

Original Research Article

Assessment of Reproductive Hormones and Antioxidant Status of Male Rats Fed With Date Juice (*Phoenix Dactylifera*)

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Abstract: Divorce cases have been recorded on the high side due to the prevalence of infertility in our society. There is believe that fruits improve fertility. The present study was carried out to evaluate *in vivo* the effect of date (*Phoenix dactylifera*) fruit juice on reproductive hormones and antioxidant status in male albino rats. Six (6) rats were divided into two (2) groups of three (3) rats each. (Control and Test). The control was administered distilled water (2ml/kg) while Test was administered 2ml/kg of the fruit juice for twenty-eight (28) days. The result showed that oral administration of the date fruit juice significantly ($P<0.05$) increased the level of antioxidant enzymes (SOD and GST) from 2.87 ± 0.15 to 3.87 ± 0.21 and 3.27 ± 0.21 to 4.73 ± 0.15 (Units/mgprotein) respectively. The level of Testosterone also increased significantly ($P<0.05$) from 0.57 ± 0.03 to 0.94 ± 0.08 (ng/ml), while the activity of FSH and LH hormones increased non-significantly ($P>0.05$) from 20.23 ± 0.86 to 21.87 ± 0.65 and 11.00 ± 0.34 to 11.57 ± 0.72 (miu/ml) respectively. There was a significant weight gain in the testes administered 2 ml/kg date fruit juice as compared to the control. Our result implies that date fruit juice had a significant effect on the testis and increased the level of testicular hormones and antioxidant enzymes. This justify the conclusion that the juice may improve fertility.

Keywords: Juice, Hormones, Antioxidant, Testis, Fertility.

INTRODUCTION

The testes (or testicles) are a pair of sperm-producing organs that maintain the health of the male reproductive system. The testes also have the distinction of being an endocrine gland because they secrete testosterone. Reproductive hormones are essential for reproduction but are not necessary for life. Examples are Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) which stimulate the gonads found in the testis and are therefore called gonadotropins (Farombi *et al.*, 2012 and Nathan *et al.*, 2016). Antioxidant enzymes form the body's endogenous defense mechanisms to help protect against free radical-induced cell damage and catalyze reactions to neutralize free radicals and reactive oxygen species. The enzymes include: superoxide dismutase, glutathione peroxidase, glutathione reductase, catalases (Amira *et al.*, 2012).

Infertility is defined as the inability of couples to achieve pregnancy after one year of continuous unprotected sexual intercourse or six months, if the

woman is 35years or older. The prevalence varies widely, being less in developed countries where resources for investigation and treatment are readily available and accessible but more in developing countries where limited resources for investigation and treatment are available, infertility is considered as a public problem. It does not affect the couple's life only, but it also affects the healthcare services and social environment (Agarwal *et al.*, 2003). The ancient Egyptians used fruits to improve fertility, to make wine and also ate them at harvest. Date fruits (*Phoenix Dactylifera*) are an important traditional crop in Iraq, Arabia, North Africa and Morocco where they are used in the treatment of various ailment/illness (Lim, 2012.). Date fruit is nutritious and it's a good source of fiber, important vitamins and minerals, including significant amounts of calcium, iron, fluorine, and selenium (Khan *et al.*, 2008). Dates have also been shown to contain antioxidants such as coumaric, ferulic acids and also display antimutagenic properties (Vayalil, 2002). Recent studies have shown that dates and their aqueous

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extracts have demonstrated the free radical scavenging activity, inhibition of free radical-mediated macromolecular damages, antimutagenic, and immunomodulatory activities (Saafi *et al.*, 2009). The outstanding ability of date fruit in treating infertility issues is not well determined nor the active principles standardized. The main aim of this study is to assess *in vivo* the effect of date fruit juice on reproductive hormones and antioxidant status in male albino rats.

MATERIALS AND METHODS

Preparation of *Phoenix Dactylifera* Juice

Date fruits were obtained from Tombia market in Bayelsa state and washed with distilled water and weighed. The weighed quantity (30 g) was then blended with 100mls of distilled water using a blending machine. The blended solution was then filtered using a sieving cloth and kept in a fridge for proper storage and also prepared frequently to avoid spoilage.

Animal/Treatment

Six (6) healthy adult male wister rats weighing between 150g to 200g were obtained from the animal house of Niger Delta University, Bayelsa State and were acclimatized for two weeks during which they were fed with standard feed (growers mash) and distilled water. A total of 6 adult albino rats grouped into two groups each having three rats. Group 1 (control) received distilled water (2 ml/kg) and growers mash for 28 days. Group 2 (Test group): received 2ml/kg per body weight (bw) of *Phoenix Dactylifera* Juice and fed with growers mash and distilled water for 28 days. The ethic regulations have been followed in accordance with national and institutional guidelines for the protection of animal welfare during experiments (Public Health Service (PHS), 1996). *Phoenix Dactylifera* Juice was administered orally with gavage for 28 days after which the animals were sacrificed using chloroform anaesthetization. The whole body was dissected and the testes were excised and washed in normal saline and part of it was placed in a sample bottle containing 10% formalin. This was subjected to histological analysis. Then 10g of the testis was weighed, homogenized using 0.1M of Phosphate buffer (p^H 7.4) and centrifuged for 10minutes at 3000rpm. This was subjected to biochemical analysis. The blood was centrifuged for 10minutes at 3000rpm and the serum was collected for hormonal analysis.

Chemicals

Epinephrine, glutathione, 5, 5-dithio-bis-2-nitrobenzoic acid, hydrogen peroxide and 1-chloro-2,4-dinitrobenzene (CDNB) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals/reagents used in the study were of analytical grade.

Biochemical Estimations

The homogenized testis and serum were used for biochemical estimations: superoxide dismutase activity (SOD), and glutathione-s-transferase activity (GST) were measured by standard spectrophotometric methods. Superoxide dismutase (SOD) was assayed by the method described by Misra & Fridovich (1972). Glutathione S-transferase (GST) was assayed by the method of Habig *et al.*, (1974). Protein concentration was determined by the method of Lowry *et al.*, (1951).

The serum testosterone, (LH), and follicle stimulating hormone (FSH) Concentrations were measured with commercial ELISA Kits (R&D), respectively.

Statistical Analysis

Data was expressed as Mean \pm Standard deviation. The significant difference between the test group and the control group were analysed using T-test. Data were analysed using SPSS (Statistical Package for Social Sciences) version 16. $P < 0.05$ was set as the level of significance.

Histopathological study

Small pieces of testis tissues were collected in 10% formalin for proper fixation. These tissues were processed and embedded in paraffin wax. Sections of 5-6 μ m in thickness were cut and stained with hematoxylin and eosin.

RESULTS

Table-1 showed that there was a significant increase ($P < 0.05$) in the activity of SOD and GST compared to the control in albino male rat testis after the administration of the *Phoenix Dactylifera* Juice. There was a significant ($P < 0.05$) increase in the level Testosterone while the increase in the levels of FSH and LH hormones were non-significant ($P > 0.05$) when compared to the control. (Table-2). Table 3 showed that there was a significant increase ($P < 0.05$) in the weight gain compared to the control in albino male rat testis after the administration of the *Phoenix Dactylifera* Juice.

The photomicrograph of transverse section of group1 rats testis showed normal seminiferous tubule. The seminiferous tubule has numerous sertoli cells, spermatogonium, and spermatocytes in the lumen of the tubule (Plate 1). Plate 2 photomicrograph showed the transverse section of group2 rats testis treated with date fruit extract showed normal arrangement of seminiferous tubules which have numerous sertoli cells and different stages of the spermatocytes.

Table 1. The effect of administration of *Phoenix Dactylifera* (PD) Juice on antioxidant enzymes (SOD and GST) in the testis of albino rats.

Test/Enzymes	SOD (unit/mg protein)	GST (unit/mg protein)
Control (distilled water)	2.87±0.15 ^a	3.27±0.21 ^a
Test PD (2ml/kg bw)	3.87±0.21 ^b	4.73±0.15 ^b

Data are Mean ± SD (n = 6). Mean in the same column with different superscript letter(s) are significantly different, (P<0.05).

Table 2. The effect of administration of *Phoenix Dactylifera* (PD) Juice on testicular hormones of albino rats.

Test/Hormones	Testosterone(ng/ml)	FSH (miu/ml)	LH (miu/ml)
Control (distilled water)	0.57±0.03 ^a	20.23±0.86 ^a	11.00±0.34 ^a
Test PD (2ml/kg bw)	0.94±0.08 ^b	21.87±0.65 ^a	11.57±0.72 ^a

Data are Mean ± SD (n = 6). Mean in the same column with different superscript letter(s) are significantly different, (P<0.05).

Table 3. Total weight gain to testis ratio as a result of administration of *Phoenix Dactylifera* (PD) on the testis.

Test/weight	Testis ratio (%)	Weight gain (g)
Control (distilled water)	0.38±0.02 ^a	5.33±1.15 ^a
Test PD (2ml/kg bw)	0.68±0.03 ^a	10.00±1.73 ^b

Data are Mean ± SD (n = 6). Mean in the same column with different superscript letter(s) are significantly different, (P<0.05).

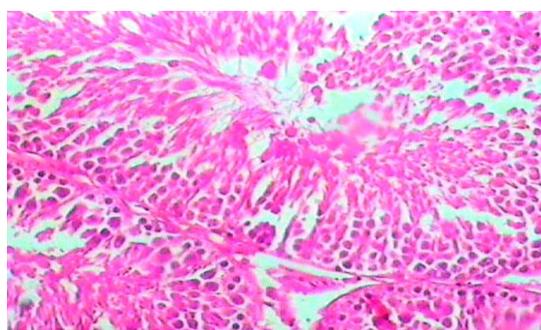


Plate 1. (Control) Photomicrograph of transverse section of testis. (H&E mag ×100)

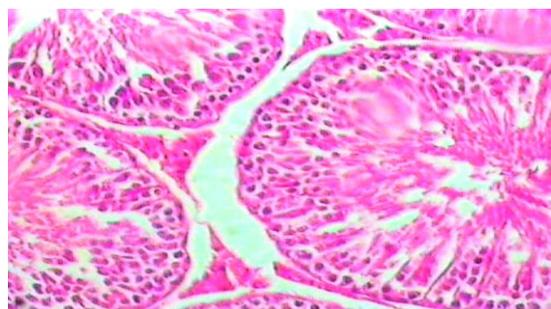


Plate 2. (Test) Photomicrograph of transverse section of rat testis treated with date fruit extract (H&E mag ×100)

DISCUSSION

The present study was conducted to assess the effect of date fruit juice on fertility and reproductive parameters of male rats. Date fruit was chosen because it is widely used in Nigeria as well as in many parts of Africa and the world. Animal studies have shown that oral feeding of date fruit increases the expression of antioxidant enzyme genes in rat cardiac tissue (Baliga *et al.*, 2011). The observed antioxidant activity of dates have, been attributed to phenolic compounds, anthocyanins, flavonoid glycosides and procyanidins present in it and that sun-drying and ripening decrease the antioxidant activity (Amira *et al.*, 2012). Selenium present in dates is also reported to contribute to the antioxidant effects. Multiple studies have shown that this essential trace element exerts its antioxidant function mainly in the form of selenocysteine residues that are an entire constituent of ROS-detoxifying selenoenzymes (thioredoxin reductases and possibly selenoprotein P) (Steinbrenner and Sies, 2009). When considered in total it is very evident that the presence of diverse phenolic compounds in date fruits confers its fertility properties (Aqil *et al.*, 2006).

Our results showed that oral administration of date juice to male rats for 28 days significantly (P<0.05) increased the level of SOD from 2.87±0.15 to 3.87±0.21 and GST from 3.27±0.21 to 4.73±0.15

(Units/mg protein) in the testis when compared to the control. The level of Testosterone when compared to the control increased significantly ($P < 0.05$) from 0.57 ± 0.03 to 0.94 ± 0.08 (ng/ml), while the activity of FSH and LH hormones when compared to the control increased non-significantly ($P > 0.05$) from 20.23 ± 0.86 to 21.87 ± 0.65 and 11.00 ± 0.34 to 11.57 ± 0.72 (miu/ml) respectively. These results are in agreement with the study of Goldman *et al.*, (1999) who reported that date fruits extract have been shown to increase sperm count in guinea pigs and to enhance spermatogenesis and increase the concentration of testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) in male rats. However, our present results are not in agreement with the study by Nathan *et al.*, (2016) which showed that administration of aqueous extract of *Phoenix dactylifera* might affect fertility.

The increase in the level of anti-oxidant enzymes may be due to the protective properties of fruits. This is in agreement with the study by Arhoghro *et al.*, (2016) who reported that the administration of low dose of plantain (*Musa paradisiaca*) stem juice had protective effect against cyclophosphamide-induced reproductive toxicity.

Our histopathological examination revealed that the examined testes of the treated rats with date fruit juice showed no marked degeneration in seminiferous epithelium, germ cells and leydig cells. There was no testicular damage in the rats given date fruit juice and this agrees with the study of Mahgoub and El-Medany, (2001). This confirms the report that the fertilizing capacity of the testis of the rats given date juice does increase.

From the results of this study, the date fruit juice had a significant effect on the testis and increased the level of testicular hormones and antioxidant enzymes. This indicates that the juice might improve the fertility status of humans.

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