

## Histological 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, Baghdad, Iraq.

### 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). Ninety Norwegian white rats (*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 histological section methods were used with the use of Haematoxylin and eosin stains. It was found there was an increase in the number of alveoli and a slight expansion in the cavities of the alveoli's mammary glands for the virgin and pregnant rats and expansion in the cavities of the alveoli's mammary glands for the lactating rats and a slight increase in the secretory substances for the pregnant and lactating. 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.*

*Key words: *Foeniculum vulgare*, herb, mammary glands*

### INTRODUCTION

The study of medical plants in terms of nutritional value and pharmaceutical are of significant economic importance to take advantage of them and a statement its medical and food role, including the (fennel) plant which belongs to the Umbelliferaea, and this plant is one of the most important plants in Iraq and around the world because of its medical and Nutritional benefits and considered as one of the medical plants that is producing milk (1). The mammary glands accomplish its unique mission by the producing and editing sufficient quantity of milk from the mother to the infant through an extensive network of subsidiary canals (2).

All mammary glands consist of (15-25) lobes, each lobe is composed from lobules and each lobule is made up out of alveolar unit, lobes are separated from each other by dense connective tissue with a lot of adipose tissue.

Each lobule has Tubulo-alvolar from which the milk is passed to mammary duct and to the Ampulla or Lactiferous sinuses then to Lactiferous duct which opens the nipple (3).

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) were used in the test which were bought from the Laboratory animal breeding center of the Faculty of Medicine in Baghdad University.

Ninety white rats, 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 (30°C 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 (4).

### *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 rats, 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 10 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 20 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 10 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 20 days.

Second phase: the study of the fennels 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 10 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 20 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 10 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 20 days.

Third phase: the study of the fennels impact on the mammary glands of the female rats during the lactation period dividing the rats as the following:

Thirty lactating female rats, 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:

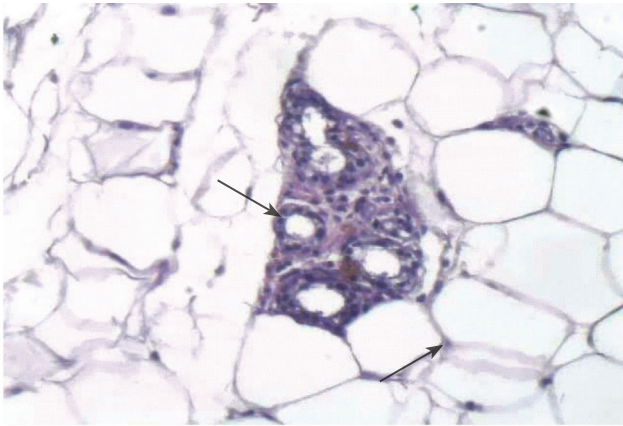


FIGURE 1: Tissue section of mammary glands of female virgin rat –control untreated – for 10 and 20 days presents that the mammary glands which include few alveoli in large amount of adipose tissue- plus 200.

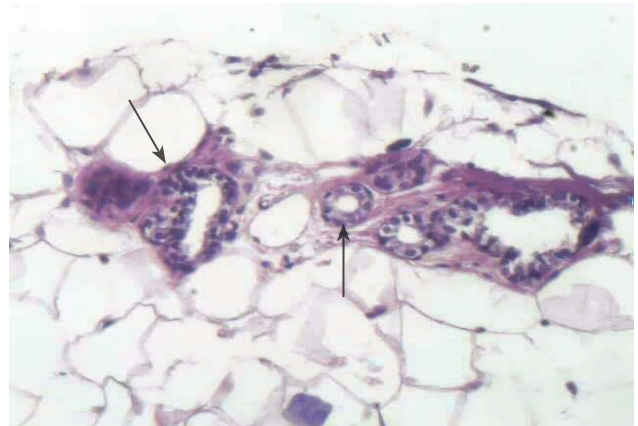


FIGURE 2: Tissue section of mammary glands of female virgin rat treated by concentration 5 percent for 10 days presents few hyperplasia and hypertrophy in the lined cells with few widening the lumens, plus 200.

### 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 10 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 20 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 10 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 20 days.

The animals (the treated and the untreated), after the lapse of 10 and 20 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.

### Fixation Fluid

### Samples of the histological study

The samples of the mammary glands have immediately anchored in the 10% formalin for 24 hours.

### Histological study

Paraffin slides were prepared according to the method (7).

## RESULT AND DISCUSSION

### Histological study

There are many histological changes with the treatment by fennel plant seed with concentration 5 and 10 percent of the daily nourishment for 10 and 20 days with the related ducts that make the effect of fennel plant seed with concentration 5 and 10 percent of the daily nourishment for 10 and 20 days, on the mammary glands possible through the nature of these histological changes as following:

### Virgin Group

#### • Control Group for 10 and 20 days

The examination of dyed the tissue sections with Hematoxyline-Eosine tincture on the control group for 10 and 20 days, presented that the mammary glands which include few of alveoli in the large amount of adipose tissue (Figure 1).

#### • Treatment Groups

#### 1. Group treated by fennel plant seed with concentration 5% for 10 days

Tissue sections of the mammary glands of this group presented few hyperplasia and hypertrophy in the lined cells of ducts and alveoli and few widening the cavities in comparing with control group (Figure 2).

#### 2. Group treated by fennel plant seed with concentration 10% for 10 days

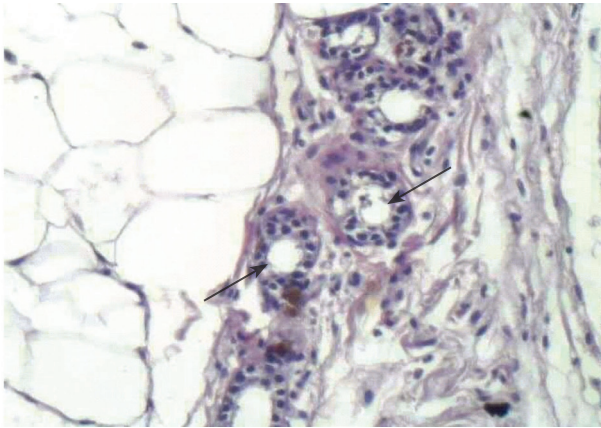


FIGURE 3: Tissue section of mammary glands of female virgin rat treated by concentration 10% for 10 days presents few hyperplasia and hypertrophy in the lined cells and few increasing the number of alveoli with widening lumens, plus 200.

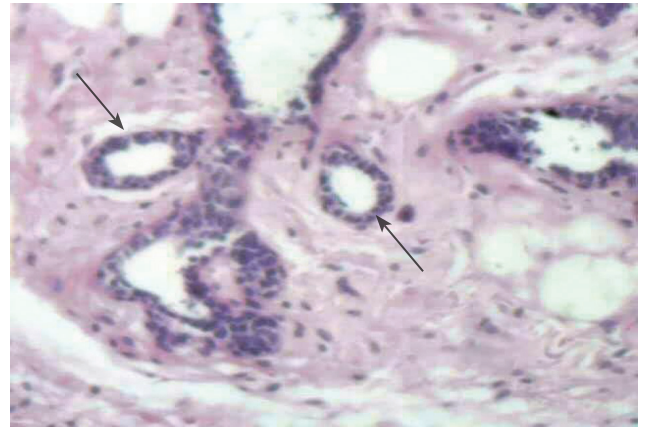


FIGURE 4: Tissue section of mammary glands of female virgin rat treated by fennel plant seed with concentration 5% for 20 days presents hyperplasia and hypertrophy in the lined cells of the alveoli and ducts with widening the lumens and ducts, plus 200.

Tissue sections of the mammary glands of this group presented few hyperplasia and hypertrophy in the lined cells of alveoli with few increasing alveoli number and few widening the lumens in comparing with control group and its counterpart group treated by concentration 5% (Figure 3).

3. Group treated by fennel plant seed with concentration 5% for 20 days

Tissue sections of the mammary glands of this group presented hyperplasia and hypertrophy in the lined cells of alveoli and ducts and few widening the lumens and ducts in comparing with control group with the concentration 5% for 10 days (Figure 4).

4. Group treated by fennel plant seed with concentration 10% for 20 days

Tissue sections of the mammary glands of this group presented significant hyperplasia and hypertrophy in the lined cells of alveoli and few increasing alveoli number and few widening the cavities including few excretions in comparing with the control group and group treated by concentration 5 and 10% for 10 days and 5% for 20 days (Figure 5).

#### Pregnant Group

• Control Group for 10 days

The tissue section of this group presents significant increasing the number of alveoli and the fats of hyperplasia in the lined epithelial cells of alveoli and widening the lumens that including few secretions with increasing the connective tissue (Figure 6).

• Treatment Group

1. Group treated by fennel plant seed with concentration 5% for 10 days

The tissue sections of the mammary glands in this group presents significant hyperplasia in the lined epithelial cells with increasing the number of alveoli to the detriment of the adipose tissue and including few secretions in comparing with the control group (Figure 7).

2. Group treated by fennel plant seed focusing 10% for 10 days

Histological sections of the mammary gland of this group showed a hyperplasia for the epithelial surrounding the lumens with an increase in the number of the lumens, increase in connective tissue located between the lobules and simple expansion in the lumens including secretory substances compared to the control group and its counterpart that is treated 5% focus (Figure 8).

• Control group for 20 days

Histological sections of this group showed an increase in the number of the lumens and the connective tissue existing between them (Figure 9).

• Treatment groups

1. Group treated by fennel with 5% concentration for 20 days

Histological sections of this group showed a vivid hyperplasia for the padded epithelial cells of the lumens as well as the number of the lumens located in the mammary gland with a simple expansion in the diameters of the lumens including secretory substances compared to the control group (Figure 10).

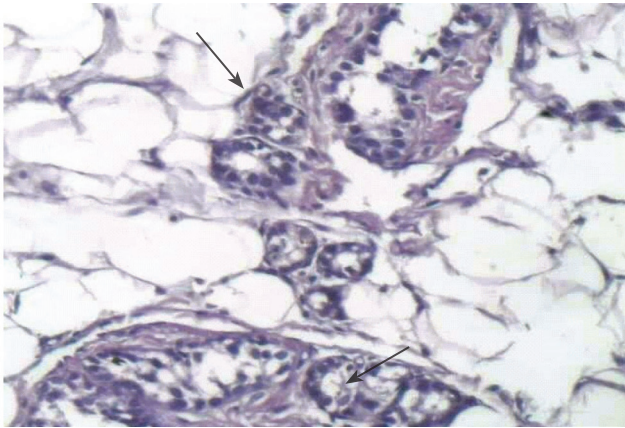


FIGURE 5: Tissue section of mammary glands of female virgin rat treated by fennel plant seed with concentration 10% for 20 days presents significant hyperplasia and hypertrophy in the lined cells of the alveoli and increasing the number of alveoli with few widening the cavities including few secretions, plus 200.

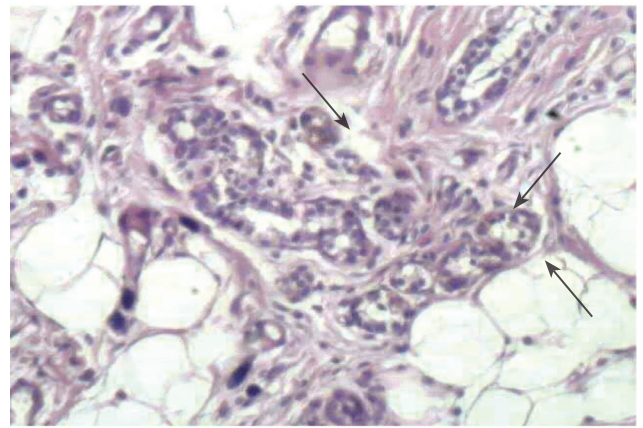


FIGURE 6: Histological section of the mammary gland of the pregnant rat (control) for (10) days shows a noticeable increase in the number of the lumens in relation to the fat with a hyperplasia in the cells and expansion in the lumens containing a few secretory substances with increase of (H&E) (200x).

2. Group treated by fennel with 10% concentration for 20 days

Histological sections of this group showed a hyperplasia in the padded epithelial cells of the lumens as well as the number of the lumens located in the mammary gland with more advent of secretory substances compared to the control group and other treated groups (Figure 11).

Lactating group

There are some histological changes that accompanied the treatment by the fennel plant seed on the lactating rats which are the following:

• Control group for 10 days

Histological sections of the mammary gland show a hyperplasia and increase in the number of the lumens generating secretory of Glycoprotein which is being watched by rosy homogeneous content inside the lumens and the ducts (Figure 12).

• Treatment groups

1. Group treated by fennel with concentration of 5% for 10 days

Histological sections showed an increase in the number of the lumens with its expansion, and the presence of secretory

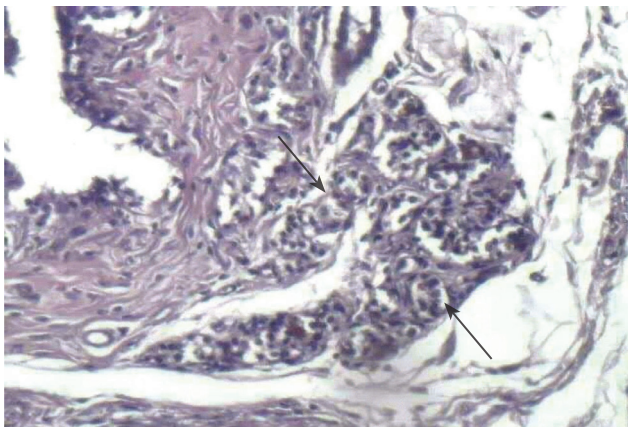


FIGURE 7: Histological section of the mammary gland of the pregnant rat treated by fennel with 5% focus for 10 days shows a vivid hyperplasia for the padded epithelial cells of the lumens as well as the number of the lumens to the detriment of the fat tissue with its containing of few secretory substances (H&E) (200x).

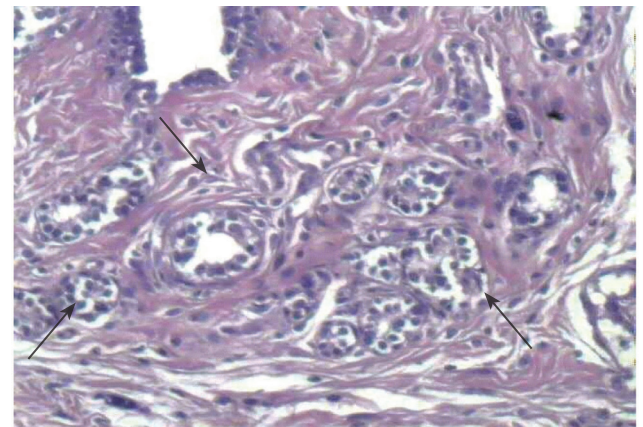


FIGURE 8: Histological section of the pregnant rat's mammary gland of this group that is treated by fennel plant seed focusing 10% for 10 days showed a hyperplasia for the epithelial surrounding the lumens and hyperplasia in the number of the lumens, increase in connective tissue located between the lobules and simple expansion in the lumens including secretory substances (H&E) (200x).

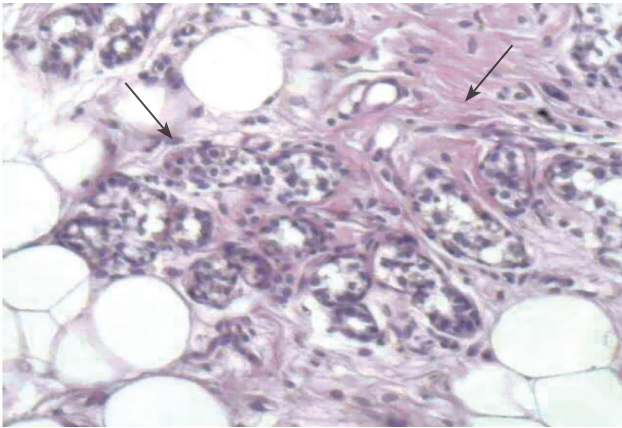


FIGURE 9: histological section of the mammary gland of the female pregnant rat (control) for 20 days shows increase in the number of the lumens and the connective tissue existing between them (H&E) (200x).

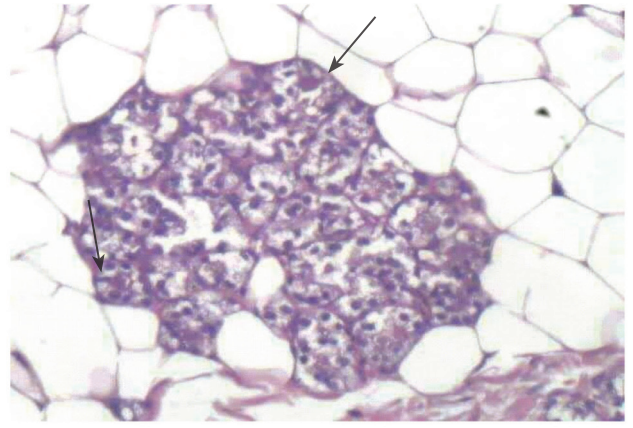


FIGURE 10: histological section of the mammary gland of the female pregnant rat that is treated by fennel plant seed with 5% focus of the daily food for 20 days shows a vivid hyperplasia for the padded epithelial cells with a simple expansion in the diameters of the lumens including secretory substances (H&E) (200x).

substances (Glycoprotein) inside the lumens and the ducts (Figure 13).

2. Group treated by fennel with concentration of 10% for 10 days

Histological sections of this group showed a hyperplasia in the padded cells of the lumens with an increase in the number of the lumens and expansion in its lumens containing few secretory substances to move some of it to the ducts compared to the control group and its counterpart that is treated by 5% focus (Figure 14).

• Control group for 20 days

Histological sections show a noticeable increase in the number of the lumens and the hyperplasia of the epithelial of the lumens with an expansion of the lumens generating secretory (Figure 15).

• Treatment group

1. Group treated by fennel with concentration of 5% for 20 days

Histological sections of this group show a hyperplasia of

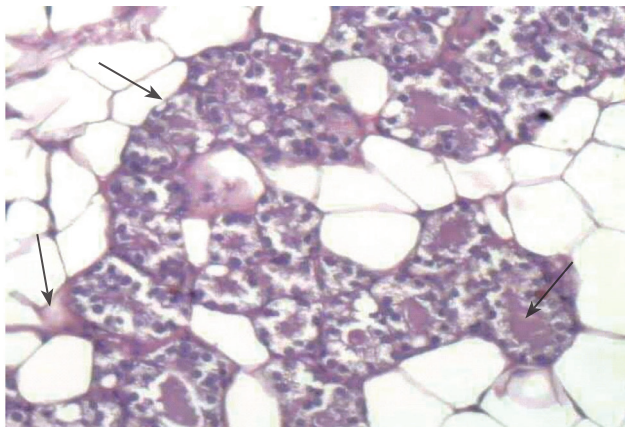


FIGURE 11: Histological section of the mammary gland of the female pregnant rat that is treated by fennel plant seed with 10% focus of the daily food for 20 days shows a hyperplasia in the padded epithelial cells as well as the number of the lumens with an expansion in the connective tissue existing between them and an increase in secretory inside the lumens and the ducts (H&E) (200x).

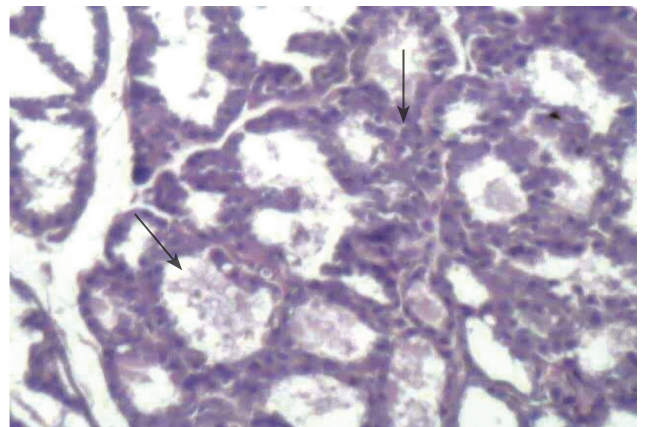


FIGURE 12: Histological section of the mammary gland of the lactating rat (control) for 10 days shows an increase the number of the lumens generating glycoprotein inside the lumens and the ducts (H&E) (200x).

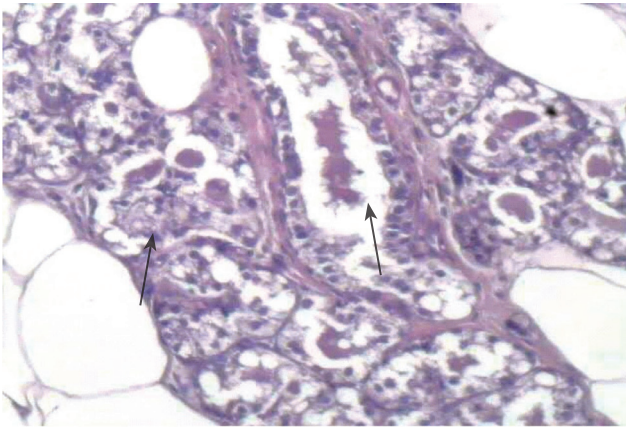


FIGURE 13: Histological section of the mammary gland of the lactating rat treated by fennel with 5% focus for 10 days shows an increase in the number of the lumens with the excretion inside the lumens and the ducts (H&E) (200x).

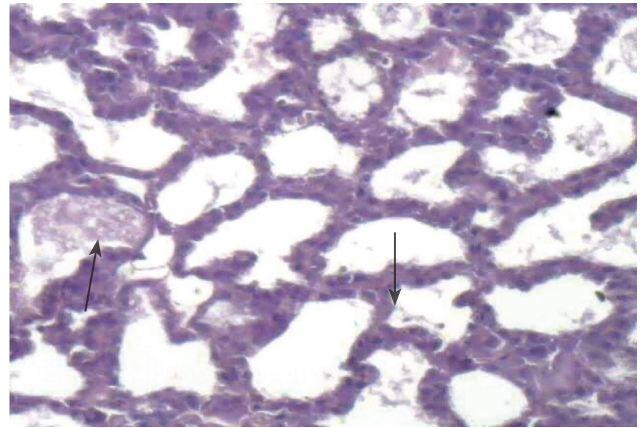


FIGURE 14: Histological section of the lactating rat's mammary gland of this group that is treated by fennel plant seed focusing 10% for 10 days showed a hyperplasia for the epithelial surrounding the lumens and increase in the number of the lumens, with the simple excretion inside the lumens and take it to the ducts (H&E) (200x).

the padded epithelial of the lumens with an increase of the lumens and an expansion of the lumens containing a lot of secretory substances inside itself and in the dilated ducts comparing it with control group and its counterpart that is treated by 5% focus for 10 days (Figure 16).

2. Group treated by fennel with concentration of 10% for 20 days

Histological changes of this group included a hyperplasia of the padded epithelial of the lumens with an increase of the lumens and an expansion of the lumens, knowing that it contains a little bit of secretory to move it out the mammary gland (Figure 17).

The results of the histological study indicated that, the fennel plant seed with 5% and 10% focus of the daily food for 10 and 20 days, stimulate to increase the chance of the mammary glands growth and its development through:

#### Virgin group

1. Simple hyperplasia of the padded epithelial cells of the lumens with a simple expansion of the lumens, 5% for 10 days.

2. A simple increase appeared in the number of the lumens, hyperplasia and hypertrophy of the padded epithelial cells with a simple expansion of the lumens, 5% for 20 days and 10% for 10 days.

3. Numerical increase and clear expansion in the lumens, a hyperplasia in the padded epithelial, and the emergence few secretory substances in the lumens of the mammary gland of the virgin treated 10% focus for 20 days.

This means that the fennel plant seed has given mammatropic effect in the growth and the development of the mammary gland, it is believed that the growth of the mammary gland in rodents depends on the glandular overlap produced topically. It may show direct stimulus to compose lumens and the breeding of the mammary cells of the lumens and the ducts under the effect of Estrogen hormone. The studies of in vivo and in vitro, indicated that the progesterone is necessary lobuloalveolar process, and this what has been proven from 27 and its group in 1995 who proved that the existence of a dense network of (estrogen and progesterone for the rats treated by this plant) distributed around the lumens and the mammary glands ducts. As well as the effect of the seed and the

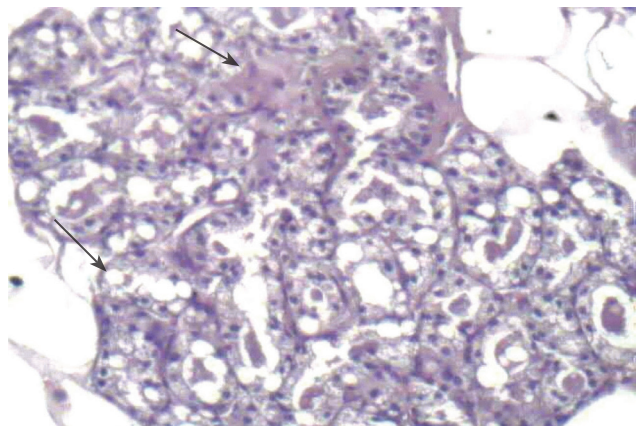


FIGURE 15: Histological section of the mammary gland of the female lactating rat (control) for 20 days shows noticeable increase in the number of the lumens (H&E) (200x).

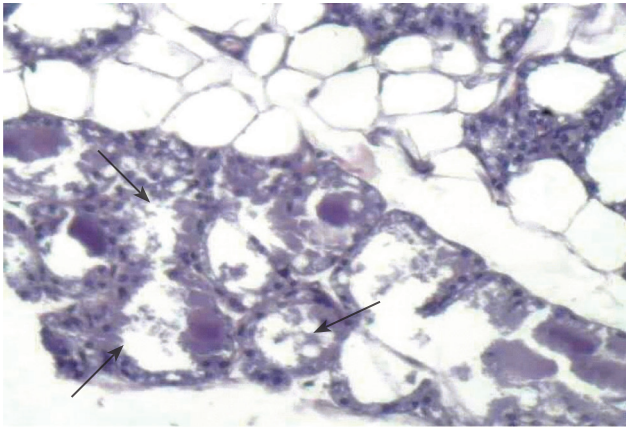


FIGURE 16: Histological section of the mammary gland of the female lactating rat that is treated by fennel plant seed with 5% focus of the daily food for 20 days shows a vivid hyperplasia for the padded epithelial cells with an expansion in the diameters of the lumens including a lot of secretory substances inside the lumens and the ducts (H&E) (200x).

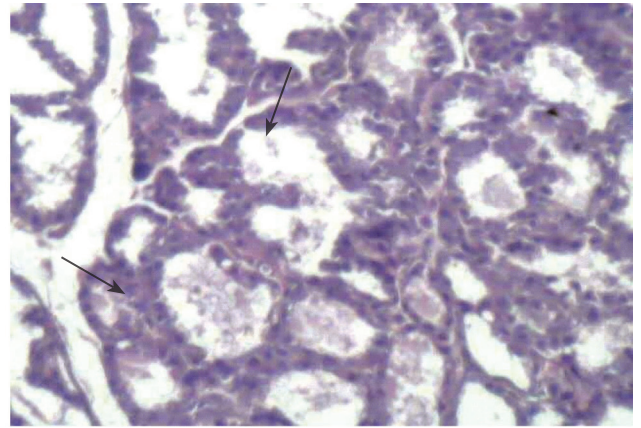


FIGURE 17: Histological section of the mammary gland of the female lactating rat that is treated by fennel plant seed with 10% focus of the daily food for 20 days shows a hyperplasia in the padded epithelial cells of the lumens as well as the number of the lumens with a clear expansion in lumens, and the existence of lumens including some excretions to move it out of the mammary glands (H&E) (200x).

effect of the estrogen and progesterone effect, the seed was given with concentration of 10% of the daily food to the virgin rat for 20 days, and lactogenic effect for what medical oil it contains which differs from the oil of leaves, it will be of lower quality in these parts.

The final phases of the mammary glands development depends on the lobules and lumens development which requires the prolactin hormone. The prolactin hormone lead a major role in the mammogenesis and lactogenesis.

The results have come through out the treatment by fennel plant seed, 5% and 10% of the daily food for 10 and 5% for 20 days, in conformity with the results obtained by 34 under the influence of water conclusion of cumin seeds, and in contravention with the treated by 10% focus of the daily food for 20 days. This might be due to the length of the time and the increase of focus.

#### Pregnant group

The results of the histological study indicated that, the fennel plant seed with concentration of 5% and 10% of the daily food for 10 and concentration of 5% for 20 days, show a hyperplasia of the padded epithelial cells of the lumens and increase in the number of the lumens and the connective tissue with the increase of secretory substances as well as the concentration of 10% for 20 days comparing to the control group.

These results are identical to the results obtained by under the effect of water conclusion of Fenugreek.

#### Lactating group

The results of the histological study indicated that, the Lactating group treated by the fennel plant seed with concentration of 5% and 10% of the daily food for 20 days, show a hyperplasia of the epithelial cells of the lumens and clear expansion in the lumens as well as the 10% focus for 20 days which gave an indication of the increase of the affectivity of the mammary gland by the increase padded epithelial tissue of lumens, whereas the increase faces a decrease the percentage of the connective and oil tissue, and this is identical to the them found the mammary gland of the female adult rat consist of epithelial, connective and oil tissue 5%, 85% and 10%, while the tissue of the lactating, the differences are divided as 62%, 30% and 2% respectively.

These results are identical to the results through the treatment by fennel plant seed with 5% and 10% of the daily food for a period of 10 and 20 days that is obtained by (5) under the effect of water conclusion of Fenugreek.

#### 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 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.



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.

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.

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. Piccaglia R, Marotti M. Characterization of some Italian types of wild fennel (*Foeniculum vulgare* Mill.). *J of Agri and Food Chem* 2001;49:239-244.
2. Sternlicht MD. Key stages in mammary gland development: the cues that regulate ductal branching morphogenesis. *Breast Cancer Res* 2006;8:201.
3. Mescher AL. Junqueira's: Basic histology. Ed by Mescher AL, 12th ed, Mc Graw Hill Companies, Singapore. 408-411, 2010.
4. PDR for herbal medicines. Medical economics company. 1st ed, 850-851, 1998.
5. Bancroft J. Enzyme histochemistry. In: Theory and practice of Histological Techniques Bancroft. Ed by Stevens A, 2nd ed, Churchill living stone, London pp 3374-3405, 1982.
6. Matsumoto M, Nishinakagawa H, Kurohmaru M, Hayashi Y. Effect of estrogen and progesterone on the parenchyma and blood vessels of the mammary gland in ovariectomized adult mice. *J Vet Med Sci* 1995;57: 39-44 (Abstract).
7. Al-Yawer MA. Evaluation of some galagtagogues in the female rat: Histological, Histochemical and morphometrical studies. PH.D. Thesis, college of medicine. University of Baghdad, 2003.
8. Al-Khateeb HMD. Some Morphological and Histochemical Studies on Rat's Mammary Gland. PhD Thesis, College of Medicine. University of Baghdad, 1996.
9. Blackburn DG. Lactation: Historical patterns and potential for manipulation. *J Dairy Sci* 1993;76:3195-3212.
10. Rees E, Eversole A. Rat mammary gland metabolism relative to epithelium and connective tissue content. *Am J Physiol* 1964;207:595-600.
11. Al-Khalisi MH. The Effect of Fenugreek seeds on mammary gland. A Histological and Histochemical Approach. PhD Thesis. College of Medicine. University of Baghdad, 2000.