Histological Effects of *Sesamum Indicum* Seeds on Mammary Gland tissue in Female white Rats

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Abstract. This study was conducted to investigate the effects of sesame seed on the growth and development of mammary glands in white females rats during the stages of (virginity, pregnancy and lactating), where the effect of 30% sesame seed on the growth and development of mammary glands was studied for 20 days. Sixty-three female Albino rats were randomly divided into three groups (virgins, pregnant and lactating) 12 rats /for each group , the three main groups were subdivided into secondary groups (control and treatment) with 6 rats /for each group. Then toe parameters were conducted including: Histological and histochemical studies for each group and through these studies the following results were obtained:

Histological specimens stain with hematoxylin and eosin stains revealed that the virgins treated with sesame seeds showed an increase in the number of the lobules filled with large numbers of expansive alveoli compared to the control group. In the pregnant groups, the lobules filled with alveoli were observed in larger numbers and diameters. In the lactation groups, the lobules are more numerous and the alveolus were more expansive and numerous in comparison with the control group.

The histochemical studies of the Periodic Acid Schiff (PAS) stain showed a positive intra-alveolar reaction in the mammary glands tissue for each animal of treated and control groups, except the virginity control group which showed a negative reaction to the stain. Whereas the Trichrome stain, which characterized only the control group of virgins with more density of collagen fibers compared to the rest of the groups which were characterized by a deficiency in the density collagen fiber groups for all stages compared to the control groups.

From the current study it can be concluded that the sesame seeds have an effective effect on the growth and development of mammary gland tissue.

1- Introduction

The mammary glands are compound tubuloalveolar gland that synthesis and secret the milk, which is the best source of nutrition for newborns.

The secretion of milk controlled by the prolactin hormone, that directly responsible for stimulating the secretion of milk which low levels indicate a decrease of breastfeeding in mothers [1]. Therefore, there is an ongoing interest in understanding and definition the milk-producing plants and their extracts [2].

Sesame seeds contains important source of vitamins, minerals and antioxidants essential for human health[3],such as vitamin B complex ( B1, thiamine, B2, riboflavin
B3, niacin, B6, pyridoxine, B9, folic acid, B12, copalamin), vitamin C, ascorbic acid and tocopherol also contains necessary minerals such as potassium, phosphorus, magnesium, calcium, iron, manganese, zinc, selenium, copper and sodium. Beside that sesame lignans of sesamol, sesamolin and sesamin important considered compounds in sesame seeds, also it contains essential fatty acids such as omega-3, omega-6, linolenic acid, valenolinic acid, phosphorus and sterols.

Sesame seeds also prescribed for treatment of menopause. In addition to increasing the expression of beta(β) estrogen receptors in the uterus, sesame seeds have ability to reduce the growth of breast tumors.

The presence of some elements such as iron, copper and cobalt increases the concentration of hemoglobin red blood corpuscles (RBC) in addition to increase the number of blood cell by stimulating the bone marrow on its composition due to the presence of vitamin B12. In the other hand, the importance of sesame seed in testicular improvement (mean number and movement of sperm in the epididymis, number and size of epithelial cell count, cavity and interstitial area as well as nuclei) and promotion of fertility and sperm production in male rats.

Thus, the main aim of this study was to investigate the effect of *Sesamum indicum* seed on the growth of mammary tissue in rat.

### 2- Materials and Methods

A total of (36) adult female were used in the experiment, their average body weight ranged between 140 -200gm. They were kept at a temperature between 20 -24 °C (room temperature). Animals were housed individually in wire-meshed stainless steel cages. The light / dark cycle was maintained as 12 hr.s /12hr.s. Rats were fed the ordinary pallet diet and maintained on free access to food and water.

The animals were randomly divided into 3 primary groups (virgins, pregnant and lactating) (12 rats\ groups) (6 rats\ treated group) and six rat served as a control. The treated groups were used as pellets with sesame seeds percent 30% (W / W) following the method.

Tissue samples were collection Immediately after death the mammary gland for histochemical study were excised and preserved in fixative solution depending on the study till the preparation of histological sections.

Fasting blood samples were drawn via intra-cardiac puncture, blood was kept into epindrof tube without EDTA, held for not more than four hours before serum collection by centrifugation 3000 rpm for 15 minutes and frozen at -20°C until analysis.

### 3- Results

Haematoxyln-eosin stained sections of the treated virgins(Fig.2) exhibited showed an increase in the size of lobules which were packed by alveolar buds and intralobular ducts, these alveoli were more dilated compared with control (Fig. 1). Mammary tissue sections from pregnant rats treated with sesame seed showed an increase in branching of alveoli with more flattened luminal epithelium (Figure 4) in comparison with control ( Figure 3). Pouring of milk from adjacent alveoli was frequently seen.

On the other hand, histological sections (figure 6) of lactating rats treated with sesame seed showed greatly dilated lobules containing more branching and more dilated alveoli with flattened epithelium in comparison with control treated groups. Pouring of secretory product was seen from adjacent alveoli (Figure 5). histochemical studies have shown the positive reaction with Periodic Acid Schiff (PAS) stain within the lumen of the alveoli in
all animals treated and controlled as in the Fig.(8,9,10,11,12), except in the group of virgins control observed negative reaction to PAS (Fig.7). The Trichrome stain showed decreased in dense of collagen fibers in the stroma in all animals (Fig.14,15,16,17,18) Except in the virgin control group showed increased in dense of it (Fig.13).

Figure(1) Histological section of mammary glands of virgin in control group .Show The presence of lobules and bronchial buds) and the interstitial ducts that are scattered in stroma Of the connective tissue and adipose tissue (X10 H & E)

A: Vesicles, D: Adipose tissue, C: Connective tissue ID: Interstitial ducts (arrow) l:terminal buds

Figure(2) Histological section of mammary gland of virgin in sesame treated group show large size of lobules (arrow) packed by alveolar buds compared to control, show is a less of stroma connective tissue and adipose tissue (X10 H & E)

Figure(3) Histological section of mammary gland of pregnant in control group shows The presence of lobules(arrow) and stroma connective tissue and adipose tissue (X10 H & E)

D: Adipose tissue, C: Connective tissue

Figure(4) Histological section in mammary gland of pregnant rat group treated with sesame shows the presence of lobules filled Vesicles with larger numbers and diameters (arrow) and stroma of connective tissue and adipose tissue is less than control (X10 H & E)

D: Adipose tissue, C: Connective tissue
Figure (5) Histological section in mammary gland of lactating rat in control group shows the expanding lobules and their number (arrow) Filled with a large of vesicles containing secretions and stroma of connective tissue (X10 H & E).
A: Vesicles, C: Connective tissue

Figure (6) Histological section in mammary gland of lactating rat group treated with sesame shows increased vesicle expansion and branching (arrow) and larger in size and full more than the control (X10 H & E).
A: vesicles

Figure (7) Histological section in mammary gland of virgin rat in control group shows negative reaction to the PAS stain (10X).

Figure (8) Histological section in mammary gland of virgin rat group treated with sesame shows positive reaction to the PAS stain (10X).
Figure (9) Histological section in mammary gland of pregnant rat in control group shows positive reaction to the PAS stain (arrow) (10X).

Figure (10) Histological section in mammary gland of pregnant rat group treated with sesame shows positive reaction to the PAS stain.

Figure (11) Histological section of mammary gland of lactating rat in control group shows positive reaction to the PAS stain (arrow) (10X).

Figure (12) Histological section in mammary gland of lactating rat in sesame treated group shows positive reaction to the PAS stain (arrow) (10X).

Figure (13) Histological section in mammary glands of virgin in control group shows large aggregation of stroma (arrow) (X10) Trichrome.

Figure (14) Histological section in mammary gland of virgin group treated with sesame shows less of stroma (arrow) (X10) Trichrome.
The present study pointed that the administration of female rats in all physiological stages with sesame seed caused an increase in the size of lobules that packed with branching dilated alveoli, especially during pregnant and lactation stages compared with control, These histological features indicate that the control mammary glands revealed features of a resting mammary gland, which match the age and physiology of this group, while the sesame seed treated groups showed that seeds had ability to induce mammogensis in the mammary glands of virgin, pregnant and lactating rats in addition to estrogen, progesterone and prolactin this results may due to the

4- Discussion

The present study pointed that the administration of female rats in all physiological stages with sesame seed caused an increase in the size of lobules that packed with branching dilated alveoli, especially during pregnant and lactation stages compared with control, These histological features indicate that the control mammary glands revealed features of a resting mammary gland, which match the age and physiology of this group, while the sesame seed treated groups showed that seeds had ability to induce mammogensis in the mammary glands of virgin, pregnant and lactating rats in addition to estrogen, progesterone and prolactin this results may due to the
presence of active components in sesame seeds like quercetin, and Sesamin which are types of phytoestrogen that promote the secretion of prolactin and development of mammary glands [14] [15].

The phytoestrogen was two types identified as (Secoisolariciresinol and matariresinol). When ingested, these compounds converted into mammalian Lignans (Enterodiol and Enterolactone), respectively, by bacterial flora in the colon of mammals. These biphenolic compounds have chemical structures that closely resemble that of endogenous 17-β estradiol and possess biphasic agonistic (estrogenic) and antagonistic (antiestrogenic) activities in vitro and in vivo [17] [16] revealed the Quercetin stimulates expression of prolactin receptors from the pituitary gland, as well as stimulates the proliferation of epithelial cells in mammary gland, while sesamin has the potential effect to stimulate estrogen E2 actions by binding to estrogen receptors [15]. The estrogen hormone acts through it is receptor ERα in mammary gland. ERα is essential for luminal epithelial proliferation, differentiation and survival during pregnancy and lactation in addition to virgin stage [18]. Furthermore, the downstream of estrogen receptor are progesterone receptor and cyclin D1. The progesterone receptor is required for epithelial differentiation and morphogenesis, while the cyclin D1 is regulated by progesterone receptor and had positive feedback on ERα transcriptional activity in mammary gland development, so that the possibility of using sesame seeds as a treatment for oligomenorrhea instead of progesterone hormone therapy [19] and [18]. Moreover, [20] pointed that the mammary stem cell might be ER positive. This cell has ability to produce complete mammary gland tissue in a manner resemble to that found in hemapoietic system, where the primary mammary stem cell gives rise to hierarchy of progenitor cell lineages to ultimately produce the different cells found in the mammary epithelium [21] Accordingly, we can postulate that the induction growth in mammary tissue constituent occurred after the estrogen hormone bind to ER in mammary stem cell. (Aupperlee and Haslam 2007)[22]

Progesterone is known synchronously effect with prolactin in promoting the development of the lobulo-alveolar system in the mammary glands [19]. Besides that, the presence of many vitamins and minerals in the seeds of sesame lead to an increase in milk production, the vitamin A and B-Carotene have a role in maintaining epithelial tissue in the mammary glands [23].

In another study indicated that folic acid has a role in increasing milk production[24] Souza and his colleagues suggest that a diet rich in zinc, magnesium [4] and selenium has improved milk production[25].

PAS stained sections of control and experimental rats paralleled and confirmed those stained by H & E stain. However, the strong positive PAS reaction in the milk secretion (seen within the lumen of alveolar and ducts of mammary glands of control and experimental rats in the pregnant and lactation groups and virgin treated group only) could be attributed to the presence of glycoprotein.

In addition, the glycoprotein together with the large lipid droplets, visualized in milk secretion, may point to the fact that there is a synthesis of the three main constituents of milk (carbohydrates, proteins and lipids) [26] [27]. The current study indicated that the mammary tissue sections of rats that are treated with spearmint and barley exhibited positive reactions to the PAS stain; these findings refer to the ability of spearmint and barley to promote lactation in these groups either by the prolactin and estrogen like actions.
or by increase the levels of these hormones. High level of prolactin hormone in the lactating mother lead to promotion of milk secretion [28] due to the active compounds that were previously discussed.

In Trichrome stained tissue showed a decrease in the proportion of collagen fibers, this is due to active compounds in sesame seeds (flavonoids and amino acids) as well as vitamins that have a role in the development and growth of the mammary glands. Besides, the increase in size of the lobules and the secretory contents of the alveoli which contribute to the decrease collagen fibers and in turn the stroma[29][30]

5. Conclusions:
The administration of sesame seeds to the female rats in the three physiological stages (virgins, pregnant and lactating) lead to promote the growth and development of mammary glands.

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