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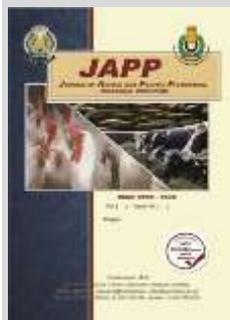
### Influence of Date Palm Pollen Administration on Reproductive Performance, Ovulatory Response, Antioxidants Capacity and Immunity of Rabbit Does

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#### ABSTRACT

This study evaluated the beneficial impact of daily oral administration with date palm pollen (DPP) on the reproductive efficiency, parameters of blood, ovarian activity and reproductive hormonal profiles in NZW rabbit does. Does (n=60) were divided into 3 treatments (20 in each) administered with 0, 250 and 500 mg DPP/doe dissolved in distilled water (2 ml) respectively, for 35 d pre-mating. Does were naturally mated with adult NZW bucks at the termination of treatment period (35 d). Both levels of DPP significantly increased pregnancy and viability rates, and litter characteristic at birth and weaning as compared to control. Oral treatment with both DPP levels significantly increased Hb, RBCs and hematocrit, serum total proteins, albumin, globulin and glucose and high-density lipoprotein, while significantly decreased WBCs serum total cholesterol, triglycerides, and low-density lipoprotein compared with control. Does receive both DPP levels had significantly improved kidney and liver functions, antioxidants capacity and immune response markers than the control group. Both levels of DPP showed significantly ( $P<0.05$ ) remarkable improvement of estrogen, progesterone and prolactin of rabbit does comparing with control. Rate of ovulation and embryo quality and quality were increased ( $P<0.05$ ) by DPP levels comparing with control group. The data showed that orally administration of DPP may help in improving the reproductive efficiency of does via adjusting the hematological and metabolites of blood, reducing lipid profile and peroxidation, improving the antioxidant capacity and immunity, and improving the ovarian activity and embryo quality.

**Keywords:** Rabbits, date palm pollen, reproductive efficiency, antioxidants, immunity

#### INTRODUCTION

In comparison with larger animals, meat production from rabbits in developing countries is a proper source covering the required needs for increasing population (Oseni *et al.*, 2014). Rabbits have a great attention due to their superiority in reproductive efficiency compared with that of other animals of farm and economic efficiency, beside short pregnancy length (El-Ratel *et al.*, 2021a; Abdelnour *et al.*, 2022). Also, increasing litter size in rabbits (4.7 litter) may fulfil needs of animal protein required for human consumption (Castellini *et al.* 2010) during her reproductive lifespan (Bakeer *et al.*, 2021). During cell metabolism, the normal production of oxidant is necessary for the regulations of redox in the cell (Kobayashi *et al.*, 2001), to support the microorganism phagocytosis (Castellini *et al.*, 2000).

Generally, oxidative stress (OS) occurred in the organism when free radicals were increased over body antioxidant defense system, leading to change in homeostasis condition of the animal and in the balance of normal the physiological processes and hormonal profiles of doe rabbits (El-Ratel *et al.*, 2017; Mutweddu *et al.*, 2020). To enhance the impaired OS effects feed additives is essential as a promising thereby. In this respect, Bakeer *et al.* (2021) and El-Ratel *et al.* (2020) mentioned that natural antioxidants were reported to eliminate the negative impacts of OS on the reproductive efficiency of doe rabbits.

In rabbits, phytochemicals affect health and reproduction as natural bioactive and non-nutritive plant chemicals (Hashem *et al.*, 2021; El-Ratel *et al.*, 2020; Abdelnour *et al.*, 2022). Date palm pollen (DPP) is a therapy-based plant and a mixture of plant base. There are male reproductive cells of flowers from (*Phoenix dactylifera*) family palmea (Hassan, 2011), and about 1000 tons of DPP are produced every year by millions of palm trees grown in the Arab Region (El-Neweshy *et al.*, 2013). The DPP is a good economical source of nutrition; it has been long used as a dietary supplement and a folk remedy for curing male infertility in traditional medicine (Arfat *et al.*, 2014). Phytochemical studies have demonstrated the presence of amino acids, vitamins (B1, B2, B12, A, E, and C), and minerals (zinc, selenium, iron and copper) in DPP. This natural product also contains volatile unsaturated fatty acid, carotenoids, tannins, saponins, and flavonoids which play a crucial role as strong antioxidant (Tahvilzadeh *et al.*, 2016 ; El-Kholy *et al.*, 2019). The DPP is comprised of steroidal compounds such as estrone, estradiol and estrol (Tuğba Tatar and Yasemin, 2018).

Beepollen protects the kidneys, and can decrease blood triglycerides, cholesterol, creatinine and urea nitrogen levels in rats (Hu *et al.*, 2003). The dietary supplementations with DPP had significantly enhanced the nutrient digestibility and immunity (Mousa *et al.*, 2018), and increased haemoglobin, total proteins, globulin in blood serum, and anti-oxidant status in tissues of poultry (Refaie

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et al., 2019). In male rabbits, consuming DPP can improve the reproductive performance (Attia et al., 2011) and incorporating DPP in diet of rabbit can enhance the reproduction by disadvantaging the oxidative damage (Wong et al., 2000), but no studies are available on impact of DPP on the reproductive efficiency of rabbit does.

Thus, this study aimed to evaluate the impact of oral administration with DPP on reproductive performance, blood parameters, ovarian activity and reproductive hormonal profiles in rabbit does.

## MATERIALS AND METHODS

The current study was conducted at a private commercial rabbit farm, Mansoura city and Laboratory of Physiology and Biotechnology, Animal Production Department, Faculty of Agriculture, Mansoura University, Egypt.

Two experiments were evaluated in this study including *in vivo* (1st experiment) and *in vitro* (2nd experiment) study.

### 1<sup>st</sup> experiment: "in vivo study"

Sixty nulliparous New Zealand White rabbit does (6-month-old and averaged body weight of  $3010 \pm 50.28$  g) were used in this experiment. Italian wire galvanized cages (60×55×40 cm) supplied with water nipples with nest-box in each cage were used for housing does individually in naturally ventilated building. Rabbits were fed *ad libitum* on a commercial basal diet (Crude protein 17.50%, Crude fibre 13.25 % and 2600 kcal/kg Digestible energy).

Total of sixty doe rabbits were divided into 3 similar groups (20does/group) in a straight run experimental design. In the 1st group (G1), animals were received an oral dose of distilled water (2 ml) and served as control. In the 2nd and 3rd groups, animals were given an oral dose of DPP dissolved in 2 ml distilled water at levels of 250 (G2) and 500(G3) mg per animal, respectively. Treatment started 35 days before mating.

### Reproductive performance of does (*in vivo*)

At the termination of experiment treatment period (35 d), does in all groups were naturally mated with adult NZW rabbit bucks (5 in each). Does were diagnosed for pregnancy 10–12 d post-mating through abdominal palpation. Proved pregnant does number was registered, and then rate of pregnancy (PR) was calculated ( $PR = \text{pregnant does number} / \text{mated does number} \times 100$ ).

Before 2 days of expected kindling date, nest boxes of doe cage were prepared for kindling. Immediately after kindling, rate of kindling (KR) was calculated ( $KR = \text{of kindled does number} / \text{pregnant does number} \times 100$ ). Total borns at birth and born alive after 12 h of kindling were counted per doe, then viability rate was calculated. At weaning (28 d of age), number and viability rate of bunnies was calculated. Also, live body weight and litter weight (at birth and weaning) were recorded.

### Blood sampling:

At the termination of the experimental period, five females in each group were carefully chosen randomly for blood collection from ear vein into one sterile test tube heparinized (for hematological parameters) and another non-heparinized tube (biochemical assessments) for each doe. The first samples (whole blood) were used to estimate some hematological parameters including levels of hemoglobin (Hb), hematocrit value (Ht), red (RBCs), white (WBCs) blood

cells and platelets counts according to Wintrobe (1967). Other samples, blood serum was obtained by centrifugation of clotted blood at 3000 rpm for 20 min. Serum sections were detached and stayed in 1.5 ml Eppendorf tubes and stored at  $-20$  °C, pending analysis. Total proteins (TP) and albumin (Alb), glucose, lipid profile (total cholesterol, triglycerides, high-density and low-density lipoprotein), kidney functions (urea and creatinine) concentrations in blood serum were determined using commercial kits (Egyptian company for biotechnology, Obour City industrial area, Cairo, Egypt). Concentration of globulin (GL) was calculated by subtracting AL from TP concentration.

Aspartate (AST) and alanine (ALT) aminotransferase and alkaline phosphatase activities were assayed in blood serum (Diamond Diagnostics, Egypt). Total antioxidant capacity (TAC), glutathione content (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) levels were determined by using commercially available kits (Bio-diagnostic Co., Recycling Crusher-SBM®) and spectrophotometer. Level of Immunoglobulin's (IgA, IgG and IgM) was assayed by using ELISA kits. Concentrations of serum estradiol-17 $\beta$  (E2) at pre-mating, progesterone (P4) at 15 day pregnancy and prolactin at 7 days' post-partum were determined by using ELISA kits.

### 2<sup>nd</sup> experiment: "in vitro study"

#### Ovulatory response

After 60–64 h of mating, 5 conceived females/group were chosen and slaughtered for *in vitro* evaluating the ovulatory response parameters. After slaughtering, ovaries were immediately excised, submerged in a flacon plastic tissue culture dishes (60 × 15 mm). Harvesting medium (saline solution) was used in Petri dishes and preserved at 38.5°C (El-Ratel et al., 2021b).

Count of all visible follicles with  $\geq 2$  mm in diameter (VF), bleeding follicles (BF) and ovulation sites (CLs) on each ovarian surface was determined, then total follicles (TF) were counted and ovulation rate (OR) was calculated.

$$TF = \text{Number of VF} + \text{BF. OR} = \text{Number of CLs} / TF \times 100.$$

After isolation of reproductive tract of each doe, embryos were flushed by phosphate buffer saline supplemented with fetal calf serum (10% FCS) and gentamycin (50  $\mu\text{g}/\text{ml}$ ). The recovered embryos were searched and counted using stereoscopic microscope, and then embryo recovery rate (ERR) was computed.  $ERR = \text{number of embryos} / \text{number of CLs} \times 100$ . Embryos were washed three times by saline solution, then count and morphological evaluation using stereoscopic microscope of morulae were done to classify to normal and abnormal embryos (El-Ratel et al., 2021b).

#### Statistical analysis:

One-way ANOVA design was used for the statistical analyzing the collected data according to SAS (2000). A completely randomized design was used according to the following statistical model:  $Y_{ij} = \mu + G_i + e_{ij}$  where,  $\mu$  = overall mean,  $G_i$  = group (1-3), and  $e_{ij}$  = the residual error. To separate the significant differences among groups, Duncan's multiple range test (Duncan, 1955) was used at  $P < 0.05$ .

Chi-Square test was used for statistical analysis of all rate values (pregnancy, kindling, ovulation, embryo recovery, normality and blastocyst production).

**RESULTS AND DISCUSSION**

**Results**

**1<sup>st</sup> experiment:**

**Reproductive performance of does**

The effects of treatment of DPP on reproductive performance of rabbit does are presented in Table 1. Reproductive measurements, in terms of pregnancy rate and

litter size at birth (total, live and at weaning), viability rate at birth, average bunny weight (at birth and at weaning) and litter weight (at birth and at weaning) significantly ( $P < 0.05$ ) increased in both treatment groups (G2 and G3) as compared to the control group (G1). While, kindling rate and viability rate at weaning in treatment groups did not differ significantly from those in the control group.

**Table 1. Effect of different levels of date palm pollen on reproductive traits of rabbit does.**

Item	Control (G1)	Date Palm Pollen levels (mg/doe)		P-value
		250 (G2)	500 (G3)	
Pregnancy rate (%)	13/20 (65.00)	17/20 (85.00)	19/20 (95.00)	-
Kindling rate (%)	13/13 (100.00)	17/17 (100.00)	19/19 (100.00)	-
Total litter size (n)	6.15±0.421 <sup>b</sup>	8.71±0.438 <sup>a</sup>	9.37±0.547 <sup>a</sup>	0.0001
Live litter size at birth (n)	5.15±0.450 <sup>b</sup>	8.36±0.498 <sup>a</sup>	8.95±0.521 <sup>a</sup>	0.0001
Viability rate at birth	83.100±4.968 <sup>b</sup>	95.33±2.125 <sup>a</sup>	95.88±1.813 <sup>a</sup>	0.0142
Litter size at weaning (n)	4.846±0.406 <sup>b</sup>	7.929±0.497 <sup>a</sup>	8.68±0.472 <sup>a</sup>	0.0001
Viability rate at weaning (n)	94.86±2.289	94.44±2.23	97.58±1.165	0.3935
Bunny weight at birth (g)	49.31±1.173 <sup>b</sup>	56.07±0.774 <sup>a</sup>	56.68±0.749 <sup>a</sup>	0.0001
Bunny weight at weaning (g)	254.77±23.805 <sup>b</sup>	469.50±29.916 <sup>a</sup>	505.05±27.451 <sup>a</sup>	0.0001
Litter weight at birth (g)	529.85±1.724 <sup>c</sup>	620.38±1.539 <sup>b</sup>	633.263±0.871 <sup>a</sup>	0.0001
Litter weight at weaning(g)	2565.85±213.453 <sup>b</sup>	4925.86±315.031 <sup>a</sup>	5502.32±302.566 <sup>a</sup>	0.0001

Different lower case letters indicate significant differences ( $P < 0.05$ ).

**Hematological variables:**

The effects of DPP on hematological parameters of doe rabbits are listed in Table 2. Oral administration with both levels of DPP significantly ( $p < 0.05$ ) increased Hb

concentration, RBCs count and HT percentage, while significantly ( $p < 0.05$ ) decreased WBCs count compared with the control. Conversely, the levels of palm pollen treatments did not affect blood platelet in rabbit does.

**Table 2. Effect of different levels of date palm pollen on hematological parameters of rabbit does**

Item	Control (G1)	Date Palm Pollen levels (mg/doe)		P-value
		250 (G2)	500 (G3)	
Hb(g/dl)	11.92±0.092 <sup>b</sup>	12.57±0.085 <sup>a</sup>	2.61±0.056 <sup>a</sup>	0.0001
RBCs (x10 <sup>6</sup> /ml)	5.80±0.079 <sup>b</sup>	6.35±0.033 <sup>a</sup>	6.45±0.053 <sup>a</sup>	0.0001
WBCs (x10 <sup>3</sup> /ml)	7.73±0.063 <sup>a</sup>	7.13±0.024 <sup>b</sup>	7.07±0.021 <sup>b</sup>	0.0001
Platelets	216.76±4.373	210.64±3.859	205.11±2.371	0.1177
Ht (%)	35.28±1.136 <sup>b</sup>	42.48±1.084 <sup>a</sup>	41.24±0.998 <sup>a</sup>	0.0010

Different lower case letters indicate significant differences ( $P < 0.05$ ).

**Biochemical parameters:**

Oral treatment with both levels of DPP in G2 and G3 significantly ( $P < 0.05$ ) affect serum metabolites of females, including increasing concentrations of total proteins, albumin, globulin and glucose comparing with G1. However, DPP significantly ( $P < 0.05$ ) reduced serum total cholesterol, triglycerides, and LDL concentrations, and significantly

( $P < 0.05$ ) increased HDL in blood serum as compared to the control (Table 3).

Rabbit does receive both levels of DPP had significantly ( $P < 0.05$ ) improved the kidney and liver functions, in terms of reducing concentration of urea and creatine, and activity of AST, ALT, and alkaline Phosphatase in blood serum as compared to control group (Table 3).

**Table 3. Effect of different levels of date palm pollen on biochemical parameters of rabbit does**

Item	Control (G1)	Date Palm Pollen levels (mg/doe)		P-value
		250 (G2)	500 (G3)	
Blood metabolites				
Total protein (g/dl)	6.22±0.040 <sup>b</sup>	6.82±0.044 <sup>a</sup>	6.88±0.040 <sup>a</sup>	0.0001
(g/dl)	3.30±0.025 <sup>b</sup>	3.45±0.060 <sup>a</sup>	3.56±0.029 <sup>a</sup>	0.0032
Globulin (g/dl)	2.92±0.039 <sup>b</sup>	3.37±0.078 <sup>a</sup>	3.32±0.032 <sup>a</sup>	0.0001
Glucose (mg/dl)	119.46±1.226 <sup>a</sup>	118.51±1.797 <sup>a</sup>	101.74±2.722 <sup>b</sup>	0.0001
Lipid profile				
Total cholesterol (mg/dl)	90.46±1.533 <sup>a</sup>	77.58±2.217 <sup>b</sup>	78.05±2.308 <sup>b</sup>	0.0011
Triglycerides (mg/dl)	78.50±1.461 <sup>a</sup>	64.73±1.565 <sup>b</sup>	63.23±1.127 <sup>b</sup>	0.0001
HDL(mg/dl)	27.63±1.282 <sup>b</sup>	32.59±1.508 <sup>a</sup>	33.91±1.575 <sup>a</sup>	0.0244
LDL(mg/dl)	20.71±0.973 <sup>a</sup>	13.26±1.321 <sup>b</sup>	13.27±1.512 <sup>b</sup>	0.0018
Kidney functions				
Urea(mg/dl)	36.30±1.079 <sup>a</sup>	29.30±1.488 <sup>b</sup>	30.03±1.561 <sup>b</sup>	0.0072
Creatine(mg/dl)	1.91±0.029 <sup>a</sup>	1.31±0.034 <sup>b</sup>	1.30±0.018 <sup>b</sup>	0.0001
Liver functions				
Aspartate transaminase (IU/l)	39.56±1.544 <sup>a</sup>	32.90±0.836 <sup>b</sup>	31.53±1.421 <sup>b</sup>	0.0020
Alanine transaminase (IU/l)	35.64±1.298 <sup>a</sup>	26.86±1.451 <sup>b</sup>	23.69±0.455 <sup>b</sup>	0.0001
Alkaline Phosphatase (IU/l)	46.72±1.009 <sup>a</sup>	41.73±0.809 <sup>b</sup>	39.65±1.369 <sup>b</sup>	0.0018

Different lower case letters indicate significant differences ( $P < 0.05$ ).

**Antioxidants capacity and immunity of rabbit does**

Data presented in Table 4 showed that levels of DPP resulted in higher ( $P \leq 0.05$ ) levels of serum TAC, GSH, GPx, SOD, and CAT as antioxidants capacity markers and lower

( $P \leq 0.05$ ) serum MDA as a lipid peroxidation marker than the control group. Regarding the immune response, also DPP levels led to increase ( $P < 0.05$ ) serum IgA, IgG, and IgM concentrations of females compared with G1 (Table 4).

**Table 4. Effect of different levels of date palm pollen on antioxidants capacity and immunity of rabbit does**

Item	Control (G1)	Date Palm Pollen levels (mg/doe)		P-value
		250 (G2)	500 (G3)	
<b>Antioxidants capacity:</b>				
TAC(mmol/l)	1.14±0.029 <sup>b</sup>	1.34±0.026 <sup>a</sup>	1.37±0.025 <sup>a</sup>	0.0001
GSH(ng/ml)	9.36±0.046 <sup>b</sup>	11.24±0.094 <sup>a</sup>	11.32±0.027 <sup>a</sup>	0.0001
GPx(IU/ml)	6.09±0.0282 <sup>b</sup>	6.14±0.036 <sup>a</sup>	6.23±0.026 <sup>a</sup>	0.0187
SOD (IU/ml)	6.23±0.034 <sup>b</sup>	6.47±0.062 <sup>a</sup>	6.51±0.036 <sup>a</sup>	0.0019
CAT(IU/ml)	107.04±1.445 <sup>c</sup>	126.13±1.412 <sup>b</sup>	132.31±1.312 <sup>a</sup>	0.0001
MDA(nmol/ml)	3.14±0.043 <sup>a</sup>	2.24±0.071 <sup>b</sup>	2.09±0.042 <sup>b</sup>	0.0001
<b>Immunity</b>				
IgA(mg/dl)	148.38±2.172 <sup>c</sup>	157.25±1.666 <sup>b</sup>	163.95±1.268 <sup>a</sup>	0.0001
IgG(mg/dl)	477.71±2.167 <sup>b</sup>	517.08±4.602 <sup>a</sup>	524.76±1.763 <sup>a</sup>	0.0016
IgM(mg/dl)	121.25±1.711 <sup>b</sup>	127.59±1.716 <sup>a</sup>	131.04±0.709 <sup>a</sup>	0.0001

Different lower case letters indicate significant differences ( $P < 0.05$ ).

**Reproductive and prolactin hormones**

Results presented in Table 5 demonstrated the impact of DPP treatments on reproductive (estrogen, progesterone)

and prolactin hormones. Levels of DPP showed significantly ( $P < 0.05$ ) remarkable improvement of estrogen, progesterone and prolactin of rabbit does compared with the control.

**Table 5. Effect of different levels of date palm pollen on estrogen, progesterone and prolactin of rabbit does**

Item	Control (G1)	Date Palm Pollen levels (mg/doe)		P-value
		250 (G2)	500 (G3)	
Estrogen (E2, ng/ml)	10.84±0.201 <sup>b</sup>	12.06±0.2013 <sup>a</sup>	11.93±0.092 <sup>a</sup>	0.0005
Progesterone (P4, ng/ml)	0.48±0.001 <sup>b</sup>	0.66±0.002 <sup>a</sup>	0.69±0.001 <sup>a</sup>	0.001
Prolactin (PRL, ng/ml)	16.85±0.498 <sup>b</sup>	19.67±0.706 <sup>a</sup>	20.31±1.1302 <sup>a</sup>	0.0261

Different lower case letters indicate significant differences ( $P < 0.05$ ).

**2<sup>nd</sup> experiment:**

**Ovulatory activity**

Treatments of both levels of DPP significantly ( $P < 0.05$ ) lowered number of hemorrhagic and total follicles, but

did not demonstrate statistical differences ( $P > 0.05$ ) of large follicles and corpora lutea numbers as compared to the control group. Ovulation rate was significantly ( $P < 0.05$ ) increased by both DPP levels as compared to the control group (Table 6).

**Table 6. Effect of different levels of date palm pollen on ovarian activity of rabbit does.**

Item	Control (G1)	Date Palm Pollen levels (mg/doe)		P-value
		250 (G2)	500 (G3)	
Large follicles (n)	18.40±1.201	15.20±0.969	15.00±1.517	0.1403
Hemorrhagic follicles (n)	3.40±0.509 <sup>a</sup>	1.20±0.200 <sup>b</sup>	0.80±0.200 <sup>b</sup>	0.0003
Total follicles (n)	21.80±1.356 <sup>a</sup>	16.40±1.122 <sup>b</sup>	15.80±1.393 <sup>b</sup>	0.0122
Corpora lutea (n)	16.20±0.583	15.20±0.860	15.00±1.225	0.6298
Ovulation rate (%)	75.32±4.796 <sup>b</sup>	93.07±2.122 <sup>a</sup>	95.253±1.212 <sup>a</sup>	0.0012

Different lower case letters indicate significant differences ( $P < 0.05$ ).

**Embryo yield and quality:**

Results presented in Table 7 showed significant ( $P < 0.05$ ) effect of DPP only on embryo quality, but yield/doe and recovery rate of embryos were not affected by DPP

administration. Both DPP levels significantly ( $P < 0.05$ ) improved quality of recovered embryos in term of increasing normality and reducing abnormality of embryos.

**Table 7. Effect of different levels of date palm pollen on embryos quality of rabbit does.**

Item	Control (T1)	Date Palm Pollen levels (mg/doe)		P-value
		250 (G2)	500 (G3)	
Number of embryos/doe	15.80±0.583	15.00±1.00	15.00±1.225	0.8015
Embryo recovery rate	97.56±1.507	98.46±1.538	100.00±0.000	0.4008
Normal embryos/doe (n)	11.00±0.447 <sup>b</sup>	14.40±0.872 <sup>a</sup>	14.60±1.288 <sup>a</sup>	0.0313
Abnormal embryos/doe (n)	4.800±0.735 <sup>a</sup>	0.60±0.2449 <sup>b</sup>	0.40±0.400 <sup>b</sup>	0.0001
Normality (%)	70.04±4.139 <sup>b</sup>	96.21±1.567 <sup>a</sup>	97.33±2.667 <sup>a</sup>	0.0001
Abnormality (%)	29.96±4.139 <sup>a</sup>	3.79±1.567 <sup>b</sup>	2.67±2.666 <sup>b</sup>	0.0001

Different lower case letters indicate significant differences ( $P < 0.05$ ).

**Discussion**

As a scope of view, antioxidants are the compounds and reactions that scavenge, dispose, and suppress ROS production, or combating their activities. Antioxidants reduce the OS by shattering the oxidative chain reaction (Niederberger, 2012; Mansuri et al., 2014). Dates, as source of antioxidants, can neutralize and destroy ROS (NO, OH, and H<sub>2</sub>O<sub>2</sub>) and its precursors to prevent lipid peroxidation by antioxidant enzymes activity stimulation (Vyawahare et al.,

2008). Aqueous date extract has proper activity as an antioxidant because it contains polyphenols particularly flavonoids (Cherubini et al., 1999; Al-Farsi et al., 2005). It is well known that the phenolic compounds have antioxidant activity as deactivator and captivator ROS and decomposing peroxides (Shahidi et al., 1992). Our results showed that DPP extract affected the reproductive traits through increasing the pregnancy rate and litter size. Increasing pregnancy rate and litter size of doe rabbits in our study is agreement with the

statement that oral treatment of doe rabbits with natural antioxidants enhanced their reproductive efficiency (El-Ratel *et al.*, 2020), and particularly, bee pollen that revealed positive impact on fertility rate of rabbits (Attia *et al.*, 2011). In this respect, Attia *et al.* (2015) showed that bee pollen, as a natural growth promoter, is equally potent for improving reproductive traits of V-line rabbits in term of increasing conception rate. It is of interest to observe that improving reproductive traits of doe rabbits is in association with increasing pre-mating levels of estrogen and prolactin levels. Moshfegh *et al.* (2016) found that DPP significantly increased levels of serum estrogen in female mice. Also, Abdel-Khalek *et al.* (2022) reported that increasing pre-mating level of PRL is in association with improving reproductive performance and PRL level at mid-pregnancy of California doe rabbits. The improvements in litter weights and viability rate were accompanied by increasing PRL due to increasing yield of milk and improving survival rate of litters (Attia *et al.*, 2015; Abdelnour *et al.*, 2022). Moreover, El-Kholy *et al.* (2019) indicated that the positive impact of DPP proven on productive traits of females could be due to the higher content of macro- and micro-nutrients such as amino acids, vitamins, and minerals), agents of protection, and phytosterols like flavonoids, carotenoids and phenolic constituents. Generally, many investigators found that DPP has physiologically anti-bacterial, anti-viral (Aamir *et al.*, 2013), anti-inflammatory, anti-proliferative (Elberry *et al.*, 2011), anti-diabetic (Miller *et al.*, 2003) and antioxidant (Saleh *et al.*, 2011a) properties.

The present results concerning the improvement in the reproductive performance of does as affected by DPP *in vivo* study were in accordance with the ovulatory response parameters. Rabbits are induced ovulatory animals, because the release of LH pre-ovulatory surge is a reflected by stimulation of the sensory and neuro-endocrine (Dufy-Barbe *et al.*, 1973). Supplementation of DPP induced higher ovulatory response by reducing the number of TF and increasing CLs numbers, subsequently increasing ovulation rate and improving quality of embryos. The noticed increase in CLs number in DPP treatment groups may be attributed to the positive impact of DPP on LH surge, reflecting higher ovulation rate in DPP treatment groups. Similarly, Al-Samarrai *et al.* (2017) showed that DPP might impact on the levels of LH in rabbits, because DPP contains estradiol and flavonoid (Robert and Krueger, 2007), cytochrome p450 enzyme and like-estrogen compounds. Cytochrome p450 enzyme is able to transfer cholesterol to progesterone and increase progesterone hormone (Moshfegh *et al.*, 2016). The containing DPP estrogen, which stimulates both FSH and LH responsible for ovulation (Hammed *et al.*, 2012). Increasing estrogen level of doe rabbits treated with DPP in our study may promote the oviduct growth and help to form proteins for the oviduct and stimulate its formation (Saleh *et al.*, 2021b). Also, improving pre-mating PRL profile in treatment groups is in agreement with El-Ratel *et al.* (2020) and Obochi *et al.* (2009), who reported that antioxidants extract stimulates the release of this hormone through anterior-pituitary activation. In this way, Abel-Khalek *et al.* (2022) found a storing correlation between pre-mating PRL profile and litter size (ovulation rate) of California rabbits.

In rabbits, blood hematology and biochemical reference values may help as markers of the physiological aspects and health status. Our study showed that DPP extract

had positive effects on blood hematology traits through increasing the Hb and RBCs and decreasing WBCs as compared to the control. Tamemy and Amen (2019) found a significant effect of DPP on blood traits of local rabbits. The significant increase in hemoglobin level may reflect a positive impact on the protection of RBCs membrane and increasing level of iron and its absorption from the digestive tract (Saleh *et al.*, 2021b). Also, DPP contain phenolic compounds and certain other substances, acting as an antioxidant and protecting red blood cells from oxidative decomposition (Maertens, 1992).

The oral administration of DPP extract increased serum total proteins, albumin, globulin and HDL concentrations and decreased total cholesterol, triglycerides, LDL, glucose, urea, creatine, AST, ALT, and ALP as compared to the control. Such results may indicate increasing the process of protein synthesis and decreasing the process of protein destruction in DPP treatment groups as compared to control. The reduction in cholesterol level in DPP groups may be due to the containment of DPP on tannin compounds which inhibit certain enzymes responsible for the synthesis of cholesterol (Al-Esawii and Abd-Alhussain, 2012). Also, containing polyphenol in DPP works to lower cholesterol (Anderson, 2004). The observed reduction in glucose level by high DPP level is attributed to that pollen grain contained insulin (Mohamed and Shanoon, 2011) which plays an important role in lowering glucose levels in the blood. The significant decrease in AST and ALT activity compared to the control treatment may be due to the containment of DPP antioxidants (flavonoids) that protect the unsaturated fatty acids in the membranes of cellular oxidation processes and retention membranes with optional permeability and cell retention and nonpermeability outside the cell body (Aydilek, 2004).

Regarding the antioxidants capacity and immunity, DPP administration significantly increased TAC, GSH, GPx, SOD and CAT of doe rabbits which may be due to high contents of bioactive volatile unsaturated fatty acid and flavonoids in DPP. These compounds have potential as antioxidants, and as dietary supplementation to promote health, nutritionally and physiologically (Saleh *et al.*, 2021a). These results are in agreement with Refaie *et al.* (2019), who showed that the average of TAC in hens was increased by treatment with DPP or DPP extract.

## CONCLUSION

Orally administration of DPP may help in improving the reproductive efficiency of doe rabbits via modifying the hematological and blood metabolites, reducing lipid profile and peroxidation, improving the antioxidant status and immunity, and improving the ovarian activity and quality of embryos. These consequences enhanced the reproductive outcomes of doe rabbits treated with DPP administration at a level of 250 mg/doe for 35 days pre-mating.

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## تأثير المعاملة بطلع النخيل على الأداء التناسلي ، الاستجابة المبيضية ، حالة مضادات الأكسدة والمناعة لامهات الارانب

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### الملخص

تهف هذه الدراسة الى تقييم تأثير تجريع امهات الارانب النيوزيلندي البيضاء يوما بمستخلص طلع النخيل على الكفاءة التناسلية خصائص الدم وحالة نشاط المبيض والهرمونات الجنسية . استخدم في هذه التجربة 60 ام قسمت الى 3 مجموعات، كل مجموعة على 20 ام، جرعت امهات المجموعة الأولى والثانية والثالثة بـ صفر ، 250 و 500 ملجم ام من مستخلص طلع النخيل لكل ام على التوالي ، والتي تم اذابتها في 2ملى ماء مقطر لمدة 35يوم قبل التلقيح ، كقترعة معاملة . لقت الامهات بذكور من نفس النوع بعد انتهاء فترة المعاملة (35 يوم). وقد أظهرت النتائج وجود زيادة معنوية في معدلات الحمل والحيوية وخصائص البطن عند الميلاد، والظلم للامهات المعاملة بمستخلص طلع النخيل مقارنة بمجموعة الكنترول. كما لوحظ زيادة معنوية في تركيز الهيموجلوبين وعدد كرات الدم الحمراء والهيماتوكريت، مع زيادة معنوية في تركيز كلا من البروتينات الكلية، الالبيومين، الجلوبيولين، الجلوكوز والليپوبروتينات عالية الكثافة في سيرم الدم، مع انخفاض معنوي في عدد كرات الدم البيضاء، تركيز الكوليسترول، الدهون الثلاثية و الليپوبروتينات منخفضة الكثافة مقارنة بالكنترول. وجد ان الامهات المعاملة بمستخلص طلع النخيل بجمع مستوياته أدت الى تحسن معنوي في وظائف الكبد والكلى، نشاط مضادات الاكسدة والاستجابة المناعية للجسم مقارنة بمجموعة الكنترول. كما لوحظ وجود تحسن معنوي في تركيز الهرمونات الجنسية ( الاستروجين، البروجستيرون و البرولاكتين) للامهات المعاملة بطلع النخيل مقارنة بالكنترول. معدلات التويض وعدد وجودة الاجنة زادت معنويا للامهات المعاملة بطلع النخيل مقارنة بالكنترول. نستخلص من هذه الدراسة ان معاملة امهات الارانب بمستخلص طلع النخيل أدت الى زيادة الاداء التناسلي وخصائص الدم الهيمولوجية والبيوكيميائية ونشاط المبيض وجودة الاجنة ونشاط مضادات الاكسدة والاستجابة المناعية.