes (g) in control and experimental rats (treated with DPP suspension). Each value represents the mean  $\pm$  SD for 12 mice with p-value ( $P < 0.05$ ).



**Figure 4.** Comparison of intra-testicular testosterone levels (ng/ml) of control and experimental male albino rats. Each value represents the mean  $\pm$  SD for 12 mice with p-value ( $P < 0.05$ ).



**Figure 5.** Scatter plot of serum testosterone (ng/ml) vs. intra-testosterone levels (ng/ml) of control and experimental group. Each value represents the mean  $\pm$  SD for 12 mice with p-value ( $P < 0.05$ ).

testosterone levels without causing any feedback inhibition. The results of this study are of significance since rats given DPP suspension in experimental group exhibited increasing body weight, testis weight, serum testosterone and intra-testicular testosterone levels at 24 and 35 days of exposure. The increase in both serum and intra-testicular testosterone levels indicates that DPP suspension altered leydig cell steroidogenesis. However, the exact mechanism of this action is not known. According to Guyton and Hall (2006), estradiol has a direct impact on the production and maturation of sperms. Future research will address the mechanisms responsible for the increase in androgen levels caused by DPP. Our results validate its utilization in the male factor infertility as an adjuvant therapy.

#### ACKNOWLEDGEMENT

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#### Conflict of interest statements

Authors declare that they have no conflicts of interest.

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