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Comparison of Folliculogenesis and Follicular Maturation in Norvegicus Rats Using *Foeniculum Vulgare* and Clomiphene Citrate

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Abstract

Objectives: The objective of this work is to understand the importance of follicular maturation in the reproductive life of the Wistar's rats (*Rattus norvegicus*) using *Foeniculum Vulgare* (Fennel) and Clomiphene Citrate as reference drug.

Methods: *Foeniculum Vulgare* (Fennel) are used in doses of (400, 800 mg/Kg), Clomiphene Citrate along with distilled water administration. The sexual hormones involved in the oestrous cycle, folliculogenesis, the role of the ovaries, the embryonic and fetal development of the ovary are addressed. An extract of Fennel was obtained using ethyl alcohol by miscreation. After successful administration, a histological study was also conducted on 40 rats grouped into four categories. Statistical analysis also applied standard parameters like chi-square, Tukey test and variance test.

Results: It was identified that after administration of Fennel citrate drug along with distilled waters, significant differences ($P < 0.05$) were identified in the rats and all rats formed primary (90% given 800 mg/kg after fennel administration), secondary and matured (80% given 800 mg/kg after fennel administration) follicles.

Conclusion: The effect of *F. Vulgare* at dose of 400 mg was not influential as compared to *F. Vulgare* at 800 mg. The clomiphene citrate increased the number of primary follicles in all of the rats studied. In contrast, the rats that ate *F. Vulgare*, had secondary and mature follicles predominating and did not have any primary follicles.

Keywords: *Foeniculum*, *Rattus norvegicus*, rats, Wistar, follicular maturation, Clomiphene Citrate

Introduction

Folliculogenesis the developmental process of ovarian follicles begining from a reserve of quiescent primordial follicles set up in early life and ending with either ovulation or follicular death by atresia.[1] Many of couples of reproductive age suffer from infertility, a severe problem which affects millions of people of reproductive age worldwide and has an impact on their families and communities. Estimates suggest that between 48 million couples and 186 million individuals live with infertility globally.[2] It can be described as the inability to carry a pregnancy to term following a suitable amount of sexual contact that contraceptive methods have not interrupted. Anovulation is defined as a condition in which follicular development and rupture are disrupted, resulting in the oocyte not being released from the follicle; numerous factors have been identified as contributing to this condition.[3] Complementary and alternative traditional medicine is extensively practised and respected throughout the world for various reasons.[4] Traditional therapies and traditional medicine practitioners provide the primary, and in some cases the only, source of health care for several millions of people. This type of treatment is convenient, easily accessible, and reasonably priced. Furthermore, it is culturally accepted and trusted by many individuals. Considering that the use of Fennel would minimise the expense of unsuccessful ovulation treatments, improving the likelihood of having planned pregnancies with fewer complications and, as a result, the birth of a new human, this is a significant topic.[5,6] The aim of this study is to evaluate the effects of *Foeniculum Vulgare* (Fennel) on folliculogenesis and follicular maturation in norvegicus rats exposed to Clomiphene Citrate, in order to supply new information that can be utilized to incorporate new understanding into health care practises and policies. Specifically, the current study investigates the effects of Fennel at doses of 400 mg/kg and 800 mg/kg on folliculogenesis and follicular maturation in norvegicus rats, as well as the effects of Clomiphene Citrate on folliculogenesis and follicular maturation in norvegicus rats, and whether there is a difference in the effects of folliculogenesis and follicular maturation between rats that consumed Fennel.

Materials and Methods

Plant Material: *Foeniculum Vulgare* (Figure 1) is a medical plant that belongs to the Umbelliferae family (Apiaceae), well known and applied by humans since ancient times because of its flavour. It is cultivated in nearly all countries. It is globally known as Fennel or other names in more than 100 countries.[7] Fennel is a herbaceous plant, erect in size that can reach 2 meters in height. The deep green leaves are long and thin, ending in needle-shaped segments, which harden on the outside in the summer to prevent water loss.[8] There are varieties of Fennel with a tan (purple) color. The yellow flowers appear in clusters of 20 to 50 small flowers on short peduncles, called umbels (inflorescence in which the pedicels of all the flowers are inserted at the same point on their axis, similar to the ribs of an umbrella).[9] They bloom in summer. The fruits are ovoid and oblong. Biological-pharmacological studies have been carried out to assess the native uses of this plant.[10] *F. vulgare* plant extracts and isolated compounds have been estimated for various activities and proved good medicine for human health issues.[11]

Norvegicus Wistar-Type Rats and Rat Cycle Identification as shown in Figure 2.

Forty rats were used as experimental animals, females between 230–280 gr of the Wistar breed, albino variety, all of them with similar feeding.

They were divided into four groups and administered distilled water, Clomifero Citrate, Fennel (400 mg/Kg) and Fennel (800 mg/kg) respectively. They were divided into four groups and administered distilled water, Clomifero Citrate, Fennel (400 mg/Kg) and Fennel (800 mg/kg). Animals in all groups were sacrificed after the sixth administration day (Figure 3, Table 1).

Fig. 1 ***Foeniculum Vulgare* plant.**

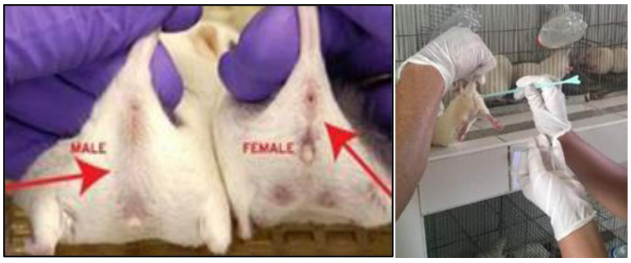

Fig. 2 **Sample of rats (left) and rat cycle identification (Right).**

The ovaries were trimmed of fat and fixed in 10% formalin. They were dehydrated in graded alcohols, pressed in xylene, immersed in paraffin, and then dyed with hematoxylin and eosin (H&E). Subsequently, histological sections were made in the microtome. The follicles were classified as primary, secondary and mature (Figure 3).

- Laboratory Materials: Beaker; 10 ml vial; Dropper; Vials
- Equipment: Percolator equipment; broken steam; Water bath; Stove; Analytical balance; Vortex
- Others: Surgical gloves; Venoclysis equipment; 1 ml tuberculin syringes; marbles; drinkers; cannula
- Reagent: Distilled water and 96° Ethyl Alcohol

Chromatographic technique is an analytical system that allows the different components of a problem sample to be separated by distribution between two phases, one stationary and the other mobile, in order to identify and/or quantify them. Uniform layer of an absorbent maintained on a plate made of glass, aluminium or another support. The substances to be separated are run using a solvent (mobile phase). The mobile phase is liquid, and the stationary phase is solid and polar (silica gel).

The mixture to be analysed is deposited a small distance from the lower edge of the plate and introduced into a cuvette containing the mobile phase. The mobile phase rises along with the plate by capillarity, displacing the mixture's components at different speeds, causing their separation. When the solvent front is close to the upper end of the plate, it is removed

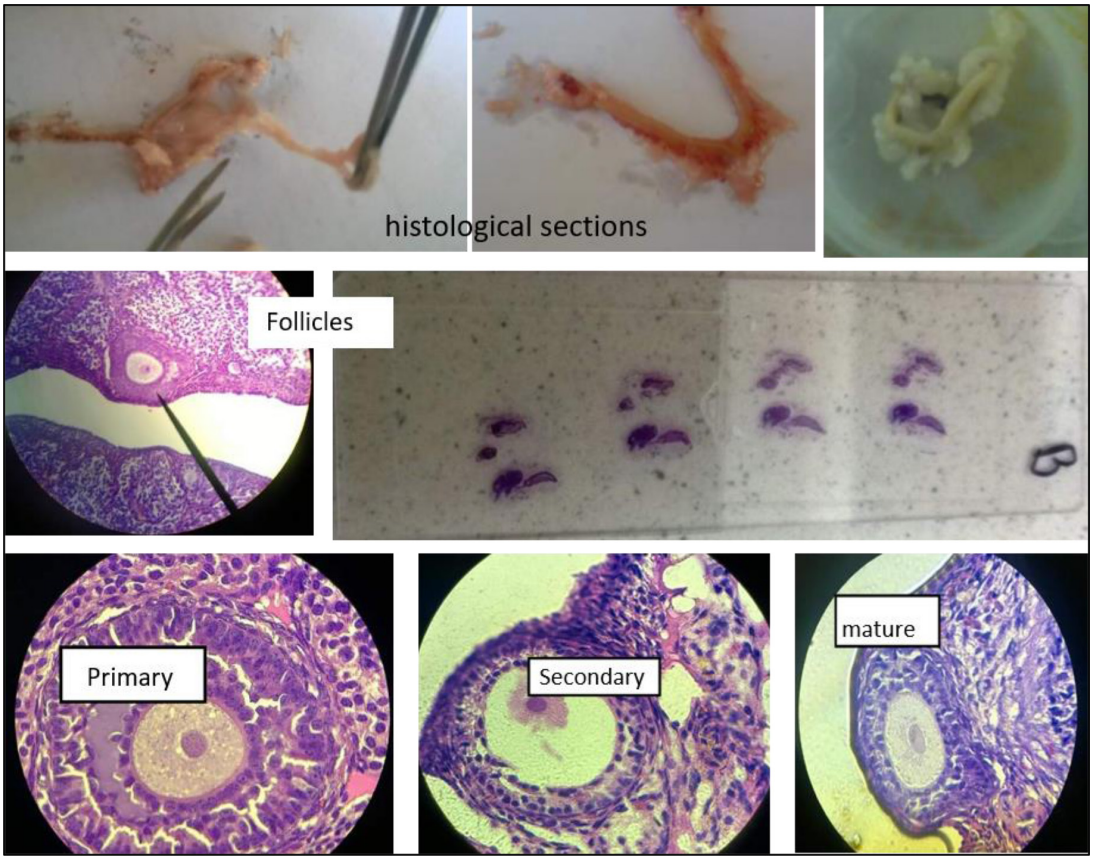

Fig. 3 **Rats histological sections and follicles.**

Table 1. **Drug administration protocol used in the current study**

Rat No	Identification	Weight (gm)	Administration	Dose
Group–1 (Fennel 400 mg)				
R1	Head and left front leg	266	Proestrous	0.13 ml of Fennel up to 1 mL
R2	2 right legs	270		0.13 ml of Fennel up to 1 mL
R3	Head and 2 hind legs	230		0.12 ml of Fennel up to 1 mL
R4	Head and two right legs	260		0.13 ml of Fennel up to 1 mL
R5	Head and 2 left legs	240		0.12 ml of Fennel up to 1 mL
R6	Head	237		0.11 ml of Fennel up to 1 mL
R7	Back	248		0.12 ml of Fennel up to 1 mL
R8	Tail	249		0.12 ml of Fennel up to 1 mL
R9	Right front leg	253		0.12 ml of Fennel up to 1 mL
R10	Right hind leg	238		0.12 ml of Fennel up to 1 mL
Group–2 (Fennel 800 mg)				
R11	Head and right front leg	252	Proestrous	0.24 ml of Fennel up to 1 mL
R12	Right front leg	246		0.24 ml of Fennel up to 1 mL
R13	Head	255		0.25 ml of Fennel up to 1 mL
R14	Head and back	275		0.27 ml of Fennel up to 1 mL
R15	Head and two right legs	260		0.25 ml of Fennel up to 1 mL
R16	Left anterior leg	260		0.25 ml of Fennel up to 1 mL
R17	Left hind leg	268		0.26 ml of Fennel up to 1 mL
R18	Right Side Legs	265		0.26 ml of Fennel up to 1 mL
R19	Left Side Legs	254		0.24 ml of Fennel up to 1 mL
R20	Front legs	238		0.23 ml of Fennel up to 1 mL
Group–3 (Clomiphene Citrate)				
R21	Head	263	Right-handed	1 mL
R22	Back	252		
R23	Tail	250		
R24	Right front leg	243		
R25	Right hind leg	262		
R26	Left front leg	252		
R27	Left hind leg	267		
R28	Two right legs	258		
R29	Two left legs	263		
R30	Front legs	266		
Group–4 (Distilled water)				
R31	Head	260	Proestrous	1 mL
R32	Back and right front leg	245		
R33	Tail	258		
R34	Back	254		
R35	2 right legs	257		
R36	Head back tail	244		
R37	Head right front leg	249		
R38	Head right hind leg	245		
R39	Left front leg head	248		
R40	Left hind leg head	239		

and visualised in a ultraviolet visible lamp with a wavelength of 366 nm. Determination of the so-called fingerprint of the *F. Vulgare* seed was used as the mobile phase. Toluene and ethyl acetate (50-50), respectively, were used as developers. An atomiser spread sulfuric acid at 5% and vanillin at 1%; then, the oven was previously conditioned at a temperature of 110°C, placing the plate for 5 minutes. Visually the Chromatographic analysis of Fennel in which substances with high polarity are observed that we see in colours that will allow us to characterise our drug so that they can later be compared.

Preparation of the Ethanolic Extract of Fennel

For the preparation of the extract, seeds of Fennel were taken, which were taken to a drying chamber with less than 40ºC for 72 hours. Then they were pulverised, obtaining a pulverised dry powder of 100 gr, which was used to prepare the alcoholic extract. The final dry powder was mixed with ethyl alcohol (96º), which was allowed to macerate for 5 days to extract polar and non-polar substances efficiently. At the end of 5 days, it was filtered in alcohol, obtaining the active extract, evaporating at 40ºC for 48 hours. The extract was used for the respective dilutions and administration in the experimental animals.

Results

The number of primary, secondary, and mature follicles in rats exposed to various doses of fennel and clomiphene citrate was determined and the results are graphically represented in Figure 4.

According to the analysis of variance (F_o = 2.96, 5.03, & 4.46) for primary, secondary and mature follicles respectively, it is shown that the number of primary follicles in the concentrations of Fennel and Clomiphene Citrate presented statistically significant differences ($P < 0.05$).

- Furthermore, according to the Tukey test (F_t = 2.88 for Primary, 2.88 for Secondary, and 2.88 for mature follicles, respectively), it is observed that the highest number of primary follicles was found when Clomiphene Citrate was applied with an average of 4.50, which is significantly higher than the other treatments.
- It was discovered that there were statistically significant variations in the number of secondary follicles in the amounts of Fennel and Clomiphene Citrate ($P < 0.05$) between the two treatments.
- A similar result was reported using the Tukey test, which revealed a significant difference between the treatments when the concentration of Fennel 800 mg/kg was given, with an average of 2.80 secondary follicles being detected, compared to the other treatments.
- Using Tukey's test, it was discovered that when Fennel 800 mg/kg was administered, the largest number of mature follicles was detected, with an average of 2.40, compared to the other treatments, and that this was significantly different from the other treatments.
- With reference to the Chi-square test (χ^2 = 22.22), the presence of primary follicles was found when different quantities of Fennel, Clomiphene Citrate, and distilled water were used, and statistically significant differences ($P < 0.05$) were found between the treatments. Similarly, it was discovered that 100% of the rats who got Clomiphene Citrate developed primary follicles (Figure 5).
- There were statistically significant changes ($P < 0.05$) in the presence of secondary follicles when varied amounts of Fennel, Clomiphene Citrate, and distilled water were employed, according to the Chi-square test (χ^2 = 12.5). According to the data, 90% of rats given 800 mg/kg Fennel produced secondary follicles.
- Different concentrations of Fennel, Clomiphene Citrate, and distilled water were found to have statistically significant differences ($P < 0.05$) in the existence of mature follicles, as determined by the Chi-square test (χ^2 = 68.69). Fennel 800 mg/kg was found to increase the follicle count in the testicles of 80 per cent of the experimental rats.

Discussion

In current study, the effects of *F. vulgare* on folliculogenesis and follicular maturation in rats were investigated. When different amounts of Fennel, clomiphene citrate, and distilled water were administered to primary, secondary, and mature follicles, statistically significant changes ($P < 0.05$) were detected. Primary follicles were found in 100% of the Clomiphene Citrate-treated rats. In contrast, primary follicles were found in 90% of the distilled water-consuming rats and 90% of the Fennel-consuming rats at an 800 mg/kg dose, respectively. The dose of 800 mg/kg of Fennel resulted in 90% of the rats showing secondary follicles and 80% of the animals showing mature follicles after the herb was administered. 80% and 70% of the rats that received 400 mg/kg Fennel showed secondary and mature follicles, respectively, with no statistically significant variations between the two groups.

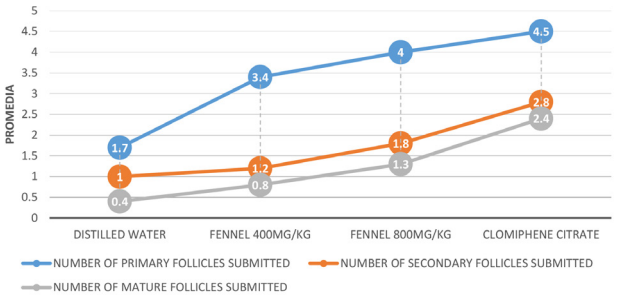

Fig. 4 **Number of primary, secondary and mature follicles in rats subjected to concentrations of fennel and clomiphene citrate.**

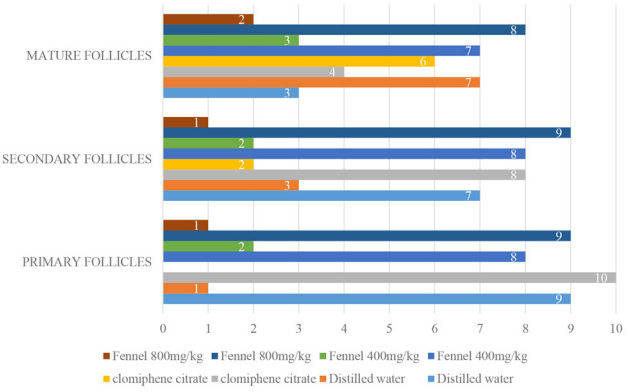

Fig. 5 **Presence of primary, secondary and mature follicles in histological sections of *Rattus norvergicus* ovary.**

Consequently, the presence of secondary and mature follicles varied substantially between Fennel dosages of 800 mg/kg and 400 mg/kg when comparing the two treatments. Statistically significant differences were seen in the number of formed follicles between the quantities of Fennel, pure water, and Clomiphene Citrate used in the experiments ($P < 0.05$). According to Tukey's test, the highest primary follicle counts were obtained when Clomiphene Citrate was used. In contrast, the highest secondary and mature follicle counts were obtained when Fennel 800 mg/kg was used. After 10 days of oral treatment with the extract, the researchers[12] discovered that female rats developed vaginal cornification and began their monthly cycle and other pharmacological characteristics such as toxicity, side effects, and drug interactions. The ovary, endometrium, myometrium, cervix, and vagina grew weight when given greater doses, whereas the mammary glands acquired weight when given intermediate amounts of the medication.

In a study conducted by Gardner et al. (2013), discovered that oral administration of an acetone extract of Fennel for 14 days boosted the estrus cycle and increased weight in the breast, endometrial, cervix, and vagina in rats.[13] An acetone extract of Fennel administered daily at doses ranging from 0.5 to 2.5 mg/kg body weight caused dose-dependent estrogenic effects in ovariectomised rats, resulting in the stimulation of the estrus phase (after 10 days, in 40% of rats at 0.5 mg/kg and 100% of rats at 2.5 mg/kg) and increased weight of the mammary glands (P 0.05 to 0.5 mg/kg, $P < 0.01$ to 2.5 mg/kg).

Dehghani et al. (2005) discovered that *F. Vulgare* had a deleterious impact on the reproductive organs of male rats after conducting a study.[14] In this experiment, 40 male rats were used in the experiment. Four groups of Sprague-Dawley rats were produced at random using a computer programme. The organic extract of *F. Vulgare* was provided to the study groups at doses of 100, 250, and 500 mg/kg for a total of 30 days, depending on their weight. The rats were killed and dissected on day 30 of the experiment to conduct histological studies. The herb *F. Vulgare* considerably elevated estradiol levels in male rats while simultaneously reducing testosterone levels in the bloodstream.

Kilic-Oakman et al. (2003) employed the rat model to investigate the effects of Letrozole and Clomiphene Citrate on ovarian follicle count, endometrium thickness, and serum levels of estradiol, follicle-stimulating hormone (FSH), luteinising hormone (LH), and testosterone (Test) in a female reproductive tract.[15] The experiment, which involved 30 female Wistar-Albino rats at twenty weeks of age and weighing 250 g, was conducted on Wistar-Albino rats. From the total of 30 rats, 3 groups of ten rats, each were formed. Vaginal smears were taken from all rats before they were given the Letrozole pill to see if they had regular menstruation for the previous 3 cycles. Diestrus, a 4-day oestrous cycle, was administered in 2 mL normal saline containing 5 mg/kg body weight daily; 100 g/kg daily in 2 mL normal saline; or 2 mL of sterilised sodium chloride saline included 5 mg/kg body weight daily. The medications were delivered via lavage for a total of two days. The creatures were terminated with ether after two days of suffering. In addition to doing an autopsy in order to collect tissue samples for histology, blood samples were collected to evaluate serum levels of oestrogen, (FSH), (LH), and (Test). When the ovary was examined under a microscope, the size, thickness of the endometrium, and ovary diameter could all be determined. If you're looking to increase follicular growth, letrozole is every bit as effective as clomiphene citrate is. Letrozole has the potential to be used to manage fertility in the future. In our study, we administered the medication Clomiphene Citrate, and a significant amount reduced the number of primary follicles.

Conclusion

The effect of *F. Vulgare* at 400 mg was ineffectual compared to *F. Vulgare* at 800 mg. It was discovered that clomiphene citrate increased the number of primary follicles in all of the rats studied. In contrast to the rats that ate *F. Vulgare*, which had secondary and mature follicles predominating, the rats that ate Clomiphene Citrate had primary follicles predominating, while the rats that ate *F. Vulgare* did not have any.

Recommendations

- Carry out further research to identify the minimum and maximum doses that can be used to obtain the same effects.
- Conduct future research comparing the effectiveness of different medicinal plants used in the South Iraqi Region, in addition to *F. Vulgare*.
- Carry out pilot studies to be carried out in human beings.

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Keywords: Foeniculum, Rattus norvegicus, Rats, Wistar, follicular maturation, Clomiphene Citrate

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