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The influence of date palm pollen (DPP) diet supplementation on various reproductive parameters in female albino rats

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ABSTRACT

The effect of Date Palm Pollen (DPP) on the hormonal profile, histological and reproductive parameters in pregnant rats and rat pups following gestation was investigated in this study. The effect of DPP on estrous cycle, pregnancy, reproductive organs, hormonal profiles and the development of pups was investigated by administering various doses of DPP pollen to pregnant albino rats for the duration of their pregnancy. DPP significantly increased ($P<0.05$) the weight of the uterus and ovaries in pregnant rats, it also significantly increased ($P<0.05$) the number of implantation sites in treated rats, as well as crown-rump length. Morphometric analysis revealed that DPP increased uterine weight and height as well as ovarian weight, height and width. Histological analysis revealed numerous secondary follicles in the ovaries and numerous glands in the uterine stroma of the treated groups. The number of glands decreased after DPP administration was discontinued. DPP increased mitotic figures in the uterine stroma, and robust collagen fibers were found in the treated groups compared to the Control Group. DPP increased serum hormone levels, the number of uterine glands in treated groups, which can be attributed to its content of steroid-like biocomponents, but it did not have any observable effect on the rat pups after gestation.

Key words: date palm; histomorphology; estrogen; ovary; pollen; progesterone; sex hormones; uterus; Wistar albino rats

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Introduction

The World Health Organization (W.H.O.) defines infertility as a disease of the male or female reproductive system defined by the failure to achieve pregnancy after 12 months or more of regular unprotected sexual intercourse (GORE et al., 2015).

Male and female infertility has risen as a major worldwide concern, with an approximate 15% of couple unable to achieve pregnancy within the space of 12 months despite frequent unprotected sexual intercourse, and with about 10% of these infertilities having no specific cause (ZHANG et al., 2013). Male infertility is usually associated with low sperm quality, mobility or motility, whereas female infertility is caused by a variety of issues, ranging from abnormality of the female reproductive organs (the uterus, Fallopian tubes and ovaries), includ- ing polycystic ovarian syndrome (PCOS), uterine fibroid, pelvic inflammatory disease, premature ovarian failure (POF), or is due to hormonal imbalances, the use of recreational drugs, consumption of high dosage non-steroidal anti-inflammatory drugs, amongst other issues (AKBARIBAZM et al., 2021). Infertility care involves the use of assisted reproductive technologies (ART) to help establish a pregnancy.

ART is the assistance provided to address fertility by combining a wide range of procedures in the attempt to overcome the natural obstacles preventing fertilization and implantation including interventions such as *in-vitro* fertilization (IVF), artificial insemination, intracytoplasmic sperm injections (ICSI), gamete intrafallopian transfer (GIFT) amongst others (BEGUM, 1970). Although these procedures have been available for more than three decades, they are mostly still largely unavailable, inaccessible and unaffordable to many childless couples in many parts of the world, particularly in low and middle-income countries (GORE et al., 2015).

Many studies have revealed the use of ethnopharmacology to treat infertility, especially in low income and middle-income countries (LMIC) where fertility treatment and procedures to assist reproduction may not be available or affordable to many couples with infertility issues. Plants used to treat both male and female infertility include *Ceratonia siliqua L., Anastatica hierochuntica L.* (JARADAT and ZAID, 2019) *Punica granatum, Matricaria chamomilla, Withania somnifera, Trifolium pratense, Camellia sinensis,* Cinnamomum, *Foeniculum Vulgare, Nigella sativa, Phoenix dactylifera* (AKBA-RIBAZ et al., 2021).

Phoenix dacylifera belongs to the palm family and has been a food source in many parts of the world, essentially in Asia and Africa (BEDAN et al., 2023). It is also found to have anticancer and anti-inflammatory properties because of its phytochemical content. Researchers have also recorded the promising fertility effects of the plant components, including the pollen. Date palm pollen (DPP) has a high moisture content (28.80%), ash (4.57%), crude fiber (1.37%), crude fat (20.74%), protein (31.11%) and carbohydrate (13.41%) (HASSAN et al., 2012). Studies carried out to determine the effect of DPP on the male reproductive organs revealed that DPP increased the epididymis– body weight ratio of the testis due to the increase in the concentrations of estradiol and testosterone secretion (ELBERRY et al., 2011). MEHRABAN et al. (2014) proved that DPP improved sperm parameters and gonads in rats, improved certain sexual behavioral parameters in male rats and increased the weight of the testicles and serum testosterone levels (SALMANI et al., 2022).

In laying hens, DPP improved egg weight, ovarian follicles' mass and quality and the frequency of laying, suggesting its fertility effects in hens, thereby improving egg yield (SALEH et al., 2021). It is proposed that DPP can create an appropriate situation for oogenesis and maintain efficient fertility in female mice by promoting oogenesis, supporting oocyte maturation, regulation of hormones, bolstering pregnancy, and preventing postpartum hemorrhage (ABDI et al., 2017; SHEHZAD et al., 2021) all while being a naturally available and affordable alternative to orthodox fertility medication.

This research was carried out to investigate the effect of DPP on the mean body and organ weight, hormone profile and histology of the female reproductive organs in pregnant rats, and also on the estrus cycle, hormonal density following gestation.

The study also investigates the effect of DPP on the rat pups.

Materials and methods

Plant materials. *Phoenix dactylifera* pollen (DPP) was obtained from Shuwarin Town, Kiyawa Local Government Area, Jigawa State, Nigeria. The plant was identified and authenticated by a botanist at the Department of Biological Sciences, University of Maiduguri. A specimen voucher (Ref No. UMM/FPH/AMA/080) was deposited in the herbarium of the Pharmacology Department. The pollen was dried and refrigerated at 4°C.

Extraction procedures. A total yield of 254. 28g of DPP crude powder was obtained from 5.5kg of date palm kernels. Aqueous extraction was performed by adding 100 g of the powder to 100 ml of distilled water and re-fluxing for one hour. The resulting solution was filtered to remove debris, and concentrated using a hot air oven at 50°C.

Experimental animals. Experimental animals were obtained from the animal facility at the Department of Veterinary Medicine, University of Maiduguri, and the National Veterinary Research Institute, Vom. The rats were housed in standard laboratory conditions at 32±4°C with access to food and water ad libitum. Ten male rats (200-240 g) and forty female rats (80-100 g) were used for the study.

Preparation of DPP suspension. An aqueous suspension of DPP crude powder was prepared at a concentration of 400 mg/ml in distilled water. Different doses were administered on the basis of the animals` body weight, using oral gavage. The stock solution was administered to all rats to ensure that the rats received constant and same amounts of bioactive ingredients.

*Experimentaldesign.*Femaleratsweredivided into four groups: the Control (Group I) and three experimental groups receiving DPP at 78, 156, and 312 mg/kg, respectively. The concentrations were determined from the results of the acute toxicity study carried out in a previous study (BEDAN et al., 2023). DPP was administered throughout estrous, in late diestrus, and the entire gestation period. The rats were sacrificed at different stages using ketamine hydrochloride (0.1 g/kg) via intramuscular injection. Five rats were sacrificed just before gestation from each group, and the rat uteri and implantation areas were determined. The remaining five rats were allowed to bear their young to determine the effect of DPP on the rat pups. The rats were euthanized by administering 0.1 mg/kg ketamine hydrochloride anesthetic (Rotexmedica, Trittau, Germany) via intra-muscular injection into the left thigh of the rats (ATTAH et al., 2022). The rats were laid in a supine position and a thoraco-abdominal dissection was performed to expose the pelvic organs. The uterus was harvested, trimmed of any adherent connective tissue and its wet weight taken. It was then fixed in formal saline / Bouin's fluid, and processed for routine tissue processing. Sections were stained with Hematoxylin and Eosin and Verhoef Van Gieson (VVG) for collagen and muscle fiber histopathological examination.

Determination of ovulation. Ovulation was assessed using daily vaginal smears, as described by (MARCONDES et al., 2002). The presence of keratinized cells and thin mucus strands indicated the late diestrus phase. 0.2 ml of normal saline (0.9 %) was drawn into the pipette and the tip of the pipette was pushed gently into the entrance of the vagina to a depth of 2-4 mm and the fluid was released and flushed into the vagina and then sucked back into the pipette. This was repeat- ed twice or three times until the fluid was cloudy. A small amount of the cell suspension ob- tained from the vagina along with the fluid (nor- mal saline) was expelled onto a labeled glass slide and a cover slip was placed on the slide.

These slides were observed under a light microscope at a magnification of 10x and 40x objective lenses. The appearance of keratinized cells, with an irregular and shrunken appearance, and a few leucocytes, vacuolated and nucleated basophilic epithelial cells, indicated the late stage of diestrus. Also, the appearance of thin mucus strand was also an indication (BEDAN et al., 2017; MARTINS et al., 2005).

Ten male rats were mated with the female rats during the proestrus phase of the estrus cycle to bring about conception. The proestrus phase of the estrous cycle was chosen because the female rats

permit mating at the end of proestrus (SUCKOW, 2005). One estrus cycle is completed within four to five days in adult rats. The greatest changes in the accessory reproductive organs are known to occur between the late diestrus and estrus phases of the estrus cycle (ASTWOOD, 1939). Pregnancy was determined by the methods described by (PARONIS et al., 2015).

DPP was administered daily and continued during gestation until parturition. On the 20th day of the gestational period, five rats each from all the groups were euthanized using ketamine hydrochloride (2mg/kg) via intra-muscular injection, cesarean sections were performed and macroscopic examination of the uterine implantation pattern was carried out, including the sites and stage of implantation, the intra-uterine growth rate (crown rump), and the ovarian/ uterine mass index and diameter. The fetuses were examined for congenital physical malformations. Blood samples were obtained by cardiac puncture from each rat and the serum density levels of the female reproductive hormones (FSH, LH, Progesterone and Estrogen) in the maternal blood were measured and analyzed, using ELISA kits.

The remaining five rats bore their young and the pups were studied to determine developmental-landmarks and whether DPP had any effect on live birth, the number of pups, malformations or developmental anomalies and the weight of the pups.

Hormonal profile. Biochemical assays were conducted to find the serum density levels of reproductive hormones (Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), progesterone, and estrogen). The assay kits were obtained from Chemux Bioscience, Inc., 385 Oyster Point Boulevard Suite5-6. South San Francisco. CA944080. Cat. No. 10004).

Histological analysis. The organs harvested from all the study segments were fixed in appropriate fixatives (Bouin's fluid and formal saline), then embedded in paraffin and sectioned at 5µm. Sections were stained with Hematoxylin and Eosin, reproductive organs (uterus) were counter stained by Verhoeff-Van Gieson (VVF) and mounted in Canada balsam. A histopathological examination of all the slides was carried out.

Histomorphometric analysis. A histomorphometric study of the primary, secondary and tertiary ovarian follicles was carried out. Oocyte structural morphology, the corpus luteum and atretic corpora were also studied according to the method described by BANCROFT et al. (1996) and MOSHFEGH et al. (2016). Analysis of the muscles and elastic fibers of the uterus was carried out using a special staining technique with VVG. Macroscopic observations conducted included recording weekly changes in body weight (g), ovarian and uterus weight per body weight (g/g), ovarian mass index and the diameter, length and weight of the uterine horn, according to the procedure outlined by MOSHFEGH et al. (2016). Biochemical assays were conducted to determine the serum density levels of reproductive hormones.

Statistical analysis. Data were presented as mean ± SD. Statistical analysis was conducted using GraphPad Prism 8. One-way ANOVA was used to analyze variations among the groups, and the t-test was used to test differences between the groups. A P-value <0.05 was considered statistically significant.

Results

The effect on maternal body weight during gestation. Oral administration of DPP the during estrus, diestrus and gestation periods led to a slight reduction in body weight in the experimental groups compared to the Control, as seen in Groups I-IV (Table 1).

The effect on the Estrus cycle and pregnancy outcome. Ten female nulliparous rats in each group were mated to achieve pregnancy. Five rats were selected for each group. There was a loss of pregnancy recorded in a single rat each in groups I and II, but none recorded in Groups III and IV. Following the sacrifice of rats prior to gestation, more implantation sites were found in Groups III and IV (6.0 and 6.7 respectively) compared to 4.7 and 4.0 in Groups I and II (Fig. 1). Implantation sites were determined by dissecting the uterine horns.

Table 1. The effects of administration of DPP on the mean body weight of female rats during gestation

Group	Treatment	Initial body weight (g)	Final body weight (g)	Body weight difference	Percentage weight gain
I	0 mg/ml DPP	141.60 ± 5.38	162.40 ± 8.03	20.8	28
II	78 mg/ml DPP	138.30 ± 5.07	155.00 ± 5.83	16.7	23
III	156 mg/ml DPP	134.50 ± 2.73	153.20 ± 4.87	18.7	26
IV	312 mg/ml DPP	148.40 ± 5.13	165.40 ± 7.59	17.0	23

Results are presented as Mean ± SEM. mg/ kg = milligram per kilogram, g = gram

The right and left ovaries were significantly heavier in rats in Groups III and IV, who received the highest concentration of the extract, and the weight of the uterus was heaviest in Group IV. There was no significant difference in placental weight between the groups of rats that bore their young, ut in the pups, the crown-rump length was longest in Group III at 0.86cm (Table 2).

Hormonal profile during gestation. The results showed that administration of DPP grains did not have any significant effect on maternal serum LH and FSH concentrations. Estrogen concentrations were significantly increased in Groups II and IV when compared to the Control Group, and serum progesterone was also significantly ($P<0.05$) elevated in Group IV when compared to the Control Group (Table 3).

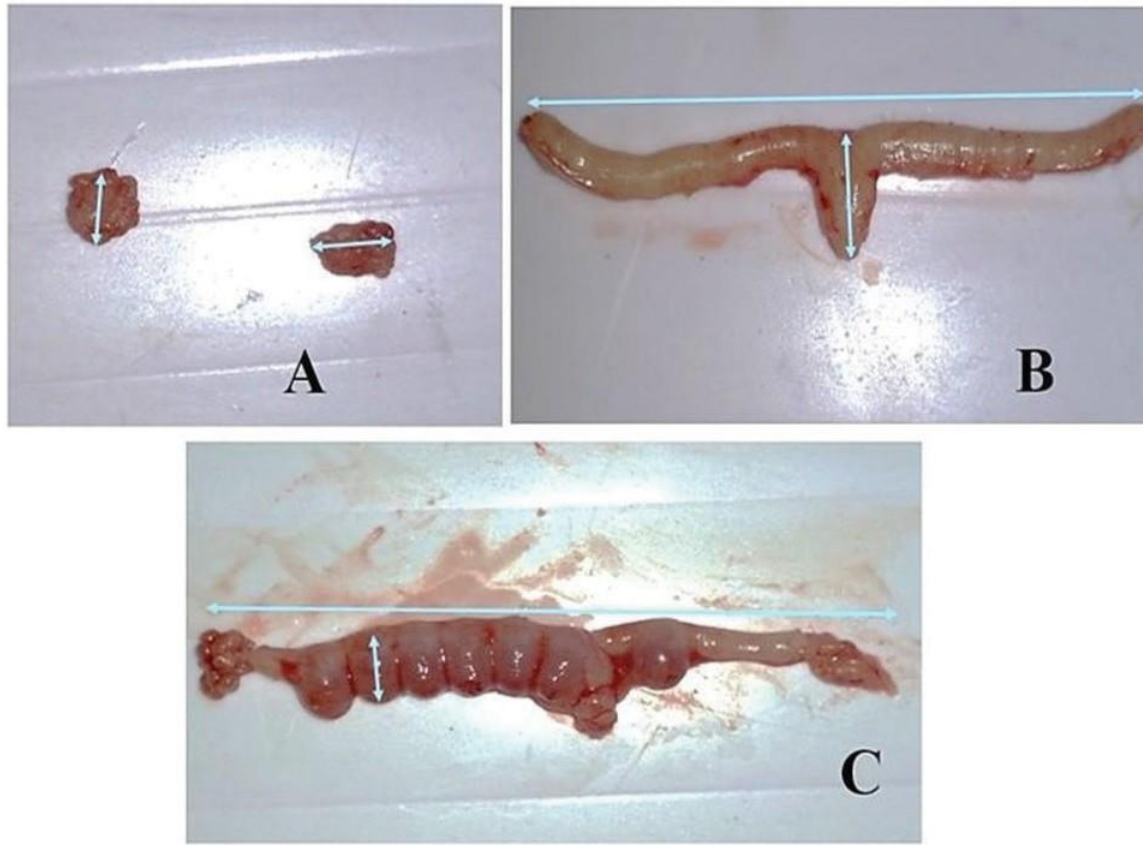

Fig. 1. Morphometric measurements of the ovaries and uterine horns

A= ovaries, B=uterine horn, C= gravid uterine horn. The horizontal line represents the length while the vertical lines measure the width

Table 2. The effect of DPP during the estrus cycle and gestation

PARAMETER	GROUP I	GROUP II	GROUP III	GROUP IV
NUMBER OF FEMALES MATED	10	10	10	10
NUMBER OF PREGNANT RATS	10	10	10	10
RATE OF PREGNANCY (%)	100	100	100	100
PERCENTAGE OF VIABLE PREGNANCY (%)	80	80	100	100
RATE OF MISCARRIAGES (%)	20	20	-	-
PLACENTAL WEIGHT (G)	0.69 ± 0.15	0.60 ± 0.05	0.58 ± 0.23	0.59 ± 0.12
MEAN NUMBER OF IMPLANTATION SITES	4.75 ± 0.75	4.00 ± 0.71	6.00 ± 0.41 *	6.70 ± 0.95 *
LEFT OVARIAN WEIGHT (G)	0.04 ± 0.01	0.03 ± 0.01	0.08 ± 0.04 *	0.14 ± 0.07 *
RIGHT OVARIAN WEIGHT (G)	0.04± 0.01	0.06 ± 0.06	0.08 ± 0.04 *	0.11 ± 0.05 *
WEIGHT OF UTERUS (G)	3.91 ± 0.05	3.46 ± 0.06	3.25 ± 0.10	4.21 ± 0.54 *
CROWN-RUMP LENGTH (CM)	0.85 ± 0.15	0.79 ± 0.12	0.86 ± 0.17	7.021 ± 0.08 *

Results are presented as Mean ± SEM. Significance relative to the Control Group. % = percentage, L= left, R= right, % = percentage, g = gram, cm = centimeter, *= P<0.05, **= P<0.01

The weight of the rat pups. DPP administration decreased the weight of rat pups following maternal exposure during the gestation period. The mean weight of rat pups in groups that were administered DPP at concentrations of 156mg/kg and 312mg/kg showed a reduced weight when compared to the Control Group (Table 4).

Histology. The morphometric measurements taken of the ovary and uterus are presented in Fig. 1, which shows the areas that were measured in the ovarian and uterine tissues. Analysis of the measurements showed that DPP increased uterine and ovarian weight (Table 2). Histological anal- ysis of the tissues revealed the presence of several Graafian follicles in all groups, with more secondary follicles observed in Group IV (Fig. 2). The uter- us of the rats in Groups II-IV showed numerous glands in the stroma of the treated groups when compared to the Control Group (Fig. 3). In Groups I-IV, the administration of the extract during the ges-

Table 3. The effect of administration of an aqueous suspension of DPP on mean hormone density level in female rats during gestation

PARAMETER	GROUP I	GROUP II	GROUP III	GROUP IV
NUMBER OF FEMALES MATED	10	10	10	10
NUMBER OF PREGNANT RATS	10	10	10	10
RATE OF PREGNANCY (%)	100	100	100	100
PERCENTAGE OF VIABLE PREGNANCY (%)	80	80	100	100
RATE OF MISCARRIAGES (%)	20	20	-	-
PLACENTAL WEIGHT (G)	0.69 ± 0.15	0.60 ± 0.05	0.58 ± 0.23	0.59 ± 0.12
MEAN NUMBER OF IMPLANTATION SITES	4.75 ± 0.75	4.00 ± 0.71	6.00 ± 0.41 *	6.70 ± 0.95 *
LEFT OVARIAN WEIGHT (G)	0.04 ± 0.01	0.03 ± 0.01	0.08 ± 0.04 *	0.14 ± 0.07 *
RIGHT OVARIAN WEIGHT (G)	0.04± 0.01	0.06 ± 0.06	0.08 ± 0.04 *	0.11 ± 0.05 *
WEIGHT OF UTERUS (G)	3.91 ± 0.05	3.46 ± 0.06	3.25 ± 0.10	4.21 ± 0.54 *
CROWN-RUMP LENGTH (CM)	0.85 ± 0.15	0.79 ± 0.12	0.86 ± 0.17	7.021 ± 0.08 *

Results are presented as mean ± SEM. Significance relative to the control group, SD = Serum density, LH= Luteinizing hormone, FSH= Follicle stimulating hormone, D = mlU/ml, E= pg/ml, F= g/ml, 0 (Group 1) = Control, 78 (treatment) = Group 2, 158 (treatment) = Group 3, 312 (treatment) = Group 4, * = P<0.05, * * = P<0.01

Table 4. The effect of maternal exposure of DPP on the mean body weight of pups at birth following 21 days of lactation

GROUPS	BODY WEIGHT (BIRTH)	BIRTH WEIGHT (DAY 21)	WEIGHT DIFFERENCE	PERCENTAGE WEIGHT GAIN (%)
I	6.31 ± 0.64	25.10 ±2.11	18.8	26
II	6.13 ± 0.27	24.36 ± 1.39	18.3	26
III	5.50 ±0.13	22.44±1.38	16.9	24
IV	5.43 ± 0.17	22.40 ± 1.69	17.0	24

Results are presented as Mean ± SEM, Unit of weight - gram

tation period reduced the number of uterine glands compared to the Control Group (Fig. 4). The endometrial stroma revealed more lymphocytes in the stroma of the treated groups, with several mitotic figures observed in the treated groups (Fig. 4), which denoted cell division in these groups. The stroma of the uterus in the treated groups had numerous and more robust collagen fibers, in Groups II-IV, compared to the Control Group (Fig. 5) which signifies the role the extract plays in maintaining the integrity of, as well as providing strength and support to the uterus.

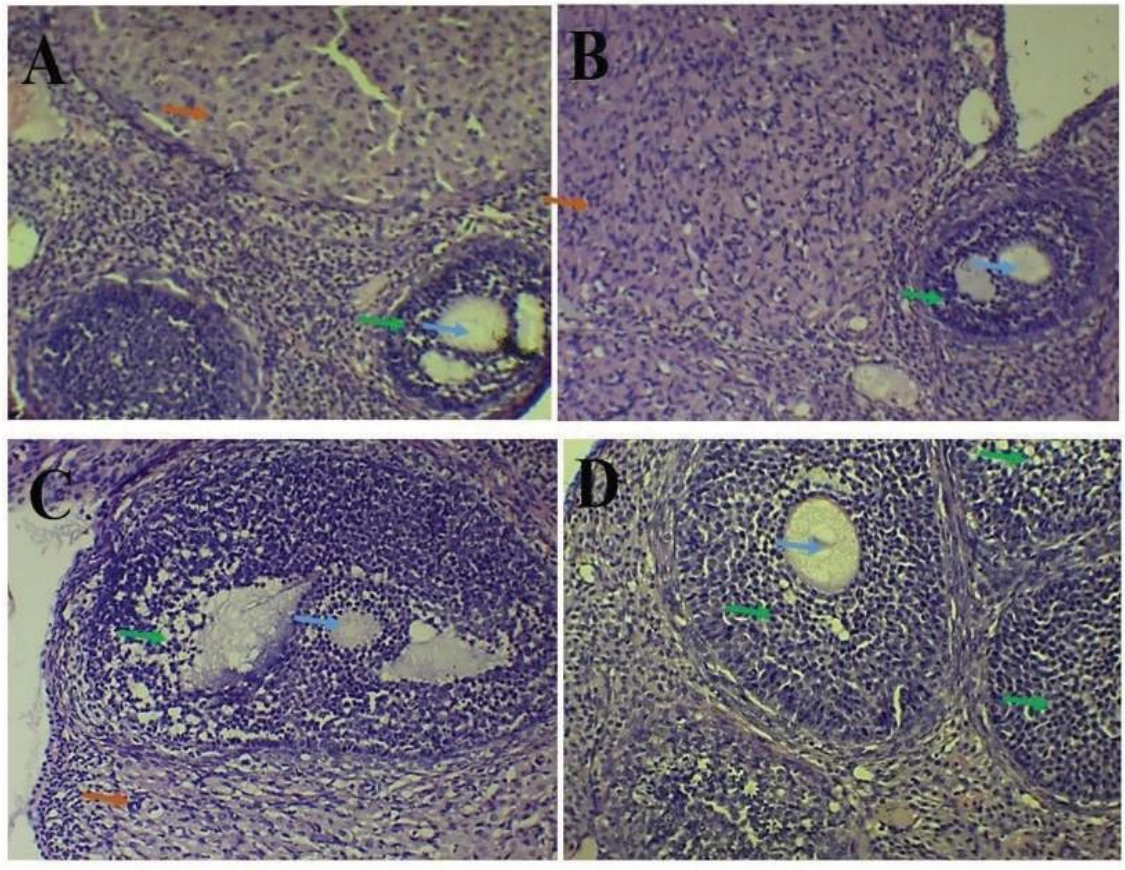

Fig. 2. Ovarian follicle at different stages of development

The Graafian follicle comprising of the oocyte (blue arrow) surrounded by granulosa cells (green arrow) was more numerous in Groups II and III (B & C). In Group IV (D) more secondary oocytes were found in the ovarian stroma along with corpus luteum (orange arrow), H and E stain, x200 magnification

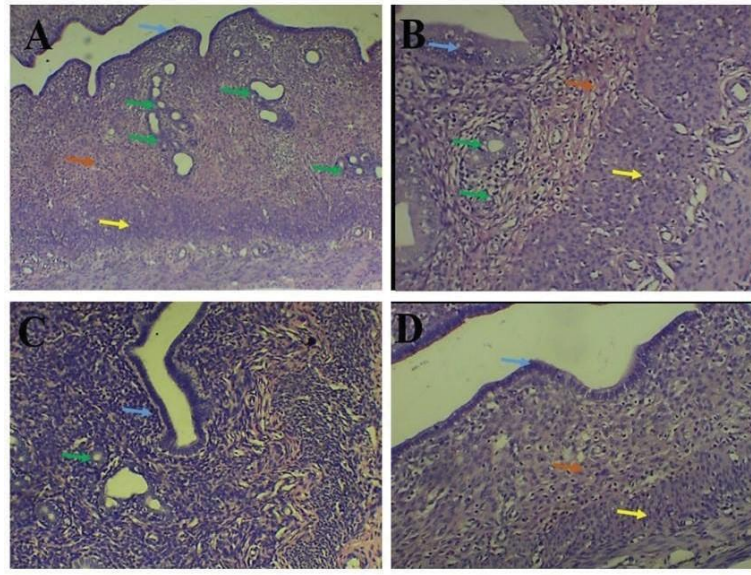

Fig. 3. Uterine histology showing fewer uterine glands (green arrow) in the groups that were administered DPP (B-D). The Control had several uterine glands (A) while the underlying smooth muscle layer (yellow arrow) was pronounced in the treated groups, and the epithelial lining (blue arrow) was thickest in Group VI (B). The uterine stroma (orange arrow) in the treated groups (B- D) were cellular and had fewer glands, H and E stain, x200 magnification.

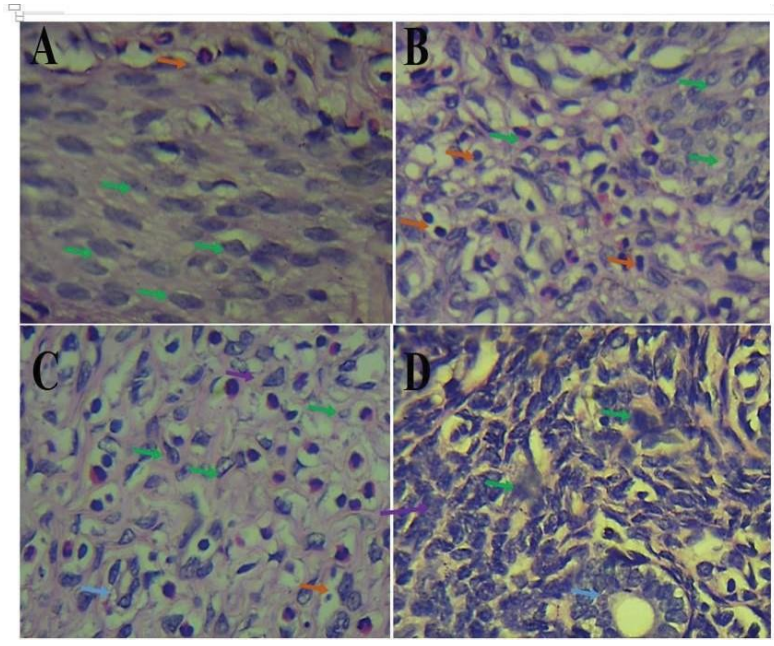

Fig. 4. The uterine stroma showing numerous endometrial stromal cells (green arrows) in the Control (A). The treated groups had more lymphocytes (orange arrow) which were undergoing active mitosis. Blood vessels (purple arrow) were found in the cellular stroma. H and E stain, x200 magnification.

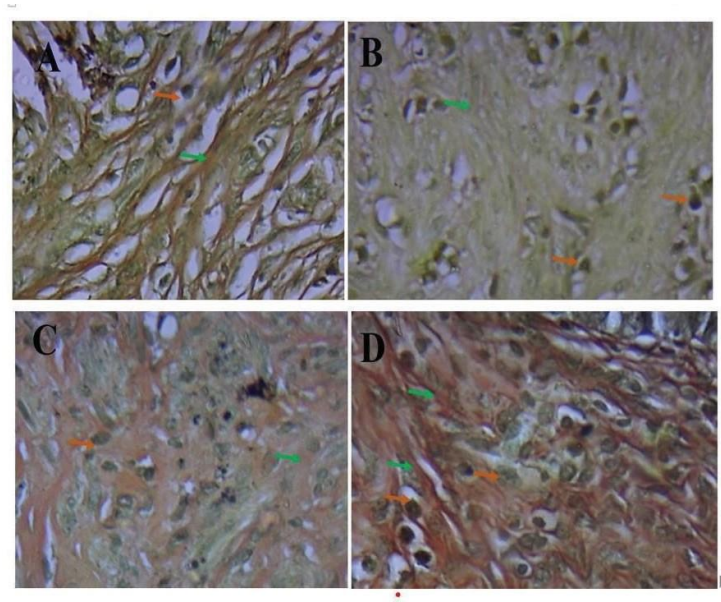

Fig. 5. Uterine histology showing the uterine cells (orange arrow) located in the stroma which has a rich deposit of collagen fibers (orange arrow). The fibers were numerous in the treated groups (B-D) and fewer in Group I (E), VVG stain, x400 magnification.

Discussion

Date palm pollen (DPP) has been used in the treatment of infertility in many cultures, so the present study investigated its effect on the fe- male reproductive organs and hormonal profile in pregnant rats and pups. Date palm pollen constituents and phytoestrogens can differ between plants in different climates and locations. The powder used in the present study was analyzed for its phytochemical content in a previous study (BEDAN et al., 2023).

Maternal weight was non-significantly decreased after administration of DPP in the treated groups. Contrary to this observation, IFTIKHAR et al. (2019) and SALEH et al. (2021) in separate studies determined that DPP increased the weight of hens, eggs produced and male rats. Also, in contrast to the present study, MAHMOUD et al. (2021) reported that palm seed extract increased body weight in treated rats compared to the Control Group.

Morphometric measurements of the female reproductive organs revealed that DPP increased uterine weight and height, and ovarian weight, height and width. Histological analysis revealed numerous secondary follicles in the treated groups, and numerous glands in the uterine stroma compared to the Control Group. DPP increased mitotic figures in the uterine stroma, and robust collagen fibers were found in the treated groups compared to the Control Group. IFTIKHAR et al. (2019) investigat- ed the effect of DPP on male rat pups for 35 days, and reported a statistically significant increase in seminal vesicle, ventral prostate and paired testes weight.

There was no significant effect on the mean relative organ weight indices when compared to the Control Group, except in the weight of the liver, which was slightly reduced. Studies conducted by other researchers have supported the data ob- tained in the current study, such as HOSSEINI et al. (2014), who investigated the effect of *Phoenix dactylifera* extract in mice and reported their activities were due to the presence of gonadotropin-like biocomponents (such as rutin, sterols, carot- enoids, and androsterone). This was confirmed by a previous study carried out to determine the phytochemical constituents of DPP (BEDAN et

al., 2023). Another study reported that the ovary was stimulated after administration of DPP, leading to oogenesis and a corresponding increase in the number of antral and secondary follicles, and also the growth and maturation of preantral folli- cles (AKBARIBAZM et al., 2021). This is in line with the findings of the current study, where sev- eral Graafian follicles and numerous secondary follicles were found in the ovaries of the treated rats. The findings from studies carried out by HOSSEINI et al. (2014) also concur with this. Likewise, SALEH et al. (2021) reported that DPP increased oviduct weight in treated hens, and attributed this effect to the high estrogen concentration of DPP, which promoted oviduct growth and production of oviduct proteins.

In the present study, DPP at all doses significantly augmented estradiol levels. Administration of DPP to pregnant and maternal rats had no significant effect on LH and FSH concentrations, but significantly increased serum estrogen and progesterone concentrations. The results imply that DPP may stimulate the ovaries directly to produce more estrogen and progesterone to mimic the action of FSH and LH without involving the hypothala- mus-pituitary-ovary feedback loop, which primari- ly regulates FSH and LH.

DPP, acting as a hormone-like substance and directly increasing serum estrogen and progesterone levels, causes a negative feedback to the hypothalamus and pituitary, thus decreasing the release of LH and FSH, as evidenced from the results obtained in the current study. JASHNI et al. (2016), also showed that the consumption of *Phoenix dactylifera* extract, due to the presence of its estrogen-like compounds, reduced the LH to FSH ratio. ABEDI et al. (2014) showed that alkaloids present in DPP have estrogenic properties which could be responsible for the increased serum concentrations of progesterone and estrogen in treated rats. MOSHFEGH et al. (2016) and HATAMIRAD et al. (2012) reported an increase in estrogen and progesterone concentrations following the administration of DPP in experimental groups. In agreement with the current study, HOSSEINI et al. (2014) and SALHI et al. (2024) reported that DPP significantly increased testosterone, estrogen and progesterone concentrations compared to the Control Group. It was proposed that DPP created an appropriate situation for oogenesis and maintained efficient fertility in female mice.

In contrast to DPP grain extracts, lyophilized date pit extract did not significantly affect body or uterine weights. The polar fraction of the lyophilized extract was shown to greatly decrease the concentration of plasma levels of estradiol. Administration of the non-polar fraction, however, non-significant- ly increased estradiol levels in Balb/c mice (ALI et al., 1999; LORIPOOR et al., 2023). JIHEEL and ARRAK (2015) revealed that DPP elevated serum levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in female rats that were administered 100 mg/kg of DPP extract.

EL-SAYYAD et al. (2018) suggested that the estrogenic compounds present in *Phoenix dactylifera* plant extract could be used to treat uter- ine disorders, as they have been shown to reduce endometrial tissue degeneration, necrotic patches, and hyperplasia in endometrial glands. This suggestion tallies with the histological observation of the uterus treated with DPP in the present study, which showed that DPP improved the integrity of the uterus by maintaining the uterine endometrial layer, and increased the stromal cells and the num- ber of endometrial glands in the treated groups. HOSSEINI et al. (2014) also reported that DPP administration at a concentration of 400mg/kg significantly increased secondary follicles and antral follicles. In addition, AL-KURAN et al. (2011) reported that date fruit provided energy, strengthened the uterine muscles, and was the best nutritional material for cervical dilutions because of its glucose content. ZHANG et al. (2013) and BAGHERZADEH KARIMI et al. (2020) reported the high calcium and tannin content of date palm fruit was responsible for the contractile activity of the smooth muscles. HOYO et al. (2011) attributed the rapid cell divisions, observed in the present study as mitotic figures in the uterine stroma, to the presence of folic acid. In the same study, it was also reported that the folic acid content of date fruit also aided the formation of the genetic structure in the cells. HASSAN et al. (2012) reported that the estradiol constituents of DPP play an effective

role in regulating the renewal of spermatogenic cells and male reproductive tissues that possess estrogen receptors.

DPP has been found to facilitate sexual function, including desire, arousal, and orgasm, as shown by SALMANI et al. (2022) who proved that administration of DPP ameliorated sexual arousal in non-menopausal women, and was found to increase dopamine release. This can be attributed to the presence of steroids, alkaloids, and flavonoids in DPP (BEDAN et al., 2023). Studies carried out using DPP suggested that the use of DPP suspension during gestation and lactation substantially improved oogenesis (SHEHZAD et al., 2021).

Date fruits are postulated to contain simple sugars that are a readily accessible energy source, strengthening uterine muscles, and providing energy to the mother during labor. They also contain hormones that help the uterus to stretch in preparation for delivery (HATAMIRAD et al., 2012; SHEHZAD et al., 2021). In female rats, date fruit played a positive role in the reproductive process by significantly improving hormonal regulation, strengthening the oocytes, and sustaining pregnancy (SARYONO and RAHMAWATI, 2016). In a related study, DANI et al. (2022) proved that *Khaya senegalenis* extract had the ability to increase the number of uterine glands and maintain the uterine endometrium, as DPP did in the present study.

Concomitant supplementation of barley and *Phoenix dactylifera* fruit to a hypercholesterolemic mice group revealed remarkable improvement in ovarian function and structure (EL-SAYYAD et al., 2018). In the current study, the results obtained were collaborated by DPP's pro-fertility effect on the uterus by increasing implantation sites in the treated groups. Maternal exposure to DPP during mpregnancy reduced the birth weight of the pups.

It was established that some plants contain certain intrinsic compounds known as phytoestrogens which can influence fertility (LEOPOLD et al., 1976). These are biologically active, non-steroidal, plant substances that mimic the effects of endogenous estrogen by binding to induce estrogen-mediated gene transcription, and thereby stimulate growth of the female genital tract or change the amount of circulating endogenous hormones. In addition, some phytoestrogens act both as agonists and antagonists of estrogen action, depending on the dose and target tissue, thus affecting growth outcome and fetal parameters, and causing adverse developmental and/or reproductive effects in both sexes (SETCHELL et al., 1987; HASSAN et al., 2012). MAHRAN et al. (1976) proved that DPP increased the weight of the uterus and gonadotrophic activity by glucoprotein B in a dose of 10 mg/kg, which is equivalent to 0.88 IU of human pituitary gonadotropins.

There was a non-significant change in the body weight during lactation (0-21 days) and post-weaning period when compared to the Control Group, which showed that maternal exposure to DPP grains during lactation did not have any significant effect on the body weight of the pups. It was noted that although DPP decreased intra-uterine growth rate, which was manifested by reduced birth weight, the rat pups did not display any delay of physical developmental landmarks, such as movement ability or hair growth rate, when compared to the pups from the Control Group.

Conclusions

The investigation revealed that DPP enhanced the hormonal profile in pregnant rats, and increased the weight of the reproductive organs in pregnant rats. It also increased size of the uterine glands while maintaining the endometrial lining, and increased the amount of stromal cells, collagen fiber deposition and implantation sites in the uterus of the treated rats. The extract, however, reduced body weight in pregnant rats as well as the pups. There was no observed change in the pups following the administration of DPP during the course of pregnancy and lactation.

Ethics approval

The study followed the University of Maiduguri Research and Ethical Committee's guidelines (UM/HA/PGP 19.20 08800), adhering to the Declaration of Helsinki, ARRIVE 2.0 guidelines, and the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8023).

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Declaration of competing interest

The authors do not have any conflicts of interest to declare.

Data availability

The data used and/or analyzed in the present study are available from the corresponding author upon request.

Authorship contribution statement

All authors contributed to the study concept and design. Helga Ishaya Bedan, Nathan Isaac Dibal and Martha Orendu Oche Attah were involved in the material preparation, data collection was done by Helga Ishaya Bedan, Nathan Isaac Dibal and Martha Orendu Oche Attah, the first draft of the manuscript was written by Helga Ishaya Bedan, Nathan Isaac Dibal, and Martha Orendu Oche Attah. The research was supervised by Sani Hyedima Garba and Tamunotonye Watson Jacks. All authors commented on the final version of the manuscript; all authors read and approved the final manuscript

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H. ISHAYA BEDAN, H., S. HYEDIMA GARBA, T. W. JACKS, M. O. OCHE ATTAH, N. I. DIBAL: Utjecaj prehrane s dodatkom peluda datulje (PD) na reproduktivne pokazatelje albino štakorica Vet. arhiv 96, 309-323, 2026.

SAŽETAK

Tijekom cijelog razdoblja gravidnosti albino štakorica istraživan je učinak različitih doza peluda datulja na estrusni ciklus, reproduktivne organe i hormonski profil te nakon toga i na razvoj njihovih mladunaca. Pelud datulje je znakovito povećao ($P<0,05$) masu maternice i jajnika u gravidnih štakorica te je također znakovito povećao ($P<0,05$) broj mjesta implantacije i dužinu tijela fetusa (engl. crown-rump length). Morfometrijska analiza pokazala je da je pelud datulje povećao masu i visinu maternice, kao i masu, visinu i širinu jajnika. Histološka analiza pokazala je brojne sekundarne folikule u jajnicima i brojne žlijezde u stromi maternice tretiranih skupina štakorica. Broj žlijezda smanjio se nakon prestanka primjene peluda datulja. Pelud datulje je povećao broj mitoza u stromi maternice, a u eksperimentalnih skupina su u odnosu na kontrolnu skupinu uočena izrazito razvijena kolagena vlakna. Pelud datulje je povećao razinu serumskih hormona i broj žlijezda u maternici tretiranih skupina, što se može pripisati činjenici da sadrži biokomponente slične steroidima. Međutim, pelud datulje nije pokazao vidljiv učinak na mladunce istraženih štakorica.

Ključne riječi: datulje; histomorfologija; estrogen; jajnik; pelud; progesteron; spolni hormoni; maternica; albino štakori Wistar