



Journal homepage:  
<http://www.bsu.edu.eg/bsujournals/JVMR.aspx>

Online ISSN: 2357-0520

Print ISSN: 2357-0512



**Original Article Research**

**Effects of some dietary supplements on the reproductive and productive performances in male rats.**

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**ABSTRACT**

The present study aimed to investigate the efficacy of three natural food supplements (water hyacinth [Eichhorniacrassipes, "EC"], Yeast [Saccharomyces cerevisiae, "S. cerevisiae"] and date seeds) on the reproductive and productive activities in male rats. Thus, 40 male albino rats were used and divided equally into 4 groups; Control group (fed normal basal diet), EC supplemented group (400 mg EC / kg body weight), S. cerevisiae supplemented group (120 mg / kg body weight) and date seed supplemented group (0.2 mg / kg body weight). Two months later, all rats were sacrificed and all samples were collected. Results revealed that date seeds supplementation increased significantly the body weight gain. Moreover, date seeds and S. cerevisiae supplementation increased significantly gonadosomatic index, serum levels of total antioxidant capacity "TAC" and all studied reproductive parameters ( $P \leq 0.05$ ) as well as it decreased serum level of malondialdehyde "MDA". On the other side, EC supplementation reduced significantly the studied reproductive parameters as well as it decreased the serum level of TAC and increased the level of MDA. Histopathologically, seminiferous tubules appeared with huge amount of spermatids in date seeds group and with moderate number of spermatids in S. cerevisiae group and with few number of spermatids in EC group.

Therefore, the present study highly recommends the usage of Saccharomyces cerevisiae as well as date seeds to minimize the ration costs, get the optimal benefit from the natural components of both supplements as well as to induce a higher productive and reproductive performance among animals.

**ARTICLE INFO**

**Article history:**

Received 11/2018  
 Accepted 12/2018  
 Online 12/2018

**Keywords:**

Eichhorniacrassipes;  
 Saccharomyces cerevisiae;  
 date seeds;  
 Rats

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## INTRODUCTION

Reactive oxygen species (ROS) are free radicals and are usually released during an unavoidable consequence of aerobic metabolism. Hydroxyl radical ( $\text{OH}^-$ ), Superoxide anion ( $\text{O}_2^-$ ) and also, non-radical molecules like hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), singlet oxygen ( $^1\text{O}_2$ ) are common forms of ROS (Heyno et al., 2011). Recently, it was evidenced that oxidative stress is one of the major factors that cause male infertility (Agarwal et al., 2014). The high levels of polyunsaturated fatty acids in Spermatozoa membranes increased their risky exposure to ROS attack. Moreover, it was reported that there are a direct correlation between teratozoospermia and oxidative stress. This is due to induction of cell apoptosis, nucleic acids impairment, and denaturation in proteins and lipids molecules (Ayaz et al., 2015). In addition, Alagbonsi and Olayaki (2017) found that ROS produced by Cannabis sativa resulted in spermatoxicity.

Many reports have been established to study the beneficial role of food additives in livestock. These studies indicated the probability that the substance will produce injury under defined conditions of exposure. The concept of risk takes into account the dose and length of exposure as well as the toxicity of a particular chemical, and is a better guide to the safety of a food. Consequently, any attempt to examine the role of the food supply should include its effect on productive and reproductive capacity of the animal (Williams, 2012).

Eichhorniacrassipes (water hyacinth) "EC" is a free-floating perennial aquatic plant which is related to tropical and subtropical areas (Aboul-Enein et al., 2011). Water hyacinth is a rich source of protein content and other nutritional values and has chelating effect. It is established that

the root of EC removes arsenic from arsenic-contaminated water (Misbahuddin et al., 2002). Moreover, methanol extract of EC leaf possesses anticoagulant activity due to presence of polysaccharides, which act on the intrinsic pathway of the coagulation cascade. In addition, crude methanolic extract showed potent activity against bacteria, fungi, and algae (Kumar et al., 2011). Furthermore, Quayum (2007) found that ethanol extract of EC ameliorates arsenic from arsenic-treated rats. The author reported that administration of different concentrations of ethanol extract of root of water hyacinth (100%, 75%, 50%, and 25%) for last two days significantly reduced the arsenic accumulation in liver, spleen, kidney, intestine, lungs and skin. Besides, it reduced the oxidative stress caused by arsenic in the aforementioned organs.

Saccharomyces cerevisiae (S. cerevisiae) is a yeast biotherapeutic agent that possess probiotic properties (Van der et al., 2005). The S. cerevisiae cell wall consists of mannoproteins,  $\beta$ -glucans and a small amount of chitin, which becomes cross-linked in a variety of ways (Orlean, 2012). The properties of S. cerevisiae offer the potential for use as a natural stimulator in animal nutrition. Therefore, yeasts may replace antibiotic growth stimulators (Linge, 2005). Many studies on pointed that the addition of brewer's yeasts into a dairy cow diet caused an increase in milk yield and a reduction of somatic cell count in milk (Dobicki et al., 2007). Moreover, Zabek et al. (2014) in sheep recorded an increase in milk yield associated with the supplementation of the ration with yeast products. In addition, Sahar-Eissa et al. (2016) found that Baker's Yeast extraction of S. cerevisiae at different doses ranged from 10, 20 & 50 mg/ml significantly increased ( $P < 0.05$ ) the human sperm vitality, motility percent, and the grade of motility. These data encouraged the authors

to conclude that yeast extraction improved male fertility.

The date seeds are commonly used in animal feeding or grinded into smaller sized particles then being heated to turn it into substitute as coffee without caffeine, which used commercially by the Arabs into two types; whether plain or mixed with coffee (Al-Farsi and Lee, 2011). Egypt is one of the Arabian countries that produce date fruit. The fruit of the date palm is composed of two parts; a fleshy pericarp and seed (Ahmed et al., 2008). The seed represent about 11-18 % of the date fruit (Amira et al., 2011). Date seed, normally, is composed of 2.3-6.4% protein, 3.1-7.1% moisture, 5.0-13.2 % fat, 0.9-1.8% ash and 22.5-80.2% dietary fiber. Also, the seeds contain high levels of phenolics (3102 - 4430 mg gallic acid equivalents/ 100 g), antioxidants and dietary fiber (78–80 g/100 g) (Al-Farsi et al., 2007). As well, it is considered a rich source for minerals as it contains considerable amounts of sodium, potassium, calcium, iron, copper, magnesium, manganese, zinc, phosphorus, lead, cadmium and chromium. (Shariati et al., 2008). Moreover, Orabi and Shawky (2014) found that oral administration of date pits palm for male rats caused a significant elevation of serum testosterone level and hemoglobin concentration as well as it decreased significantly the serum levels of ALT, creatinine and malondaldehyde.

Thus, the current study aimed to investigate the effects of EC, *S.cerevisiae* or date seeds as dietary supplements on the reproductive and productive activities in male rats.

## 1- Material and methods:

### 2.1: Rats:

The current study included 40 mature male Albino rats of an average body weight 170 - 190 g. These animals were received from Animal Experimental Unit,

Department of Physiology, Faculty of Veterinary Medicine, BeniSuef University. Animals were left for 2 weeks for acclimatization to the laboratory environment before the experiment. Throughout the experimental period, rats were kept under constant environmental and hygienic conditions as well as offered food and water ad libitum. The basal food consisted of cereal standard diet supplemented with minerals and vitamins mixture.

### 2.2:Preparation of dietary supplements:

The fresh plants of EC were collected at Nile River, Beni-Suef city, Egypt. The plant was identified by a botanist, in the Department of Botany, Faculty of Science, Beni-Suef University, Egypt. After authentication, the plants were cleaned and shade dried, the leaves were separated and milled into coarse powder by a mechanical grinder (Dineshkumar et al., 2013). On the other side, date fruits were obtained from dates shop in El-Minia Government; the seeds (pits) were collected, rinsed well in water then left to dry and roasted. Then the dried seeds were heated (for 15 minutes) then ground into a fine powder by using a mechanical grinder (El-Fouhil, 2010). In the same respect, the live yeast (*S. cerevisiae*) was obtained from HolwElsham Company, Egypt.

### 2.3: Experimental design:

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Rats under experiment were equally divided into 4 groups (10 rats each); the 1<sup>st</sup>(control) was fed the normal basal diet while those of the 2<sup>nd</sup> group were fed the same basal diet plus EC leaves powder at a rate of 400 mg / kg body weight as outlined by Dineshkumar et al. (2013). Rats of the third group were fed basal diet plus active dried yeast at a rate of 120 mg / kg body weight (Eze et al., 2012) while rats of the fourth group were fed normal diet plus date

seed powder ( 0.2 mg / kg body weight) as reported by **Shariati et al., 2012**). In all groups, animals were fed their corresponding diet daily, in the morning, for 8 successive weeks.

#### **2.4: Sample collection:**

At the end of the 8<sup>th</sup> week, individual blood samples were collected from rats of all groups. Moreover, individual tissue samples of the testis were obtained and prepared for histopathological examination, as mentioned by **Narayana et al. (2005)**.

#### **2.5: Calculation of the gonadosomatic index (GSI):**

The gonadosomatic index was calculated from the following formula as mentioned by **Adebiyi (2013)**.

#### **2.6: Determination of serum total antioxidant capacity (TAC) malondialdehyde (MDA):**

Estimation of serum malondialdehyde ( MDA ) in male Albino rats was implemented using commercial kits ( Sigma-Aldrich, USA ) according to **Qiao et al. ( 2016 )** and **(Koracevic et al., 2001)**.

#### **2.7: Immunoassay of serum testosterone (T) level:**

The serum level of testosterone(T) was measured using Testosterone ELISA Kit (Catalog Number KA 0309309, Abnova Manufacturing and SPF Facility, Taiwan) according to **Chard (1990)**.

#### **2.8: Semen evaluation:**

Semen samples were collected on clean glass slide by maceration of the tail of the epididymis using sterile scalpel. Sperm count was determined using the hemocytometer as described previously by **Rathje et al. (1995)**. Individual motility was examined microscopically according to method described by **Beardeu and Fuqcy (1980)**. Live / dead percentage of sperms was determined by using eosin nigrosine stain as performed by **Bloom (1950)**. The sperm cells abnormalities was determined

by using alkaline methyl violet according to **Bloom (1943)**.

#### **2.9: Evaluation of spermatogenic activity:**

A total of 100 seminiferous tubules was scored / animal under ordinary microscope (400 X) to determine the relative frequency of the final stage (elongated spermatozoa) as a clue for testicular activity **(Saidapur and Kamanth, 1994)**.

#### **2.10: Histological examination:**

In this respect, the testis specimen was prepared and stained by haematoxylin and eosin "H and E stain" as described by **Drury and Wallington (1980)**. Stained tissue sections were used for histological studies as outlined by **Bancroft and Stevens (1996)**.

#### **2.11: Statistical analysis of the data:**

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Throughout the present study, the obtained data were subjected to the statistical analysis as outlined by **Snedecor and Cochran (1987)** as well as **SAS Program (1994)**.

### **2- Results:**

#### **3.1: Effect of supplementation of rat's diet with Eichhorniacrassipes, Saccharomyces cerevisiae or date seeds on body weight gain and gonadosomatic index:**

Table 1 showed that there was no significant difference in the body weight gain among all treatments except the significant increase with date seeds in comparison with the control group value (P < 0.05). In addition, Table 1 clarified that all treatment increased significantly the gonadosomatic index when compared with control group (P < 0.05).

#### **3.2: Effect of supplementation of rat's diet with Eichhorniacrassipes, Saccharomyces cerevisiae or date seeds on serum level of testosterone and semen parameters.**

It was evident from Table 1 that serum T level and sperm concentration

increased significantly ( $P < 0.05$ ) when the diet is supplemented with either *S.cerevisiae* or date seeds in comparison to corresponding values of control group. On the other side, supplementation of diet with EC did not induce any significance difference in the aforementioned studied parameters than the control group. In addition, primary abnormalities % was reduced significantly ( $P < 0.05$ ) with date seed supplementation but they are similar with EC or *S.cerevisiae* supplementation in comparison with control values. Furthermore, *S. cerevisiae* or date seeds supplementation increased significantly the frequency of the final stage with mature sperm cells while it was reduced significantly with EC supplementation in comparison to control values ( $P < 0.05$ ).

**3.3:Effect of supplementation of rat's diet with Eichhorniacrassipes, Saccharomyces cerevisiae or date seeds on total antioxidant capacity (TAC) and malondialdehyde (MDA) (Mean  $\pm$ SE):**

Table 1 demonstrated that serum level of total antioxidant capacity increased significantly ( $P < 0.05$ ) with either the supplementation of *S.cerevisiae* or date seeds treatments while it was decreased significantly ( $P < 0.05$ ) with EC in comparison to the control group. Moreover, *S.cerevisiae* or date seeds treatments supplementation in rat's diet reduced the serum level of MDA ( nmol. / ml ) whereas it was increased significantly with EC treatment when compared to control group.

**3.4: supplementation of rat's diet with Eichhorniacrassipes, Saccharomyces cerevisiae or date seeds on histopathological findings in testicular tissues.**

Tissue specimens of the testis related to control group showed normal testicular architecture. It showed normal seminiferous tubules containing normal spermatogenic cells and huge amount of spermatid (Fig. 1, 2). The histological finding in *S.cerevisiae* and date seed group showed similar histological finding as control group (Fig. 5, 6, 7, 8). On the other side, the testes in EC supplemented group revealed few numbers of spermatids within the seminiferous tubules (Fig. 3, 4).

**Table 1: Effect of supplementation of rat's diet with Eichhorniacrassipes, Saccharomyces cerevisiae or date seeds on all studied parameters (Mean  $\pm$ SE):**

Estimated parameters	Control	EC	S. cerevisiae	Date seeds
Body weight gain	21.60 $\pm$ 0.93 <sup>a</sup>	21.20 $\pm$ 1.77 <sup>a</sup>	23.20 $\pm$ 0.97 <sup>a</sup>	37.60 $\pm$ 1.12 <sup>b</sup>
Gonadosomatic index	0.29 $\pm$ 0.09 <sup>a</sup>	0.39 $\pm$ 0.08 <sup>b</sup>	0.40 $\pm$ 0.05 <sup>b</sup>	0.38 $\pm$ 0.04 <sup>b</sup>
Serum testosterone levels	1.86 $\pm$ 0.04 <sup>a</sup>	2.06 $\pm$ 0.32 <sup>a</sup>	2.95 $\pm$ 0.06 <sup>b</sup>	4.08 $\pm$ 0.13 <sup>c</sup>
Primary sperm abnormalities %	3.60 $\pm$ 0.50 <sup>a</sup>	4.40 $\pm$ 0.50 <sup>a</sup>	2.80 $\pm$ 0.37 <sup>ab</sup>	1.60 $\pm$ 0.24 <sup>b</sup>
Sperm concentration X 10 <sup>6</sup> / mm <sup>3</sup>	39.00 $\pm$ 1.18 <sup>a</sup>	38.80 $\pm$ 2.31 <sup>a</sup>	45.60 $\pm$ 2.73 <sup>b</sup>	49.40 $\pm$ 2.95 <sup>c</sup>
Frequency of final stage with mature sperm cells (%)	79.80 $\pm$ 0.86 <sup>a</sup>	69.60 $\pm$ 1.26 <sup>b</sup>	86.20 $\pm$ 1.74 <sup>c</sup>	92.40 $\pm$ 0.92 <sup>c</sup>
TAC (Mmol. / L)	3.26 $\pm$ 0.03 <sup>a</sup>	2.96 $\pm$ 0.09 <sup>b</sup>	4.09 $\pm$ 0.08 <sup>c</sup>	5.45 $\pm$ 0.04 <sup>d</sup>
MDA (nmol. / ml)	2.17 $\pm$ 0.05 <sup>a</sup>	4.16 $\pm$ 0.04 <sup>b</sup>	2.61 $\pm$ 0.08 <sup>c</sup>	1.89 $\pm$ 0.03 <sup>d</sup>



Fig (1): A photomicrograph of testis in adult male albino rats of control group showing normal seminiferous tubules containing normal spermatogenic cells and huge amount of spermatid. Note, interstitial tissue containing blood capillaries and Leydig cells. (H&E) stain X200.

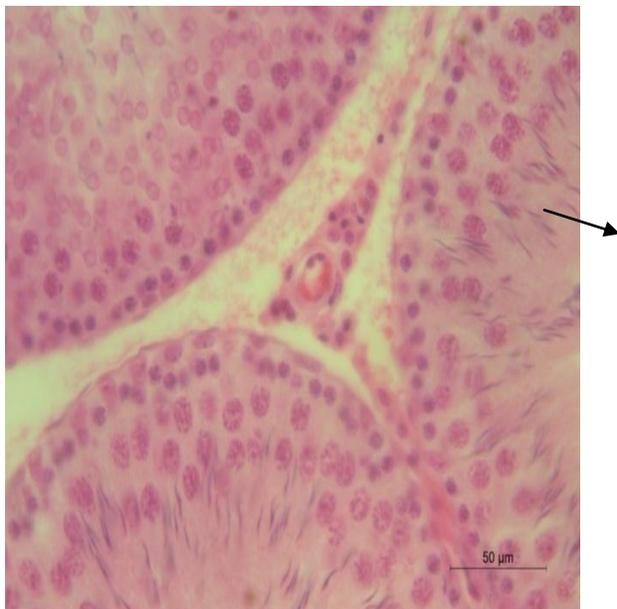


Fig (2): A higher magnification of fig (1) normal spermatogenic cells and huge amount of spermatid. Note, Leydig cells (arrow). (H&E) stain X400.



Fig (3): A photomicrograph of testis in adult male albino rats of Eichhorniacrassipes group showing normal seminiferous tubules containing normal spermatogenic cells but few numbers of spermatids. Note, interstitial tissue containing blood capillaries and Leydig cells. (H&E) stain X200.

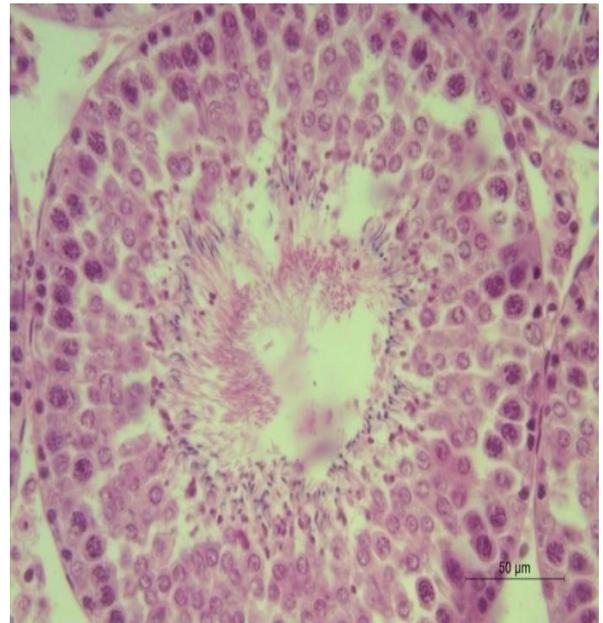


Fig (4): A higher magnification of fig (3) normal spermatogenic cells and few number of spermatids. Note, Leydig cells (arrow). (H&E) stain X400.

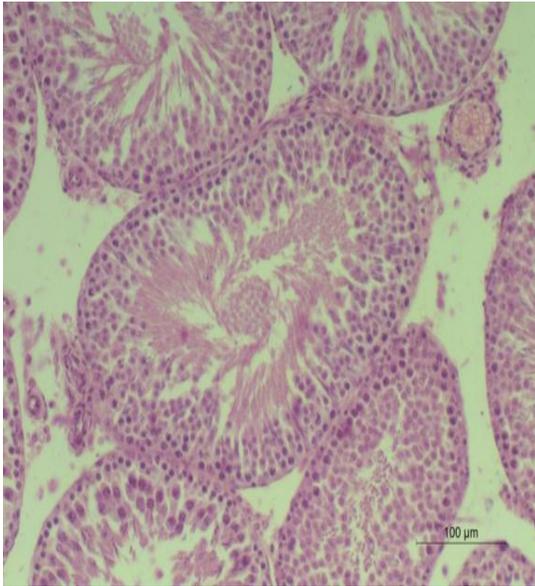


Fig (5): A photomicrograph of testis in adult male albino rats of *Saccharomyces cerevisiae* group showing normal seminiferous tubules containing normal spermatogenic cells and moderate amount of spermatid. Note, interstitial tissue containing blood capillaries and Leydig cells. (H&E) stain X200.

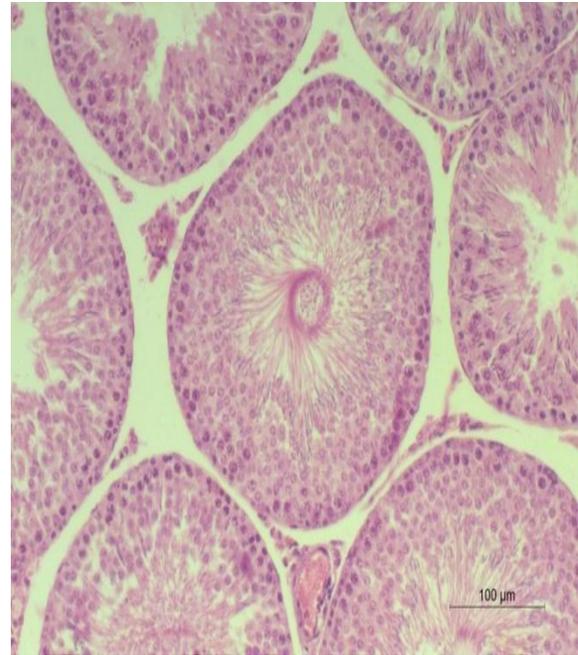


Fig (7): A photomicrograph of testis in adult male albino rats of date seed group showing normal seminiferous tubules containing normal spermatogenic cells and huge amount of spermatids. Note, interstitial tissue containing blood capillaries and Leydig cells. (H&E) stain X200.

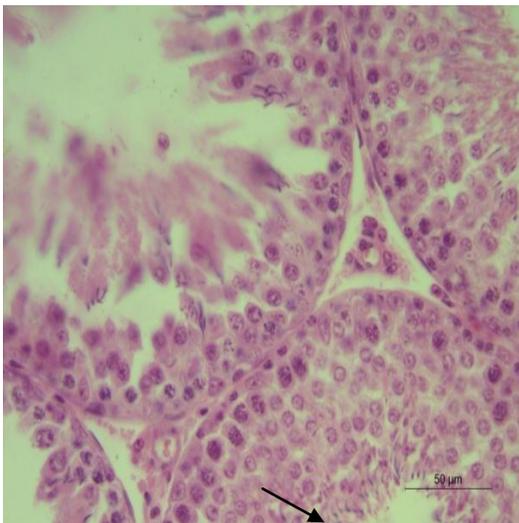


Fig (6): A higher magnification of fig (5) normal spermatogenic cells and moderate amount of spermatid. Note, Leydig cells (arrow). (H&E) stain X400.

### 3- Discussion:

From the results of the current study, table 1 disclosed that there was no significant difference in the body weight gain among all supplemented treatments except the significant increase with date seeds in comparison to the control group value. In addition, Table 1 clarified that all treatment increased significantly the gonadosomatic index when compared to control value. The significant increase of body weight gain could be attributed to the higher nutritive value of date seed. In this concern, **Al-Farsi et al. (2007)** found that the nutritive value of date seeds could be referred to the seed composition as it is composed of 3.1–7.1% moisture, 2.3–6.4% protein, 0.9–1.8% ash, 5.0–13.2 % fat and 22.5–80.2% dietary fiber. Also, the seeds contain high levels of phenolics (3102 - 4430 mg gallic acid equivalents / 100 g), antioxidants and dietary fiber (78–80 g/100 g). The use of date seed in fiber-based foods and dietary supplements are suggested due to the excellent content of dietary fiber in the seed (**Habib and Ibrahim, 2011**). The total dietary fiber found in date seed was 58 %, with 53% of it was insoluble dietary fiber namely as hemicelluloses, cellulose and lignin (**Al-Farsi and Lee, 2008**). Also, proteins (albumin, globulin, prolamin and glutelin) had been found to present in date seed in considerable amount reached to 5 - 6 % of total protein content (**Hamada et al., 2002**). As well, it is considered a good source for minerals as it contains considerable amounts of sodium, potassium, calcium, iron, copper, magnesium, manganese, zinc, phosphorus, lead, cadmium and chromium. (**Shariati et al., 2008**). Therefore the present data emphasizes those of **Ali et al. (2011)** who recommended the addition of date seeds to animal feed to enhance growth. On the other side, **Hamza (1998)** found that the addition of EC into rat diet led to a significant

reduction in body weight concomitant with many pathological and clinicopathological side effects in the liver and kidneys and these alterations were dose dependent which come in agreement with the results of the current study.

It was evident from Table 1 that serum T level and sperm concentration increased significantly ( $P < 0.05$ ) when the diet is supplemented with either *S.cerevisiae* or date seeds in comparison to corresponding values of control group. On the other side, supplementation of diet with EC did not induce any significance differences in the aforementioned studied parameters than the control group. Furthermore, primary sperm abnormalities % was reduced significantly ( $P < 0.05$ ) with date seed supplementation but they are similar with EC or *S.cerevisiae* supplementation in comparison to control values. These results clarified the beneficial roles of date seeds to improve all studied reproductive parameters. In this respect, (**El-Mougy et al., 1991**) have recorded that date extracts increased the concentrations of T in rats as well as levels of follicle stimulating hormone and luteinizing hormone which are responsible for regulating testis functions. Moreover, **Orabi and Shawky (2014)** found that oral administration of date pits palm for male rats caused a significant increase of serum T level. Also, experimentally, date extracts have been shown to increase sperm count in Guinea pigs and to enhance spermatogenesis and increase the concentration of testosterone, follicle stimulating hormone, and luteinizing hormone in rats (**El-Mougy et al., 1991**). Moreover, **Fatma et al. (2009)** found that date seed oil improved sperm motility after 24 h. of incubation ( $P < 0.05$ ) and protected spermatozoa against the deleterious effects of  $H_2O_2$  on motility, viability, acrosome reaction and lipid peroxidation. Upon these data of

spermiogram, date seed oil could be considered a potent treatment for male infertility. In addition, **Sahar-Eissa et al. (2016)** found that Baker's Yeast extraction of *S.cerevisiae* at different doses (10, 20, 50 mg / ml) significantly increased ( $P < 0.05$ ) the sperm vitality, motility percent, and grade of motility. These data encouraged the authors to conclude that Yeast extraction to improve male fertility.

It is well known that dietary antioxidants are important in controlling and ameliorating the harmful effects of oxidative stress therefore high intake of ration with high antioxidant content contributes to reduce risk of oxidative stress-mediated diseases and infertility. In this concern, date seeds and yeast have been shown to contain significant amounts of antioxidants (**Al-Farsi and Lee, 2008**). The current results in Table 1 demonstrated that serum level of total antioxidant capacity increased significantly ( $P < 0.05$ ) with either the supplementation of *S.cerevisiae* or date seeds treatments while it was decreased significantly ( $P < 0.05$ ) with EC in comparison to the control group. Moreover, *S.cerevisiae* or date seeds treatments supplementation in rat's diet reduced the serum level of MDA whereas it was increased significantly with EC treatment when compared to control group. **Joseph et al. (2005)** reported that phenolic and flavonoids content of the date seeds were found to act as antioxidants by chelating redox-active metal ions, inactivating lipid free radical chain reactions, preventing hydro peroxide conversion into reactive ox-radicals, and have an anti-inflammatory properties. The effects of polyphenols is reinforced by experimental studies on animals and human cell lines, which demonstrated that they can contribute to the prevention of degenerative diseases, cardiovascular diseases and obesity as well (**Wojcik et al., 2010**). Moreover, **Orabi and**

**Shawky (2014)** found that oral administration of date pits palm for male rats caused a significant increase of serum testosterone level and hemoglobin concentration (MCH and MCHC) as well as caused a significant decrease in ALT and creatinine. Also, the daily oral administration of seeds extract decreased malondaldehyde level in testicular tissue. Regarding *S.cerevisiae*, it is considered a yeast biotherapeutic agent that possess probiotic properties (**Van der et al., 2005**). Also, previous records clarified that they contain anti-infective effect of  $\beta$ -glucans which is based on their ability to activate leukocytes by stimulating their phagocytic activity and the production of inflammatory cytokines (**Xiao et al., 2004**). In addition, yeast may replace antibiotic growth stimulators (**Erasmus et al., 2005**). A prominent evidence shows that probiotics can influence the metabolic processes of pathogens which lead to infection, and thus confer some type of protection against diseases (**Ravel et al., 2011**).

#### **Conclusions:**

Therefore, the present study highly recommends the use of *S. cerevisiae* as well as date seeds or a mixture of both natural food supplements in order to minimize the ration costs, get the optimal benefit from the natural components of both supplements as well as induce a higher productive and reproductive performance among animals.

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