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Research Article

ANTI-INFERTILITY EFFECTS TEST OF DATE PALM FRUIT EXTRACT (*PHOENIX DACTYLIFERA* L.) IN FEMALE MICE (*MUS MUSCULUS*) COMPARED WITH PROPOLIS

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ABSTRACT

Objective: The date palm (*Phoenix dactylifera* L) is one of the plants empirically used to increase fertility. The aim of this study was to compare the effect of ethanol extract of date palm fruit and propolis on fertility in female mice.

Methods: Five groups were assigned to 1 control and 4 experimental groups. The experimental group treated by oral administration of 100, 200, and 400 mg/kgBW of khalal date fruit extract and 200 mg/kgBW of propolis for 5, 10, and 15 days. Control groups received no extract. After 5, 10, and 15 days, the mice were deeply anesthetized with anesthetic ether and sacrificed. Histological changes in ovary and uterine were measured. The data were analyzed using two-way ANOVA and Duncan test.

Results: The results of the study of the effect of the dose and duration of the ethanol extract of khalal date fruit on the histology of ovaries and uterine mice showed that there was an increase in the number of primary, secondary, tertiary, de Graaf, and corpus luteum follicles but did not affect the follicle of atresia and myometrial and endometrial thickness. In propolis a dose of 100 mg/kgBW increase occurs only in primary, secondary, and corpus luteum follicles.

Conclusion: Ethanol extract of khalal date fruit and propolis can increase the number of ovarian follicles. Ethanol extract of khalal date fruit dose can increase the number of ovarian follicles higher than propolis.

Keywords: Propolis, Date palm fruit, Fertility, Histology.

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INTRODUCTION

The date palm (*Phoenix dactylifera* L) is one of the plants empirically used to increase fertility [1]. Phytochemical test dates show phenolic content, sterols, carotenoids, anthocyanins, procyanidin, and flavonoids [1,2]. The pollen suspension can improve the quality of sperm, increase fertility in female mice and improves the reproductive performance of female embryos [3].

In addition, propolis is a substance produced by honeybees. Propolis has antioxidant activity against oxidants and free radicals (H_2O, O_2, and OH) compared with other bee products [4]. Propolis can be used as anti-tuberculosis [5], and this substance also has hepatoprotective effects [6]. Research shows that antioxidants in propolis can protect ovarian follicles from oxidative damage and prevent these follicles from becoming atresia. Propolis provides an ameliorative effect on ovarian toxicity in female rats induced by methoxychlor [7].

This work was conducted to study the comparison of the effect of ethanol extract of khalal date fruit and propolis on the fertility of female mice.

METHODS

Plant materials

Khalal date fruits (*P. dactylifera* L.) were collected from dates distributor, Jakarta, Indonesia. The plant specimen was identified and examined the phytochemicals in Pharmaceutical Chemical Analysis Laboratory, Faculty of Pharmacy, Andalas University.

Chemicals

Propolis, aquadest, ether, ethanol 70%, 70–100% alcohol series, xylol, paraffin, hematoxylin-eosin, Mayer's albumin, Na CMC, ammonia, chloroform, H_2SO_4 2 N, Mayer reagent, magnesium powder, concentrated HCl, and distilled water were used.

Preparation of plant extract

Preparation of fruit ethanol extract of *P. dactylifera* L. in Pharmacy Laboratory, Andalas University. The preparation was done by taking a date (without seeds) and then date cut into small pieces, was added to the jar and then soaked with ethanol 96% and then stirred and jar closed for 3 days (maceration) and then filtered. The clear extract was concentrated using a rotary evaporator at 70. The concentrated samples were packed in sealed glass bottles and stored at room temperature (20°C) until used for the experiment.

Ethical clearance

This study was approved by the committee of the research ethics of the Faculty of Medicine, Andalas University.

Experimental design

The animals divided into 5 groups of 5 each (4 experimental and 1 control groups). The experimental group treated by oral administration of 100, 200, and 400 mg/kgBW of khalal date fruit extract and 200 mg/kgBW of propolis for 10 days. Control groups received no extract. After 10 days, the mice were deeply anesthetized with anesthetic ether and sacrificed. Histological changes in ovary and uterine were measured.

Histopathological evaluation

A total of 25 left ovaries and uterine were collected on the sacrifice and fixed with 10% formaldehyde and processed routinely for paraffin embedding technique. Embedded tissue was sectioned at 5 μm and stained with hematoxylin and eosin for routine histological examination. They were examined for ovarian and uterine histology by light microscope.

Data analysis

Qualitative data on structural changes of ovarian and uterine histology were analyzed descriptively. Quantitative data on the histologic preparations of the ovaries are the number of ovarian follicles and corpus luteum, and in uterine histology preparations are uterine diameter, endometrial thickness and myometrium are analyzed using variance analysis (ANOVA) there was a significant difference between treatments, followed by Duncan test with 95% confidence interval (α=0.05).

RESULTS

The ethanol extract of 100 mg/kgBW dose of khalal dates does not affect the number of ovarian follicles of both primary (Table 1), secondary (Table 2), tertiary (Table 3), de Graaf follicles (Table 4), corpus luteum (Table 5), and follicle atresia (Table 6), and endometrial and myometrial thickness (Tables 7 and 8).

Giving ethanol extract of 200 mg/kgBW and 400 mg/kgBW dose of khalal dates increase the number of ovarian follicles of both primary (Table 1), secondary (Table 2), tertiary (Table 3), de Graaf (Table 4), and corpus luteum follicles (Table 5). Giving ethanol extract of 200 mg/kgBW and 400 mg/kgBW doses of khalal dates does not affect on the number of follicle atresia (Table 6) and thickness of endometrium and myometrium (Tables 7 and 8). Giving propolis 100mg/kgBW increases the number of primary (Table 1), secondary (Table 2), and corpus luteum follicles (Table 5). The duration of extract giving (10 and 15 days) has effect on the number of primary follicles (Table 1) and secondary follicles (Table 2). The duration of the extract of 5 days, 10 days, and 15 days has no effect on the number of tertiary follicles (Table 3), de Graaf (Table 4), corpus luteum (Table 5), follicular atresia (Table 6), and endometrial thickness and myometrium (Tables 7 and 8). Giving ethanol extract of khalal dates dose 200 mg/kgBB gives the highest increase of ovarian follicle amount compared to dose 100 mg/kgBW and 400 mg/kgBW and propolis 100 mg/kgBW.

Table 1: Effect of ethanol extract of khalal date fruit and propolis on primary follicles

Duration of Administration (days)	The average number of primary follicles±SD				Propolis 100 mg/kgBW	Average±SD
	Control	100 mg/kgBW	200 mg/kgBW	400 mg/kgBW		
5	2.67±0.58	2.33±0.58	2.67±0.58	4.67±0.58	5.33±0.58	3.53^{a}±1.36
10	3.00±1.00	3.00±1.00	5.67±1.16	4.33±0.58	4.33±1.16	4.07^{ab}±1.34
15	3.67±0.58	6.00±1.00	4.33±0.58	4.33±0.58	3.33±0.58	4.33^{b} ±1.13
Average±SD	3.11^{a}±0.79	3.78^{ab}±1.86	4.22^{b}±1.48	4.44^{b}±0.53	4.33^{b}±1.12	

Different alphabet (a, b, or ab) in the same column or row means there are significant differences (p<0.05). SD: Standard deviation

Table 2: Effect of ethanol extract of khalal date fruit and propolis on secondary follicles

Duration of administration (days)	The average number of secondary follicles±SD				Propolis 100 mg/kgBW	Average±SD
	Control	100 mg/kgBW	200 mg/kgBW	400 mg/kgBW		
5	1.33±0.58	0.67±0.58	8.33±1.53	6.00±1.00	2.67±1.16	3.80^{a}±3.14
10	1.33±0.58	5.67±0.58	9.67±2.08	5.00±1.00	5.33±1.53	5.40^{b}±2.95
15	2.00±1.00	7.00±1.00	7.33±0.58	3.33±0.58	6.00±1.73	5.13^{b}±2.36
Average±SD	1.55^{a}±0.726	4.45^{b}±2.96	8.44^{c}±1.67	4.78^{b}±1.39	4.67^{b}±2.00	

Different alphabet (a, b, or ab) in the same column or row means there are significant differences (p<0.05). SD: Standard deviation

Table 3: Effect of ethanol extract of khalal date fruit and propolis on tertiary follicles

Duration of Adm (days)	The average number of tertiary follicles±SD				Propolis 100 mg/kgBW	Average±SD
	Control	100 mg/kgBW	200 mg/kgBW	400 mg/kgBW		
5	0.33±0.58	2.00±1.00	2.33±0.58	2.00±1.00	1.24±0.43	1.17^{a}±0.56
10	1.33±0.58	0.67±0.58	4.33±0.58	0.67±0.58	1.19±0.24	1.14^{a}±0.63
15	1.00±1.00	0.67±0.58	4.00±1.00	2.67±0.58	1.61±0.34	1.61^{a}±0.34
Average±SD	0.89^{a}±0.78	1.11^{ab}±0.93	3.55^{c}±1.13	1.78^{b}±1.09	1.33^{b}±0.37	

Different alphabet (a, b, or ab) in the same column or row means there are significant differences (p<0.05). SD: Standard deviation

Table 4: Effect of ethanol extract of khalal date fruit and propolis on de Graafian follicles

Duration of Adm (Days)	The average number of de Graafian follicles±SD				Propolis 100 mg/kgBW	Average±SD
	Control	100 mg/kgBW	200 mg/kgBW	400 mg/kgBW		
5	0.67±0.58	0.33±0.58	2.67±0.58	2.67±1.16	1.00±1.00	1.47^{a}±1.25
10	0.33±0.58	1.67±1.16	2.67±0.58	1.33±0.58	1.33±0.58	1.40^{a}±1.07
15	0.67±0.58	1.00±1.00	1.67±0.58	0.67±0.58	0.33±0.67	0.87^{a}±0.74
Average±SD	0.56^{a}±0.53	1.00^{ab}±1.00	2.33^{c}±0.71	1.56^{b}±1.13	0.78^{ab}±0.83	

Different alphabet (a, b, or ab) in the same column or row means there are significant differences (p<0.05). SD: Standard deviation

Table 5: Effect of ethanol extract of khalal date fruit and propolis on corpus luteum

Duration of Adm. (Days)	The average number of corpus luteum±SD				Propolis 100 mg/kgBW	Average±SD
	Control	100 mg/kgBW	200 mg/kgBW	400 mg/kgBW		
5	3.67±1.53	3.33±1.53	6.33±1.53	4.67±1.16	6.33±0.58	4.50^{a}±1.73
10	4.00±1.00	3.33±0.58	7.00±2.00	5.67±0.58	6.00±1.00	5.00^{a}±1.81
15	2.67±1.53	4.00±1.00	9.33±1.53	7.00±1.73	8.00±1.00	5.75^{b}±2.99
Average±SD	3.45^{a}±1.33	3.55^{a}±1.01	7.55^{c}±2.01	5.78^{b}±1.48	6.78^{bc}±1.20	

Different alphabet (a, b, or ab) in the same column or row means there are significant differences (p<0.05). SD: Standard deviation

Table 6: Effect of ethanol extract of khalal date fruit and propolis on atretic follicles

Duration of Adm. (Days)	The average number of atretic follicles±SD				Propolis 100 mg/kgBW	Average±SD
	Control	100 mg/kgBW	200 mg/kgBW	400 mg/kgBW		
5	1.00±1.00	0.00±0.00	1.67±0.58	1.00±1.00	0.33±0.58	0.80^{a}±0.86
10	0.67±0.58	2.00±1.00	0.33±0.58	1.00±0.00	0.67±0.58	0.93^{a}±0.79
15	1.33±0.58	1.67±0.58	0.33±0.58	0.67±0.58	1.33±1.16	1.07^{a}±0.80
Average±SD	1.00^{a}±0.71	1.22^{a}±1.09	0.78^{a}±0.83	0.89^{a}±0.60	0.78^{a}±0.83	

Different alphabet (a, b, or ab) in the same column or row means there are significant differences (p<0.05). SD: Standard deviation

Table 7: Effect of ethanol extract of khalal date fruit and propolis on endometrium

Duration of Adm (days)	The average thickness of endometrium(µm)±SD				Propolis 100 mg/kgBW	Average±SD
	control	100 mg/kgBW	200 mg/kgBW	400 mg/kgBW		
5	172.97±26.04	106.63±64.13	162.99±39.18	103.46±18.26	184.72±119.88	146.16^{a}±65.29
10	102.82±6.35	141.44±9.55	187.69±46.72	116.01±22.28	103.99±42.94	130.39^{a}±41.85
15	146.46±21.73	95.97±18.11	116.00±71.29	123.62±26.90	158.45±36.53	128.02^{a}±40.82
Average±SD	140.75^{a}±35.19	114.55^{a}±39.53	155.56^{a}±56.52	114.36^{a}±21.59	149.05^{a}±75.23	

Different alphabet (a, b, or ab) in the same column or row means there are significant differences (p<0.05). SD: Standard deviation

Table 8: Effect of ethanol extract of khalal date fruit and propolis on myometrium

Duration of Adm (days)	The average thickness of myometrium (µm) ±SD				Propolis 100mg/kgBW	Average±SD
	Control	100 mg/kgBW	200 mg/kgBW	400 mg/kgBW		
5	67.61±18.36	67.40±26.97	94.35±40.11	69.28±27.64	167.14±113.34	93.16^{a}±62.48
10	79.50±34.57	114.68±17.03	122.19±11.99	71.66±4.01	63.95±25.21	90.40^{a}±30.32
15	81.44±12.52	62.34±3.14	73.35±34.51	81.43±23.04	104.22±16.93	80.56^{a}±22.66
Average±SD	76.18^{a}±21.55	81.48^{a}±29.70	96.63^{a}±34.44	74.12^{a}±18.94	111.77^{a}±73.97	

Different alphabet (a, b, or ab) in the same column or row means there are significant differences (p<0.05). SD: Standard deviation

DISCUSSION

The aim of this study was to compare the effect of ethanol extract of date palm fruit and propolis on fertility in female mice. The parameters observed were the number of primary, secondary, tertiary, de Graaf, and corpus luteum follicles as well as the thickness of the endometrial and myometrial walls. Results of observations of ovarian histologic preparations in the cortical section of the treatment group were obtained by primary, secondary, tertiary, de Graaf, and corpus luteum follicles compared to the control group (Fig. 1).

The results of the study of the effect of the dose and duration of the extract of ethanol on the histology of ovaries and uterine mice showed that there was an increase in the number of primary, secondary, tertiary, de Graaf, and corpus luteum follicles but did not affect the follicle of atresia and myometrial and endometrial thickness.

Propolis dose 100 mg/kgBW increase occurs only in primary (Table 1), secondary (Table 2), and corpus luteum follicles (Table 5). The average number of ovarian follicles of both primary, secondary, tertiary, de Graaf, and corpus luteum follicles increased from the control group, especially in the group given the 200 mg/kgBB dose extract. While the duration of extract giving (5, 10 and 15 days) does not affect the number of ovarian follicles and the thickness of the uterine wall significantly. The fruit of khalal dates contains many important compounds such as steroids, anthocyanins, procyanidins, carotenoids, flavonoids, phytoestrogens, and various other compounds [1]. In this study, the compounds that play a major role in increasing the number of ovarian follicles are phytoestrogens. Flavonoids in plants or also called phytoestrogens are one group of active compounds in plants that have a chemical structure similar to estradiol. Phytoestrogens consist of three main compounds, namely isoflavones, coumestants, and lignans. According to research, dates contain the highest phytoestrogens. These compounds have the ability to bind to estrogen receptors and provide both estrogenic and antiestrogenic effects [8]. Phytoestrogens can increase estrogen levels according to previous studies which show that dates can increase estrogen and progesterone levels [2] while propolis has the strongest antioxidant activity against oxidants and free radicals (H_2O, O_2, and OH) compared with other bee products [4]. Provision of propolis aims to prevent the formation of free radicals. Propolis which is an beekeeping product contains Caffeic Acid Phenethyl Ester. Research has shown that antioxidants in propolis can protect ovarian follicles from oxidative damage and prevent them from becoming atresia. Propolis with a dose of 200 mg/kgBB has an ameliorative effect on ovarian toxicity in female rats induced by methoxychlor [7]. The success of follicular differentiation depends on the presence of steroids and growth factors that stimulate follicular differentiation. The growth of the ovarian

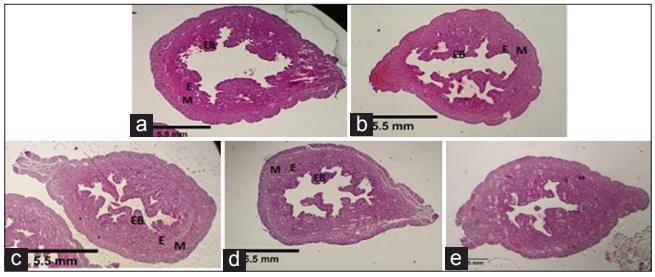

Fig. 1: Effect of ethanol extract of khalal date fruit and propolis on uterine walls in control and various experimental groups (×40, H and E); E: endometrium; and M: Myometrium. (a) Control group, (b) treatment Group 1 (extract dose 100 mg/kgbw), (c) treatment Group 2 (extract dose 200 mg/kgbw), (d) treatment Group 3 (extract dose 400 mg/kgbw), and (e) treatment Group 4 (propolis dose 100 mg/kgBW)

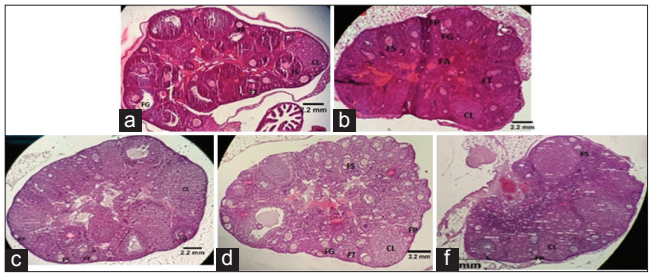

Fig. 2: Effect of ethanol extract of khalal date fruit and propolis on ovarian follicles in control and various experimental groups (100x, H and E); FP: Primary follicle; FS: Secondary Follicle; FT: Tertiary Follicle; FG: Graafian Follicle; CL: Corpus Luteum; and FA: Atretic Follicle.(a) Control group (b) treatment Group 1 (extract dose 100 mg/kgBW), (c) treatment Group 2 (extract dose 200 mg/kgBW), (d) treatment Group 3 (extract dose 400 mg/kgbw), and (e) treatment Group 4 (propolis dose 100 mg/kgBW)

follicle is achieved through the proliferation and differentiation of granulosa cells. One of the strong follicular growth stimulants is estrogen. Estrogen consists of three types of estriol, estrone, and estradiol. Estradiol increases the proliferation of granulosa cells and promotes the growth of preantral follicles and antrum formation [9]. The result showed that the average number of treatment group atresia follicles did not differ significantly with the control group (sig>0.05). The main mechanism that causes cells to be atresia is apoptosis in granulosa cells. Estrogen plays an important role in the regulation of growth, development, homeostasis, and cell death (apoptosis) in the ovaries. Estrogens prevent apoptosis in granulosa cells by inhibiting endonuclease activity in granulosa cells [9]. The average number of follicular atresia of the treatment group did not differ significantly with the control group (sig>0.05) also showed that elevated estrogen levels were not so high that they did not cause negative feedback and inhibited the secretion of follicle-stimulating hormone and luteinizing hormone in the hypothalamus resulting in follicular atresia.

Based on Fig. 2, it is seen that the histology structure of the uterus in the control group and the treatment group found no damage to the lining of the uterine wall. The epithelial epithelium juggle indicates that the mice are in the estrous cycle while the unmarked epithelium indicates that the mice are in the proestrus phase at the time of sacrifice.

Based on the result of statistical analysis, it is not seen the effect of dose and duration of extract and propolis administration on myometrial thickness and endometrium of mice. The thickness of the endometrium and myometrium of the treatment group mice did not differ significantly with the control group (sig>0.05), suggesting that steroidal compounds that have a role such as the estrogen hormone (phytoestrogens) in the extract do not cause too high estrogen levels which can lead to increased thickness endometrium and myometrium. Estrogen is used for cell proliferation of the uterus, working by binding to estrogen receptors present in the uterus [10]. It also shows that propolis only provides protection and does not increase the amount of estrogen in mice, so there is no increase in uterine wall thickness of mice in accordance with previous studies. From the overall data processing, it was found that the extract of ethanol of khalal dates had an effect on the number of ovarian follicles except for the atresia follicle but had no effect on endometrial thickness and myometrium, and propolis only increases primary, secondary follicles, and corpus luteum. Propolis only gives protection effect [11].

CONCLUSION

Ethanol extract of khalal date fruit and propolis can increase the number of ovarian follicles. Ethanol extract of khalal date fruit dose can increase the number of ovarian follicles higher than propolis.

AUTHOR'S CONTRIBUTION

All authors have equal contribution toward this manuscript, the study conception, and experimental design by Dwisari D and Ria A, plant identification by Skunda D, analysis and interpretation of statistical data by Almahdy, interpretation of histological data by Biomechy OP and Noverial.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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