



## ORIGINAL ARTICLE

# Cellular study of Fennel's (*Foeniculum vulgare*) Effect on female rat's Mammary glands

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### ABSTRACT

Many of the herbs and plants, during the past centuries, were described as milk producing. Fennel (*Foeniculum vulgare*) was one of these herbs in which the medical prescriptions were mentioned by herbal medicine practitioners in various parts of the world. The reason of this study is to shed the lights of the fennel's effect on female rats' mammary glands in the three physiological cases (virgins, pregnant and lactating). 90 Norwegian white rat (*Sprague-Dawley*) were used and divided into four groups treated by fennel plant focusing 5% and 10% of the daily food for a period of 10 and 20 days, two sets of control for each focus for a period of 10 and 20 days, and have took its allocated animal feed and each group includes five animals. Then measuring weights process has been conducted for these animals after 10 days and 20 days. It was found that there is a significant increase  $p < (0.05)$  in alveoli diameters of the mammary glands, also an increase appeared in the number of the padded epithelial cells for the alveoli of the mammary glands. In the treated groups focusing (10%) for 10 and 20 days period which was the highest for the virgin rats, slight increase for the pregnant and a significant increase ( $P < 0.05$ ) for the lactating rats focusing (10%) for 10 and 20 days period. This means that the seed has given an incentive to increase the growth and the development of the mammary gland in virgin animals as well as the ability of the seed to increase the composition of milk in the duration of the pregnancy and increase the secretion of milk in lactating animals. All these effects were the greatest, biggest and most in all physiological cases in the case of concentration of (10%) for (20) days with the emergence of hyperplasia state. This gives an indication that the seed of fennel plant has worked to increase the chance of growth and development of the mammary glands of the female rats.

Keywrds: *Foeniculum vulgare*, Cellular Study

Received 10.09.2014

Revised 11.11.2014

Accepted 28.12.2014

### INTRODUCTION

The study of medicinal plants in terms of their nutritional value and medicinal with great economic importance to take advantage of them and the statement of the role of medical and food. Following that used many of the medicinal plants in folk medicine as generator of milk in women, including fennel *vulgare Foeniculum* [1, 2], which is belongs to the family Umeblifearae.

The mammary glands is skin modified glands exist in both males and females, but they become functional in females where they provide the milk, and so for being a key material in the nutrition newborns and provide antibodies [3]. There are mummies' tissues in both sexes, but become functional in females where are the lining of the mammary gland (mammary epithelium) production and secretion milk [4]. In most species, mammary gland consists of one pair, as in humans, goats, guinea pigs in either consist of [9-7] pairs, while in rats consists of six pairs [5]. The structure of the epithelial cells in the mammary glands be similar in different species of mammals animals, no matter the big difference in the chemical composition of the milk Manufacturer in which [6].

### MATERIAL AND METHODS

#### *Animals used in the experiment*

The white rats (*Spargue-Dawley*) were used in the test which were bought from the Laboratory animal breeding center of the Faculty of Medicine in Baghdad University.

Ninety white rat, virgin of two months with 60g, pregnant from the first third of the pregnancy with 200g weights, and lactating after the first day of birth with 190g weights, were divided into four groups treated according to the focus of the fennel plant which is (5% and 10%) of the daily food of the virgin rats for 10 and 20 days for each focus. A concentrated fodder was loosely served to the animals during the whole period of the experiment for the control animal for 10 and 20 days, each group included 5 animals, and it was put in special plastic cages for raising rats equipped with a nipple at its end, and the lighting period was 10 hours throughout the study period, the temperature ranged between (30C° during the day and 10 C° at night).

#### **Preparation of food of plant material**

Plant material was used in order to provide practical method economically inexpensive and as follow

#### **Preparation of ration food from fennel seed**

The focus was given to the fennel seeds for animals (5% and 10%) From the daily diet for each rat that is compatible with the method of giving the fennel plant seeds under the U.S. Constitution for Medical Herbs [7].

#### **Experience Design**

- **First phase:** the impact of the fennel plant seed (*Foeniculum vulgare*) on the mammary glands of the female rats during the puberty dividing the virgin rats as the following:

Thirty female rat, sexually adult of two months old, were used and put in big cages and divided randomly into two groups of control animals and four groups treated by the seed of the plant, and each group include 5 animals then treated as follow:

- **Control group**

The animals of this group have freely continued eating the concentrated animal feed throughout the duration of the experiment, in which the first group has continued for 10 days and the second group for 20 days.

- **Treated groups**

**First group:** the animals of this group have been given the fennel plant seed with a five percent focus of the daily food for each rat for a period of ten days.

**Second group:** the animals of this group have been given the fennel plant seed with a five percent focus of the daily food for each rat for a period of twenty days.

**Third group:** the animals of this group have been given the fennel plant seed with a ten percent focus of the daily food for each rat for a period of ten days.

**Fourth group:** the animals of this group have been given the fennel plant seed with a ten percent focus of the daily food for each rat for a period of twenty days.

**Second phase:** the study of the fennel's impact on the mammary glands of the female rats during the period of pregnancy dividing the rats as the following:

Thirty pregnant female rat, during the first third of the pregnancy, were used and put in big cages and divided randomly into two groups of control animals and four groups treated by the seed of the plant, and each group include 5 animals then treated as follow:

- **Control group**

The animals of this group have freely continued eating the concentrated animal feed throughout the duration of the experiment, in which the first group has continued for 10 days and the second group for 20 days.

- **Treated groups**

**First group:** the animals of this group have been given the fennel plant seed with a five percent focus of the daily food for each rat for a period of ten days.

**Second group:** the animals of this group have been given the fennel plant seed with a five percent focus of the daily food for each rat for a period of twenty days.

**Third group:** the animals of this group have been given the fennel plant seed with a ten percent focus of the daily food for each rat for a period of ten days.

**Fourth group:** the animals of this group have been given the fennel plant seed with a ten percent focus of the daily food for each rat for a period of twenty days.

**Third phase:** the study of the fennel's impact on the mammary glands of the female rats during the lactation period dividing the rats as the following:

Thirty lactating female rat, after the first day of birth, were used and put in big cages and divided randomly into two groups of control animals and four groups treated by the seed of the plant, and each group include 5 animals then treated as follow:

**Control group**

The animals of this group have freely continued eating the concentrated animal feed throughout the duration of the experiment, in which the first group has continued for 10 days and the second group for 20 days.

- **Treated groups**

**First group:** the animals of this group have been given the fennel plant seed with a five percent focus of the daily food for each rat for a period of ten days.

**Second group:** the animals of this group have been given the fennel plant seed with a five percent focus of the daily food for each rat for a period of twenty days.

**Third group:** the animals of this group have been given the fennel plant seed with a ten percent focus of the daily food for each rat for a period of ten days.

**Fourth group:** the animals of this group have been given the fennel plant seed with a ten percent focus of the daily food for each rat for a period of twenty days.

The animals (the treated and the untreated), after the lapse of the ten and twenty days period, have been anesthetized by Chloroform, then set on an anatomy cork to eradicate the mammary glands after removing the hair surrounding the nipples, then the nipple was lifted by the tongs, and a piece of tissue, then the mammary tissue was removed from the overlaying skin (8).

### **Cellular Study**

Measurements of histological slides prepared from mammary glands were calculated using the ocular micrometer "OM" that is fixed in the stage micrometer "SM", Macroscopic scale is a special circular bottle that Vertically and horizontally scalar from 0 to 100 unit, but the SM is a glass slide containing in a horizontal line with a length of 2 mm in its center divided on 200 unit, each unit equals 0.01 mm or 10 micron and used to while using macroscopic scale histological measurements under the following steps:

- 1- Putting the OM in the middle of the sheft between the two lenses; the upper and the lower of the ocular lens.
- 2- Putting the SM on the microscope platform, then we move till the clearly appearance of the horizontal line in the microscopic field.
- 3- We have rotated the ocular lens, included in the OM, with moving the cell until it matches the zero mark of the OM with the zero mark of the SM.
- 4- We recorded other identical numbers (5 which is on the OM and 7 which is on the SM in this case).
- 5- We extracted the value of each unit of the OM's units in the selected objective lens as the following equation:

$$\text{Each line of OM value} = \frac{\text{The matching number of (SM) X 10 Micron X magnification power}}{\text{The matching number of (OM) X magnification power measured}}$$

$$= \frac{7 \times 10 \text{ Micron} \times 10}{5 \times 100} = 1.4 \text{ micron}$$

- 6- Lifting the SM and putting glass slide to be measured instead.
- 7- After conducting the histological measurements that include the measurement of the alveoli diameters of the mammary glands and calculating the nucleus per alveolar section multiplying it with the constant factor (f) (1.4 micron). (9)

## **RESULT AND DISCUSSION**

### **Cellular study**

#### **Virgin group**

#### **Alveoli mammary glands (Micrometer)**

When the average of alveoli glands was 9.8 and 8.1 respectively in the virgin rats in the control group for 10 - 20 days to rise a little high (11.4 and 10.6)  $\mu$  by treatment the seeds with concentration (10 - 5) % for 10 days and (12.8 and 12.7)  $\mu$  for 20 days respectively see the table 4-4.

#### **Preparation of nucleus in the alveoli section (Nucleus\Alveoli)**

The effect of seeds with concentration (5%) for 10 days presented a little high in the average of preparing nucleuses to be 12 ones while the significant high  $p < (0.05)$  in the average of preparing nucleuses reached to 18 nucleuses in the virgin rats with concentration (10%) for 10 days in comparing with the average of preparing the nucleuses is 10 nucleuses in the virgin rats for 10 days, while the effect of the seeds focusing (5 and 10)% of the daily food for 20 days, the results showed that the significant high  $p < (0.05)$  in the average of preparing the nucleuses reached to 17 and 18 nucleuses in the virgin rats with

concentration (5 and 10)% of the daily nourishment for 20 days in comparing with the average of preparing the nucleuses is 10 nucleuses in the virgin rats for 20 days.

There was a significant difference  $p < (0.05)$  when comparing the average of preparing the nucleuses, that reached to 17 nucleuses of virgin rats with concentration (5%) for 20 days, with the its average is 12 nucleuses with the same concentration for 10 days – see the table 4-4.

Table 4-4 presents the average of the cavities of alveoli glands by micrometer and the average of preparing the nucleuses in the only one alveoli section of the virgin rats.

Groups	Duration			
	10 days		20 days	
Animals	Diameters cavities average $\pm$ SD	Nucleus average $\pm$ SD	Diameters cavities average $\pm$ SD	Nucleus average $\pm$ SD
control virgin	8.1 $\pm$ 4.2	10.0 $\pm$ 3.0	9.8 $\pm$ 4.2	10.0 $\pm$ 3.0
Virgin treated ( 5% )focus	10.6 $\pm$ 3.2	12.0 $\pm$ 6.0	12.7 $\pm$ 1.9	17.0 $\pm$ 4.0* $\theta$
Virgin treated ( 10% )focus	11.4 $\pm$ 4.3	18.0 $\pm$ 8.0*	12.8 $\pm$ 2.3	18.0 $\pm$ 3.0*

-Significant Differences are less than 0.05 between the controlling animals and the animals treated by the pip plant.

-Significant Differences are less than 0.05 between the animals treated by 10 days and the animals treated by 20 days.

#### **Pregnant Group**

##### **Alveoli mammary glands – Micrometer**

When the average of alveoli glands was 12.3 and 13.8 respectively in the pregnant rats in the control group for (10 – 20) days to rise a little high (14.1 and 14.5)  $\mu$  for 20 days by treatment the seed with concentration (5 – 10)% for 10 - 20 days respectively see the table 5-4.

##### **Prepare nucleus in the alveoli section (Nucleus\Alveoli)**

When the gland average of the preparing the nucleuses was (11 and 13) nucleuses respectively in the pregnant rats in the control group for (10 and 20) days, slight high presented in the average of preparing nucleuses reached to (14, 14, 15 and 17) nucleuses respectively of the pregnant rats treated by the seed with concentration (5 – 10)% for (10 and 20) days, See the table 5-4.

Table 4-5 presents the average of the cavities of alveoli glands by micrometer and the average of preparing the nucleuses in the only one alveoli section of the pregnant rats.

groups	Duration			
	10 days		20 days	
animals	Diameters cavities average $\pm$ SD	Nucleus average $\pm$ SD	Diameters cavities average $\pm$ SD	Nucleus average $\pm$ SD
Control pregnant	12.3 $\pm$ 2.5	11.0 $\pm$ 3.0	13.8 $\pm$ 3.1	13.0 $\pm$ 4.0
Pregnant treated with concentration of ( 5% )	12.4 $\pm$ 2.3	14.0 $\pm$ 4.0	14.1 $\pm$ 2.5	15.0 $\pm$ 3.0
Pregnant treated with concentration of ( 10% )	14.1 $\pm$ 5.2	14.0 $\pm$ 5.0	14.5 $\pm$ 3.7	17.0 $\pm$ 4.0

#### **Lactating group**

##### **Alveoli mammary glands ( Micrometer)**

The results showed three significant and non-significant differences  $p < (0.05)$  in the average of alveoli glands of the lactating group through the following

-Significant high  $p < (0.05)$  in the average of alveoli glands that is 25.3 and 30.6 micrometer, respectively, of the lactating group treated by Sham mar with concentration 5 – 10 percent for 10 days in comparing with the average of alveoli glands that is 17.5 micrometer of lactating rats for 10 days.

- Significant high  $p < (0.05)$  in the average of alveoli glands that is (30.6)  $\mu$  of the lactating group treated by the seed with concentration (10)% for 10 days in comparing with the average of alveoli glands that is (25.3)  $\mu$  that treated by concentration (5) % for the same duration.

- A little high in the average of alveoli glands that is (32 and 38.5)  $\mu$  respectively of the lactating group that treated by the seed with concentration (5 and 10) % for 20 days in comparing with the average of alveoli glands that is (30.2)  $\mu$  of the lactating group for 20 days.

- Significant high  $P < (0.05)$  of alveoli glands average when comparing the average of cavities glands that is (38.5 and 32)  $\mu$  respectively of lactating rats treated by the seed with concentration (5 and 10) % for 20 days and its average that is (25.3 and 30.6)  $\mu$  respectively for 10 days.

-Significant differences  $P < (0.05)$  of alveoli glands average of lactating control group when comparing the average of alveoli glands that is (30.2) $\mu$  of lactating control group for 20 days with the average of alveoli glands that is (17.5) $\mu$  of lactating control group for 10 days, see the table 6-4.

**Preparing the nucleus in the alveoli section – Nucleus\Alveoli section**

The results of statistic analysis present the effect of the seed with concentration (5 and 10) % for (10 and 20) days, on the average of preparing the nucleuses in the only one alveoli section as following

- The effects of the seed with concentration (5) % for 10 days of lactating rats treated by it lead to little high in the average of preparing the nucleuses (12) nucleuses while presenting significant high  $P < (0.05)$  in the average of preparing the nucleuses to be (18) nucleuses in the lactating rats treated by concentration (10) % for the same duration when comparing with the average of preparing the nucleuses (10) nucleuses of the lactating control rats for 10 days.
- Significant high  $P < (0.05)$  when comparing the average of preparing the nucleuses that is (18) nucleuses of lactating rats treated with concentration (10) % for 10 days, with the average of preparing the nucleuses that is (12) nucleuses treated by concentration (5) % for the same duration.
- The effect of the seed with concentration (5 and 10) % of the daily nourishment for 20 days of the rats caused little high in the average of preparing nucleuses (21 and 21) nucleuses when comparing with the average of preparing the nucleuses that is (20) nucleuses of the control lactating rats for 20 days.
- There is significant differences  $P < (0.05)$  in the average of preparing the nucleuses(21) nucleuses of the control lactating rats treated by concentration(5) % for 20 days when comparing with the average of preparing the nucleuses that is (12) nucleuses with the similar treated by the same concentration for 10 days.
- There is significant differences  $P < (0.05)$  in the average of preparing the nucleuses (20) nucleuses of the control lactating rats for 20 days, from the average of preparing nucleuses (10) nucleuses in the control group for 10 days, see the table 6-4.

groups		Duration		
Animals	Diameters cavities average $\pm$ SD	Nucleus average $\pm$ SD	Diameters cavities average $\pm$ SD	Nucleus average $\pm$ SD
Control lactating	17.5 $\pm$ 4.3	10.0 $\pm$ 1.0	30.2 $\pm$ 7.7«	«20.0 $\pm$ 4.0
Lactating treated with concentration of ( 5% )	25.3 $\pm$ 6.9*	12.0 $\pm$ 2.0	32.0 $\pm$ 9.5 $\emptyset$	21.0 $\pm$ 2.0 $\emptyset$
Lactating treated with concentration of ( 10% )	30.6 $\pm$ 3*#	18.0 $\pm$ 5.0*#	38.5 $\pm$ 11.7 $\emptyset$	21.0 $\pm$ 3.0

Table 4-5 presents the average of the cavities of alveoli glands by micrometer and the average of preparing the nucleuses in the only one alveoli section of the pregnant rats.

-Significant differences are less than 0.05 between treatment and control animals.

-Significant differences are less than 0.05 percent between the animals treated by the seed with concentration (5)% of the daily nourishment and the animals treated with concentration (10) %.

-Significant differences are less than 0.05 between control animals for 10 days and control animals for 20 days.

The increasing number of the lined epithelial cells of lumens and increasing the glands lumens for the three physiological cases (virgin, pregnant and lactating) but the fennel plant seed with its different concentrations and the two durations [10 and 20] days has significant effect on the significant increasing the number of epithelial cells and lumens glands in the two control groups. The results of virgin rats that the seed with the concentrations of (10)% of the daily nourishment for 20 days effect significantly which may caused by its high concentration with treatment duration which lead to increase of the nucleuses of cells and there are few secretions that caused few increasing the lumens with concentration [5] % of the daily nourishment for 20 days and [5 and 10] % for 10 days for that the seed increases possibility growth the mammary glands in the virgin rats through the cellular proliferation which is the basic factor of the natural growth the mammary gland this provided by study for rats 13 for 14 and 15 days to increase the epithelial cells number are responsible of milk subsequently increasing secretions and widening the lumens to be 18 to present significant role

On the seed in the lactating rats with the different concentrations and the two durations (10 and 20) days, this caused by the increasing of epithelial cells number that play a role in increasing of secretions in the alveoli cavities (glycoprotein and fats) that means increasing milk manufacturing to transfer subsequently in to the ducts then into out of mammary gland, this agree with [10-11] for that the increasing play a role in widening the alveoli and ducts cavities. Mammary gland growth and differentiation its cells increasing during the first days of lactating, this caused by divisions, highs, increasing the number and size the epithelial cells at the same time the connective tissues lactate vessel that feed the alveoli. All these changes contribute to increase the effectiveness of glands secretions of rats (12-13). This produce a reason that there are significant differences  $P < (0.05)$  between the average of alveoli cavities and the average of preparing control lactating nucleuses for 20 days so between the average of alveoli cavities and the average of preparing control lactating nucleuses for 10 days.

These results of treatment by fennel plant seed with concentration (5 and 10) % of daily nourishment for 10 days and with concentration (5) for 20 days . which similar the results of (9) under pressure the water extract of Harmal plant seeds while the results of treatment by fennel plant seed with concentration (10)% for 20 days with the results of (9) under pressure the water extract of *Borago officinalis*.

## CONCLUSIONS

- 1- It becomes so clear, from the current study, that the fennel plant seed with its 5% and 10% focus of the daily food and for the periods 10 and 20 days has clear affect on the mammary glands of the female rat in which it can be used in the growth and development process of the mammary glands and increase in milk secretion.
- 2- The fennel plant seed, 10% of the daily food for 10 days and 5% of the daily food for 20 days, is characterized by its high ability in the growth and development process of the mammary glands of the female rats, i.e., it has the same effect as the estrogen and progesterone, as well as its ability in increasing milk secretion in the pregnant rats and in lactating rats which means it has the same effect of the prolactin hormone which is a measure to the effectiveness of the mammary glands.
- 3- The fennel plant seed, 10% of the daily food for 20 days, is characterized by its dynamic growth of the mammary gland and it has the same effect of the prolactin hormone which helps to increase the secretion of the milk in the lactating animals.
- 4- The possibility of using the fennel plant seed in the increase of growth chances of the mammary glands and its development is by 5% and 10% of the daily food for 10 days, and 5% for 20 days, while the 10% use of the fennel in the daily food for 20 days can help to increase the secretion of the milk.

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#### **CITATION OF THIS ARTICLE**

Noori M Al-S, Salem R Al-O, Olfat Q A J. Cellular study of Fennel's (*Foeniculum vulgare*) Effect on female rat's Mammary glands. *Bull. Env. Pharmacol. Life Sci.*, Vol 4 [2] January 2015: 101-107