

## Cellular Study of Fennel's (*Foeniculum Vulgare*) Effect on Female Rats' Mammary Glands

Noori M. Al-Sudany<sup>1</sup>, Salem R. Al-Oubaidei<sup>2</sup>, Olfat Q. Abdul-Jabbar<sup>3</sup>

<sup>1</sup>Department of Biology, College of Science, Al-Mustansiriya University, Baghdad, Iraq.

<sup>2</sup>Department of Pathology, College of Medicine, Baghdad University, Baghdad, Iraq.

<sup>3</sup>Department of Studies, Planning and Follow-up, Ministry of Higher Education and Scientific Research, Iraq.

### ABSTRACT

*During the past centuries, many herbs and plants, were described as milk-producing plants. Fennel (*Foeniculum vulgare*) was one of these herbs which was mentioned in medical prescriptions by herbal medicine practitioners in various parts of the world. The reason of this study is to shed the light of the fennel's effect on female rats' mammary glands in the three physiological cases (virgins, pregnant, and lactating). A total of 90 Norwegian white rats (*Sprague-Dawley*) were included in the study and were divided into 4 groups. These groups were treated with fennel plant focusing 5% and 10% of the daily food for a period of 10 and 20 days; two sets of control were used for each focus for a period of 10 and 20 days, who took their allocated animal feed. Each group includes five animals. Then measuring weight process was conducted for these animals after 10 days and 20 days. A significant increase in  $p$  ( $<0.05$ ) was observed in alveoli diameters of the mammary glands. Also an increase in the number of the padded epithelial cells for the alveoli of the mammary glands was observed. In the treated groups focusing 10% of the daily food for a period of 10 and 20 days (the highest for the virgin rats), a slight increase in  $p$  for the pregnant rats, and a significant increase in  $p$  ( $P<0.05$ ) for the lactating rats focusing (10%) for a period of 10 and 20 days period were observed. This means that the seed has given an incentive to increase the growth and development of the mammary glands in virgin animals as well as to increase the composition of milk during pregnancy and increase the secretion of milk in lactating animals. All these effects were pronounced in all physiological states in the case of a concentration of 10% and duration of 20 days with the emergence of hyperplasia state.*

*This indicates that seeds of the fennel plant increase the chance of growth and development of the mammary glands of female rats.*

*Key words: Fennel, *foeniculum vulgare*, mammary gland*

### INTRODUCTION

The study of medicinal plants in terms of their nutritional and medicinal values are of great economic importance, and one should take advantage of them. Many of the medicinal plants have been used in folk medicine as generator of milk in women, including fennel *Vulgare foeniculum* (1, 2), which belongs to the family *Umebliferae*.

The mammary glands are skin-modified glands that exist in both males and females, but they become functional in females where they produce milk—a key material in the nutrition of newborns providing antibodies (3). There are mammary tissues in both sexes, but these become functional in females, where the lining of the mammary gland (mammary epithelium) produce 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 it consists of six pairs (5). The structure of the epithelial cells in the mammary glands is similar in different species of mammals; however, there is a big difference in the chemical composition of the milk in these species (6).

Correspondence:

Noori M. Al-Sudany

Department of Biology, College of Science, Al-Mustansiriya University, Baghdad, Iraq.

e-mail: ihsanalsaimary@gmail.com

## MATERIAL AND METHODS

### Animals used in the experiment

The white rats (Spargue-Dawley) used in the test were bought from the Laboratory Animal Breeding Center of the Faculty of Medicine in Baghdad University.

Ninety white rats, virgin of 2 months with 60 g weight, pregnant from the first third of the pregnancy with 200 g weights, and lactating after the first day of birth with 190 g 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 five animals, and they were put in special plastic cages for raising rats equipped with a nipple at its end. The lighting period was 10 hours throughout the study period, and the temperature ranged between 30°C during the day and 10°C at night).

### Preparation of food of plant material

The plant material was used to provide a practical method economically inexpensive and as discussed in the following sections.

*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 US Constitution for Medical Herbs (7).

### Experience Design

*First phase:* To note the impact of the fennel plant seed (*F. vulgare*) on the mammary glands of the female rats during the puberty, 30 female rats (sexually adult of 2 months of age) were put in big cages and divided randomly into 2 groups of control animals and 4 groups of animals treated with the seeds of the plant. Each group included five animals and were treated as follow:

- Control group

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

- Treated groups

*First group:* The animals of this group were given the fennel plant seeds with a 5% focus of the daily food for each rat for a period of 10 days.

*Second group:* The animals of this group were given the fennel plant seeds with a 5% focus of the daily food for each rat for a period of 20 days.

*Third group:* The animals of this group were given the fennel plant seeds with a 10% focus of the daily food for each rat for a period of 10 days.

*Fourth group:* The animals of this group were given the fennel plant seeds with a 10% focus of the daily food for each rat for a period of 20 days.

*Second phase:* To study the fennel's impact on the mammary glands of the female rats during the period of pregnancy, 30 pregnant female rats, during the first third of the pregnancy, were used and put in big cages and divided randomly into 2 groups of control animals and 4 groups of animals treated with the seeds of the plant. Each group included five animals and were treated as follow:

- Control group

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

- Treated groups

*First group:* The animals of this group were given the fennel plant seeds with a 5% focus of the daily food for each rat for a period of 10 days.

*Second group:* The animals of this group were given the fennel plant seeds with a 5% focus of the daily food for each rat for a period of 20 days.

*Third group:* The animals of this group were given the fennel plant seeds with a 10% focus of the daily food for each rat for a period of 10 days.

*Fourth group:* The animals of this group were given the fennel plant seeds with a 10% focus of the daily food for each rat for a period of 20 days.

*Third phase:* To study the fennel's impact on the mammary glands of the female rats during the lactation period, 30 lactating female rats, after the first day of birth, were used and put in big cages and divided randomly into 2 groups of control animals and 4 groups of animal treated with the seeds of the plant. Each group included five animals who treated as follow:

- Control group

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

- Treated groups

*First group:* The animals of this group were given the fennel plant seeds with a 5% focus of the daily food for each rat for a period of 10 days.

*Second group:* The animals of this group were given the fennel plant seeds with a 5% focus of the daily food for each rat for a period of 20 days.

*Third group:* The animals of this group were given the fennel plant seeds with a 10% focus of the daily food for each rat for a period of 10 days.

*Fourth group:* The animals of this group were given the fennel plant seeds with a 10% focus of the daily food for each rat for a period of 20 days.

The animals (the treated and the untreated), after the lapse of the 10- and 20-day period, were anesthetized by chloroform; they were then set on an anatomy cork to eradicate the mammary glands after removing the hair surrounding the nipples. The nipple was then lifted by the tongs, and a piece of tissue was extracted. 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. The OM was placed in the middle of the shaft between the two lenses: the upper and the lower of the ocular lens.
2. The SM was placed on the microscope platform, and it was adjusted till a horizontal line appeared in the microscopic field.
3. The ocular lens present in the OM was rotated with moving the cell until the zero mark of the OM matched with the zero mark of the SM.
4. Other identical numbers were recorded (5 which is on the OM and 7 which is on the SM in this case).

The value of each unit of the OM in the selected objective lens was extracted using the following equation:

Each line of OM value = The matching number of (SM) x 10 $\mu$  x Magnification power / The matching number of (OM) x Magnification power measured

$$= 7 \times 10 \mu \times 10 / 5 \times 100$$

$$= 1.4 \mu$$

1. The SM was lifted and the glass slide was put to be measured instead.
2. The histological measurements were conducted that included 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).

## RESULTS AND DISCUSSION

### Cellular study

There are three groups in this study such as Virgin group, Pregnant group and Lactating group.

#### Virgin group

*Alveoli mammary glands (micrometer):* When the average sizes of alveoli glands were 9.8 and 8.1, respectively, in the virgin rats and in the control group for 10–20 days, the rats were treated with the seeds (with the concentrations of 5% and 10% for 10 days to increase the size of the alveoli glands by 11.4 and 10.6  $\mu$ m, respectively, and

**TABLE 1:** Presents the average size of the cavities of alveoli glands in micrometer, and the average number of prepared nucleuses in a single alveolus section of the virgin rats.

Groups	Duration		Duration	
	10 days		20 days	
Animals	Diameter cavities average $\pm$ SD	Nucleus average $\pm$ SD	Diameter 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 with the pip plant.  
 $\Theta$  Significant differences are less than 0.05 between the animals treated with the pip plant for 10 and 20 days.

with the same concentration for 20 days to increase the size of the alveoli glands by 12.8 and 12.7  $\mu$ m, respectively (Table 1).

*Preparation of nucleus in the alveoli section (nucleus/alveoli):* At a concentration of 5% of seeds for 10 days, the average number of prepared nucleuses reached to 12 ( $p < 0.05$ ). The average number of prepared nucleuses reached to 18 in the virgin rats at a concentration of 10% of seeds for 10 days compared with the average number of prepared nucleuses that is 10 nucleuses, in the virgin rats for 10 days at the same concentration. However, the effect of the seeds focusing 5% and 10% of the daily food for 20 days showed a high significance ( $p < 0.05$ ), and the average number of prepared nucleuses reached to 17 and 18 nucleuses in the virgin rats with concentration 5% and 10%, respectively, of the daily nourishment for 20 days compared with the average number of prepared nucleuses (10 nucleuses) in the virgin rats for 20 days.

A significant difference ( $p < 0.05$ ) was observed when the average number of prepared nucleuses was compared, which reached to 17 nucleuses at the concentration of 5% for 20 days in virgin rats and 12 nucleuses at the same concentration for 10 days (Table 1).

#### Pregnant Group

*Alveoli mammary glands – Micrometer:* The average diameters of alveoli glands were 12.3 and 13.8  $\mu$ m, respectively, in the pregnant rats and in the control group for 10 and 20 days. To

increase the diameter to 14.1 and 14.5  $\mu$ m in the pregnant rats and the control group, respectively, the rats were treated for 20 days with the seeds of fennel at concentrations of 5% and 10% for 10 and 20 days (Table 2).

*Prepare nucleus in the alveoli section (Nucleus/Alveoli):* The gland average of preparing the nucleuses was 11 and 13  $\mu$  nucleuses in the pregnant rats and in the control group, respectively, for 10 and 20 days. When the pregnant rats were treated with fennel seeds at the concentrations of 5% and 10% for 10 and 20 days, the number of nucleuses slightly increased to 14 and 14, respectively, for the concentration of 5% and 10% in 10 days and the size of the nucleuses increased to 15 and 17, respectively, for the concentration of 5% and 10% in 10 days (Table 2).

#### Lactating group

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

-A significantly high  $p < (0.05)$  in the average size of alveoli glands, that is 25.3 and 30.6  $\mu$ m, respectively, of the lactating group treated with Sham mar at the concentrations of 5% and 10% for 10 days compared with the average size of alveoli glands, that is 17.5  $\mu$ m of lactating rats for 10 days.

TABLE 2: Presents the average size of the cavities of alveoli glands in micrometer, and the average number of prepared nucleuses in a single alveolous section of the pregnant rats.

Groups	Duration			
	10 days		20 days	
Animals	Diameter cavities average $\pm$ SD	Nucleus average $\pm$ SD	Diameter cavities average $\pm$ SD	Nucleus average $\pm$ SD
Control pregnant	13.0 $\pm$ 4.0	13.8 $\pm$ 3.1	11.0 $\pm$ 3.0	12.3 $\pm$ 2.5
Pregnant rats treated with the concentration of 5%	15.0 $\pm$ 3.0	14.1 $\pm$ 2.5	14.0 $\pm$ 4.0	12.4 $\pm$ 2.3
Pregnant rats treated with the concentration of 10%	17.0 $\pm$ 4.0	14.5 $\pm$ 3.7	14.0 $\pm$ 5.0	14.1 $\pm$ 5.2

- A significantly high  $p < (0.05)$  in the average size of alveoli glands, that is 30.6  $\mu\text{m}$  of the lactating group treated with the seed at a concentration of 10% for 10 days compared with the average size of alveoli glands, that is 25.3  $\mu\text{m}$ , treated at a concentration of 5% for the same duration.

- A little high in the average of alveoli glands, that is 32 and 38.5  $\mu\text{m}$ , respectively, of the lactating group treated with the seed at the concentrations of 5% and 10% for 20 days compared with the average of alveoli glands, that is 30.2  $\mu\text{m}$ , of the lactating group for 20 days.

- A significantly high  $P < (0.05)$  in the average size of alveoli glands when compared with the average of cavity glands, that is 38.5 and 32  $\mu\text{m}$ , respectively, of lactating rats treated with the seed at the concentrations of 5% and 10% for 20 days and its average, that is 25.3 and 30.6  $\mu\text{m}$ , respectively, for 10 days.

- Significant differences  $P < (0.05)$  in the average size of alveoli glands of the lactating control group when compared with the average of alveoli glands, that is 30.2  $\mu\text{m}$  of the lactating control group for 20 days with the average of alveoli glands, that is 17.5  $\mu\text{m}$  of the lactating control group for 10 days (Table 3).

*Preparing the nucleus in the alveoli section – Nucleus\Alveoli section:* The results of statistical analysis present the effect of the seed with concentrations 5% and 10% for 10 and 20 days,

on the average of preparing the nucleuses in a single alveolous section as follows:

- The effects of the seed at a concentration of 5% for 10 days on lactating rats lead to slight increase in the average number of nucleuses prepared, that is 12 nucleuses, presenting a significant high  $P < 0.05$ . However, the average number of nucleuses prepared was 18 in the lactating rats treated at a concentration of 10% for the same duration when comparing with the average of nucleuses prepared, that is 10 nucleuses, of the lactating control rats for 10 days.
- A significantly high  $P < 0.05$  was observed when the average number of nucleuses prepared (18 nucleuses) in lactating rats treated at a concentration of 10% of seeds for 10 days was compared with the average number nucleuses prepared (12 nucleuses) in lactating rats treated at a concentration of 5% for the same duration.
- The inclusion of the seed with concentrations of 5% and 10% of the daily nourishment for 20 days in the rats resulted in a slight increase in the average number of nucleuses prepared (21 and 21 nucleuses, respectively) when compared with the average number of prepared nucleuses (20 nucleuses) in the control lactating rats for 20 days.

TABLE 3: Presents the average size of the cavities of alveoli glands in micrometer and the average number the nucleuses prepared in a single alveolous section of the pregnant rats.

Groups	Duration			
	10 days		20 days	
Animals	Diameter cavities average $\pm$ SD	Nucleus average $\pm$ SD	Diameter 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 the concentration of 5%	25.3 $\pm$ 6.9*	12.0 $\pm$ 2.0	32.0 $\pm$ 9.5 $\Theta$	21.0 $\pm$ 2.0 $\Theta$
Lactating treated with the concentration of 10%	30.6 $\pm$ 3*#	18.0 $\pm$ 5.0*#	38.5 $\pm$ 11.7 $\Theta$	21.0 $\pm$ 3.0

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

$\Theta$  Significant differences are less than 0.05% between the animals treated with the seed at concentrations of 5% and 10% of the daily nourishment.

# A significant difference is less than 0.05 between control animals for 10 days and control animals for 20 days

- There is a significant difference ( $P < 0.05$ ) observed in the average of nucleuses prepared (21 nucleuses) in the control lactating rats treated at a concentration of 5% for 20 days when compared with the average nucleuses prepared (12 nucleuses) in the control lactating rats treated at the same concentration for 10 days.
- There is significant difference ( $P < 0.05$ ) observed in the average nucleuses prepared (20 nucleuses) in the control lactating rats for 20 days when compared with the average nucleuses prepared (10 nucleuses) in the control group for 10 days (Table 3).

The increasing number of the lined epithelial cells of lumens increases the lumen glands for the three physiological cases (virgin, pregnant, and lactating), but the fennel plant seed with its different concentrations and two durations (10 and 20 days) significantly increases the number of epithelial cells and lumen glands in the two control groups. The results of virgin rats demonstrate that the seed at a concentration of 10% of the daily nourishment for 20 days affects significantly. This may be attributed to the combination of the high concentration of the seed and treatment duration, which leads to the increase

of nucleuses of cells. The size of the lumen slightly increases using a concentration of 5% of the daily nourishment for 20 days. At concentrations of 5% and 10% of the seed for 10 days, the size of the mammary glands in the virgin rats increases through the cellular proliferation, which is the basic factor of the natural growth of the mammary glands. The findings of this study demonstrate that the use of the seed in 13 rats for 14 and 15 days increases the number of epithelial cells subsequently increasing milk secretion and widening the lumens (18  $\mu$ m), thus presenting a significant role of the seed in the lactating rats with the different concentrations and the two durations (10 and 20 days). The increase in the number of epithelial cells plays a role in increasing the secretions in the alveolous cavities (glycoprotein and fats), which means increase in milk manufacturing to transfer subsequently into the ducts, and then out of the mammary glands. This agrees with (10-11) that the increase in the number of epithelial cells plays a role in widening the alveoli and duct cavities. The growth of the mammary glands and increased differentiation of its cells during the first days of lactation are caused by divisions, high, and increase in the number and size of the epithelial cells; at the same time the connective tissues lactate the vessel that

feed the alveoli. All these changes contribute to increase the effectiveness of gland secretions of rats (12-13), thus revealing that there are significant differences ( $P < 0.05$ ) between the average size of alveoli cavities and the average number of lactating nucleuses prepared 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 are similar to 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

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 a effect 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.

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 those of the estrogen and progesterone, as well as its ability in increasing milk secretion in the pregnant rats and in lactating rats means it has the same effect as that of the prolactin hormone which is a measure to the effectiveness of the mammary glands.

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 that helps increase the secretion of the milk in the lactating animals.

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.

## REFERENCES

1. A. Roiyh. Herbal medical treatments scientific method include modern medicine and ancient. 7th ed, Pen house, Beirut, Lebanon, 1978, p. 197-198.
2. Husein, F. Q. (1979). Cultivation of medicinal plants and their components. Arab House Book ,Libya - Tunisia. 1st (Ed.). P. 240, 358.
3. Anderson, S.M., Rudolph, M.C., McManaman, J.L. and Neville, M.C.(2007). Key stages in mammary gland development. Secretary activation in the mammary gland: it's not just about milk protein synthesis! *Breast Canc. Res.* 9(1): 204.
4. Medina, D. (1996). The mammary gland: a unique organ for the study of development and tumorigenesis. *J. of Mamm. Glan. Biol. and Neopl.* 15-19.
5. Hebel, R. and Strombery, M.W. (1986). Female reproductive organs In: *Anatomy and embryology of the laboratory rat*. Bio. Med. Vertage worthsee. 231-257.
6. Burgoyne, R. D. and Duncan, J. S. (1998). Secretion of milk proteins *mamm. Glan. Biol. Neopl.* 3(3): 275-286.
7. PDR for herbal medicines (1998). Medical economics company. 1st (ed.), 850-851.
8. Al-Khalisi, M.H. (2000). The Effect of Fenugreek seeds on mammary gland. A Histological and Histochemical Approach. PhD Thesis. College of Medicine. University of Baghdad.
9. Al-sady, W.A.(2005). The Effect of some seeds on mammary gland. A Histological, Histochemical, and Histochemical-Immunology approach. PhD Thesis. College of Science. University of Mustansiriya.
10. Leeson, T., Leeson, C. and Paparo, A. (1988). Mammary gland. In: *Text / Atlas of Histology*. Leeson T, Leeson C and Paparo A (Eds.). Saunders Co., Philadelphia, London. 631 – 634.
11. Junqueira, L. C., Carneio, J. and Long, J. (1986). Mammary glands. In: *Basic Histology*. Junqueira L C, Carneio J and Kelley R O (eds.). 5th (ed.), Lange Medical Publication, Los Altos, California, 507-511.
12. Pei, R.J., Sato, M., Yuri, T., Danbara, N., Nikaido, Y., and Tsubura, A. (2003). Effect of prenatal and prepubertal Genistein Exposure on N-Methyl-N nitrosourea induced mammary Tumorigenesis in female Sprague-dawley rats. *In vivo, Japan.* (17):349-358.
13. McFarlin, D.R. (2002). Characterizing oncogenic Ras effectors in a rat mammary gland model. Athesis PH.D/university of Wisconsin-Madison.