Mammogenesis Effect of Hot Aqueous Extract of *Mentha spicata* Leaves on Mammary Tissue of Ovariectimized Rabbits

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Abstract

Background: Plants are highly rich in phytochemicals being used to treat various conditions, and a rich source of polyphenols is Spearmint (Mentha spicata L). Such polyphenols have demonstrated multiple bioactivities. This study aims to investigate the effects of the intake of hot-aqueous extracts of spearmint leaves on mammary gland development and growth

Method: Thirty six female rabbits were divided into two main groups (18 rabbits/group): (virgin and lactating). Each group was further subdivided into three subgroups (Control, Ovariectomized (OVX) and Spearmint, (6 rabbits/group).All three groups tissues were examined for histological, histochemical and biochemical enzymatic analysis.

Result: Spearmint-treated ovariectimized virgin animals demonstrated an increase in the size of lobules that affected the alveolar bud. In the ovariectimized lactating group treated with spearmint, it was shown that the lobules were enlarged and produced more branching alveoli. The stain (PAS) and ALP showed positive reaction in the spearmint group treated with lactation.

Conclusion: The hot methanol extract of the spearmint Mentha spicata leaves have a beneficial effect on mammary gland growth and development.

Keywords: *Mentha spicata, Ovariectomy, mammary gland virgin, lactating,rabbit.*

Introduction

The mammary glands are complex tubuloalveolar glands which main function is to produce and secrete milk, it plays a major role in human nutrition either through oral feeding or via the food industry[1]. Mother's milk plays an significant role in neonatal survival, proper development and growth[2]. Mammary glands begin to develop in females at puberty and achieve maximum functional status after pregnancy. Ovarian steroids (estradiol and progesterone) play a crucial role as regulators of the different stages of mammogenesis and mammalian development[3]. Menopause is defined as the point in time after consecutive months of amenorrhea and refers to changes occurring in the hypothalamic-pituitary-ovarian axis [4].

Many females actively looking for alternative menopausal medications such as herbal nutritional supplements in attempting for reliefng the weakness, depression anxiety, extreme fatigue, joint pain and insomnia[5]. Herbal plants are an important source of herbal care and often form the basis of indigenous or traditional healing systems, which are still commonly used by the majority of populations in many countries[6,7]. The Lamiaceae family of Mentha is widely distributed in Europe, Asia, Africa, Australia and North America[8,9]. Extracts of this genus are traditionally used foods and

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are highly valued due to the presence of large amounts of antioxidant such as phenolic bioactive compounds[10]. Bazii *et al*, 2013 showed that the *Mentha spicata* leaves have mammagens activity without any toxicityeffect[11], Probably, it is because of the anti-androgen spearmint action, which increased serum oestrogen levels, luteinizing hormone (LH) and follicle stimulating hormone (FSH)[12,13]. Accordingly, The aim was to shed light on the stimulating ability of spearmint in the mammary tissue of ovariectimised rabbits.

Material and Method

Animals preparation: Thirty-six healthy virgin and lactating female rabbits from the ages of approximately 5-12 months old were used. The animals were housed 4/cage in a facility with a constant temperature of 22°C with consistent humidity and 12/12 hours light/dark cycle in Animal Housing Department/vet. Collage/ Karbala University. The rabbits were fed on green vegetables and tab water ad libitum. All female rabbits were divided into two main groups (18 rabbits/group): The G1 group contained the virgin rats. While the G2 group examined the lactating rabbits. Each group was further divided into three subgroups (6 rabbits per sub group):The control group was intubated tap water orally while the bilateral ovariectomy group were intubated orally with 5.25 ml/kg b.w [14], serving as spearmint group for the duration of one month.

Preparation of the hot Aqueous Extracts: Spearmint leaves had been bought from the local market. Soaking method was used to produce plant extracts[15].

Surgical operation: After routine preparation of the site of operation (midline region), the general anesthesia was induced with diazepam in dose 1mg/kg b.w. after 10 minutes as a preanesthetic. Then xylazine was injected in doses of 10 mg/kg b.w. while ketamine, in 50 mg/ kg b.w. Both injections were performed intramuscularly according to open operation [16]

Tissue sampling and processing: The tissue samples obtained from the mammary gland of all rabbits from both groups were anesthetized with chloroform directly after being sacrificed. Routinally stained histological slides with hematoxyl, eosin stain and the Periodic Acid Schiffs Stain Was achieved through the procedure that described by Lawrence [17]

Alkaline Phosphatase Activity: Most mammary gland ALPs are in the myoepithelial cells, Epithelial secretory cells have low levels of ALP activity, which could play a role in milk secretion. This enzyme relates to mammaryfunction and may be concerned with nutrient transport. Therefore the activity of ALP was measured to demonstrate the alkaline phosphatase activity based on modified procedure described by[18.]

Results

Histological Studies:

Virgin Rabbits: In the Control group Haematoxyln-eosin stained tissues illustrated the lobules were packed with alveolar buds and intralobular ducts. While small lobules scattered among huge amount of adipose tissue were examined in the OXV group. In Spearmint group there was an increase in the size of lobules when compared with the OVX group Fig.1(a,b and c) respectively.

Lactating Rabbits: The histological examination in OXV group showed an increase in the lobular size with corresponding decrease in the adipose tissue. The alveoli and ducts were dilated and filled with secretory products. Figure 2band2c illustrated thespearmint group which showcased that lobules were increased in size, packed with branching alveoli and filled with secretory products. In the OXV and Spearmint groupsgreatly, narrow lobules containing little branching and non-dilated alveoli in compared withControl group(Fig.2a).

Periodic Acid Schiffs Staining: Staining with Periodic Acid Schiffs (PAS) stain gives identical features to those stained with Hematoxylin and Eosin stain, these results included:

Virgin Rabbits: The histological examination of mammary gland sections in control, OXV and Spearmint groups showed negative reaction to the PAS stain as illustrated in (Fig.3a, b, and c)respectively.

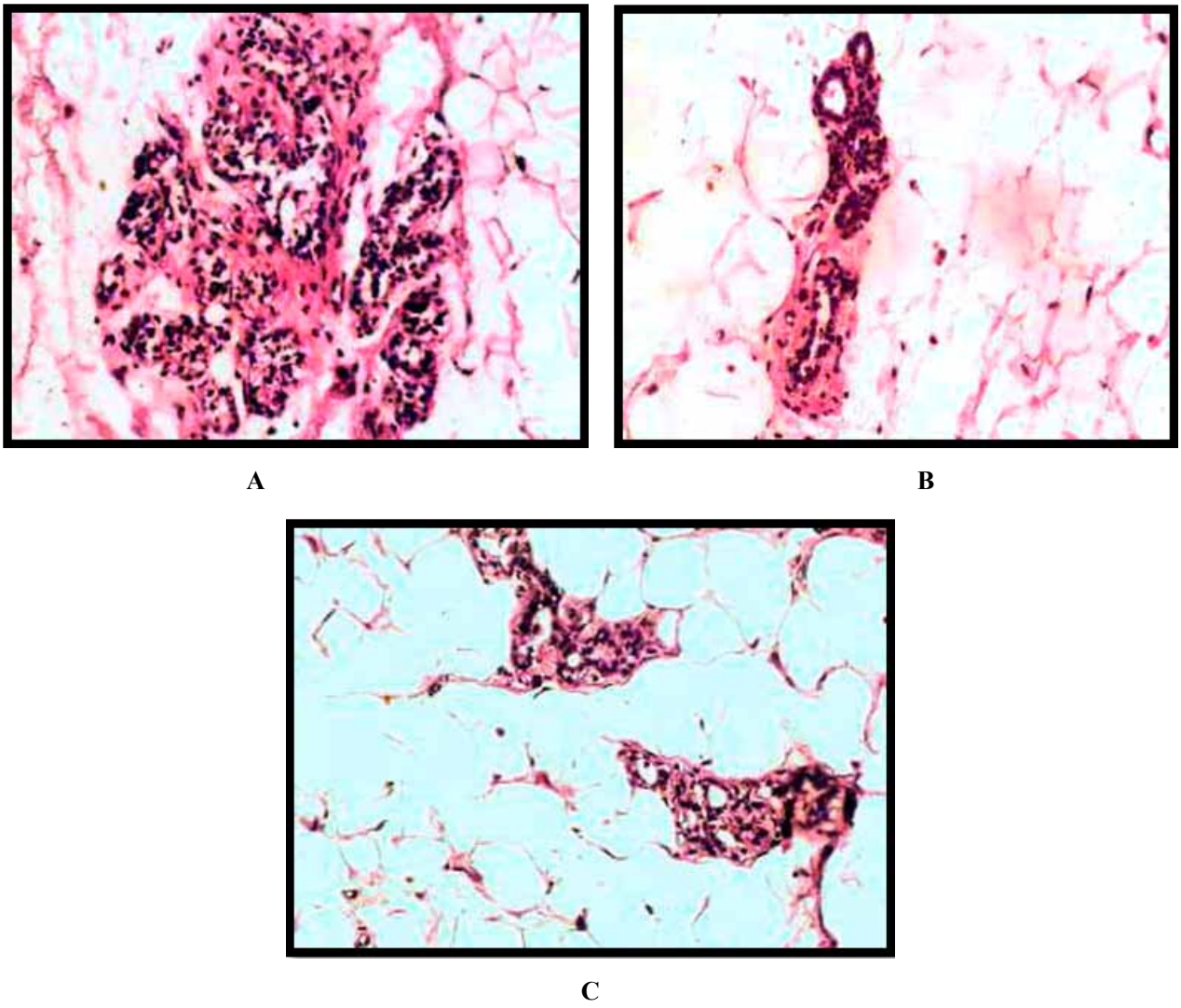
Lactating Rabbits: Positive reactions to the PAS stain were noticed in the histological sections of mammary gland that were obtained from the Control group. Similar results were reported in the mammary tissue of lactating rabbits treated with OXV and Spearmint as seen in (Fig.4a, b, and c)respectively.

Alkaline Phosphatase:

Virgin Rabbits: Mammary tissue sections of rabbits treated with hot aqueous extract of Spearmint, OXV and Control group were showed negative expression of

ALP activity around the intralobular ducts (i.e. positive activity of ALP in the myoepithelial cells) as illustrate on (Fig.5a, b and c).

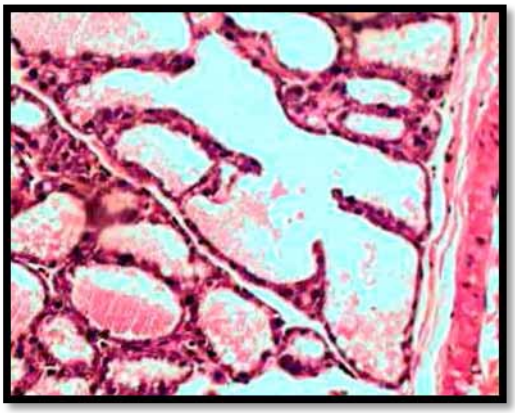
Lactating Rabbits: Microscopic examination to sections of mammary gland of rabbits in spearmint showed moderate activity of ALP of the myoepithelial cell (Fig.6c). On the other hand, the mammary tissue of lactating rabbits of control group showed more dense activity to ALP (Fig.6a) compared to OXV group which showed weak expression (Fig.6b).



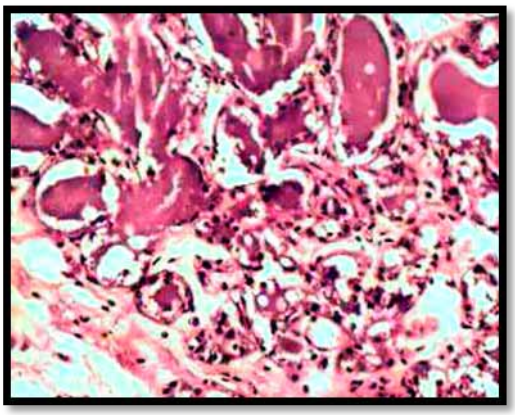
A **B**

C

Figure 1: Virgin groups. A. Sham group showed lobules (arrow) with alveolar buds. B. OVX group show small lobules (arrow) scattered among huge amount of adipose tissue C. Spearmint group show an expansion in the size of lobules (arrow) in comparison with the OVX group (H & E) (200X).



A



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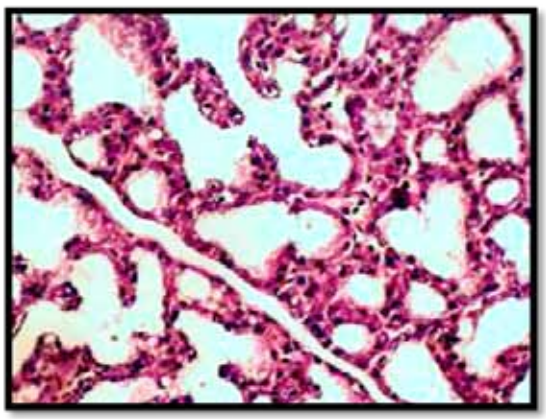

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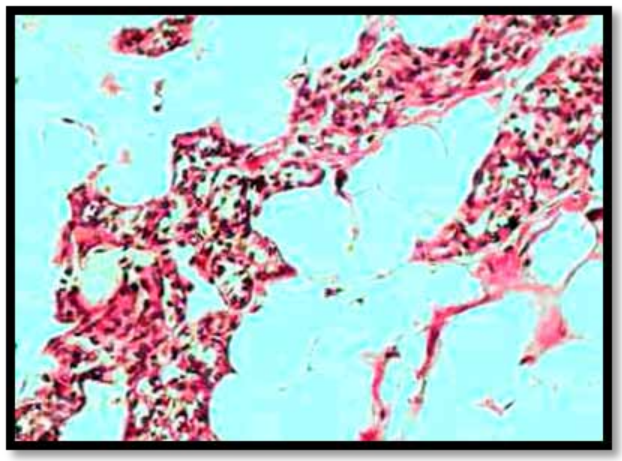
Figure 2:Lactating groups. A. Sham group noted expanded lobules (arrow) Contains dilated and branched alveoliwith low epithelial tissue (curved arrow). B. OXV group showed an increase in the lobular size (arrow) with corresponding decrease in the adipose tissue. The alveoli and ducts were dilated and filled with secretory products) C. Spearmint group showed more increased in the size of lobules, packed with branching alveoli (H & E) (200X).



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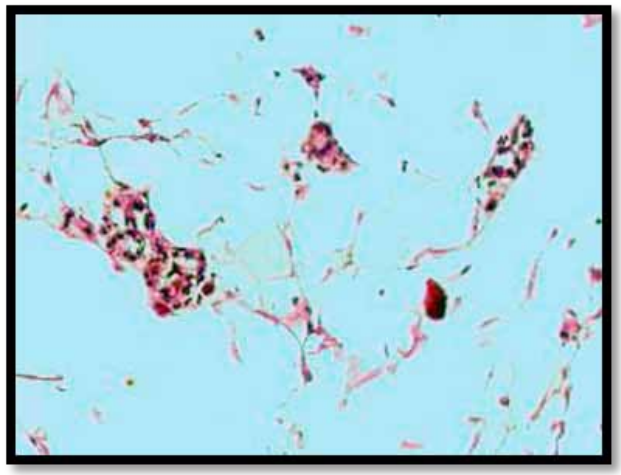

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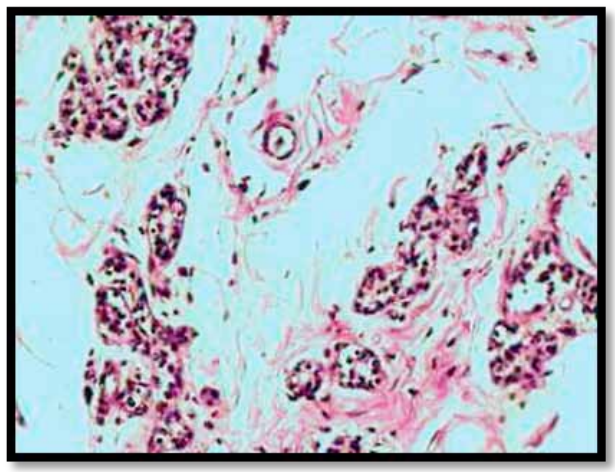

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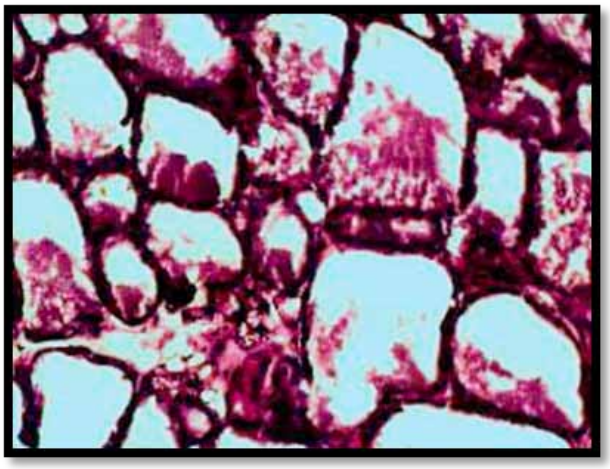
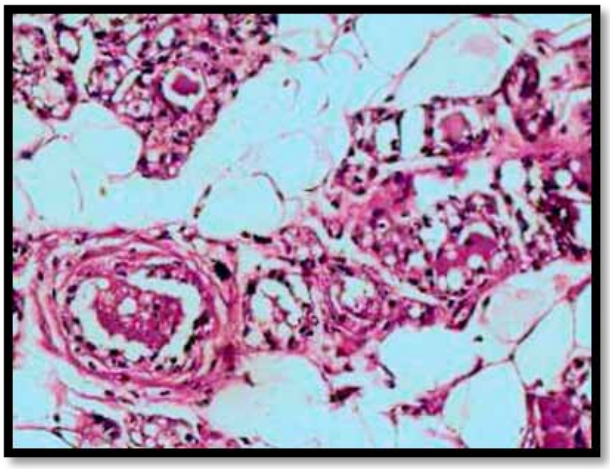
Figure 3: Virgin groups. Sham group (A), OXV group (B) and Spearmint group (C) showed negative reaction (PAS)(200X).



A



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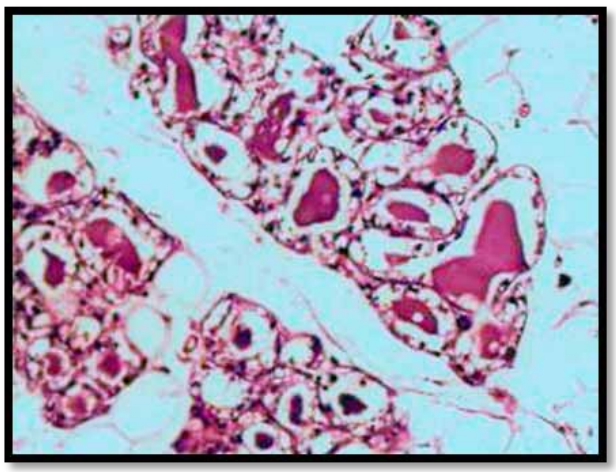

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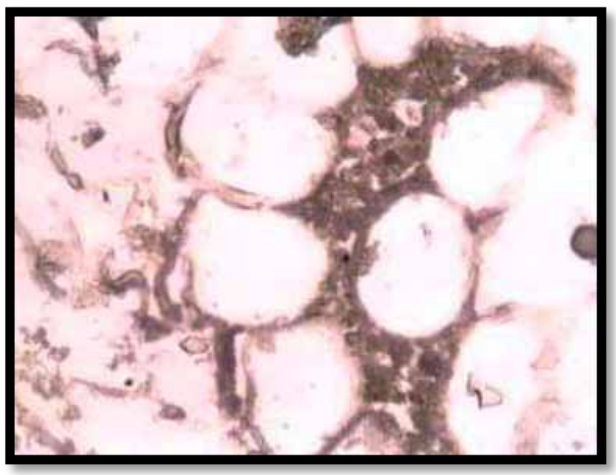
Figure 4: lactating groups. sham group (A), OXV group (B) and Spearmint group (C) showed positive reaction (head of arrow) (PAS) (200X).

A



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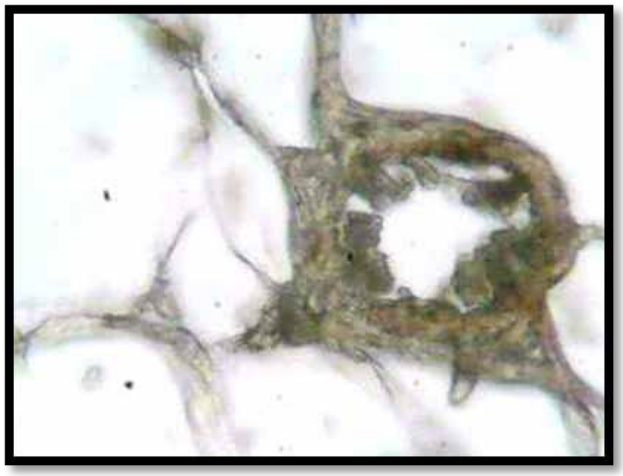

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Figure 5: Virgin Rgroups. Sham group (A), OXV group (B) and Spearmint groups (C) showed negative reaction of myoepithelial cells to the ALP (400X).

A

B

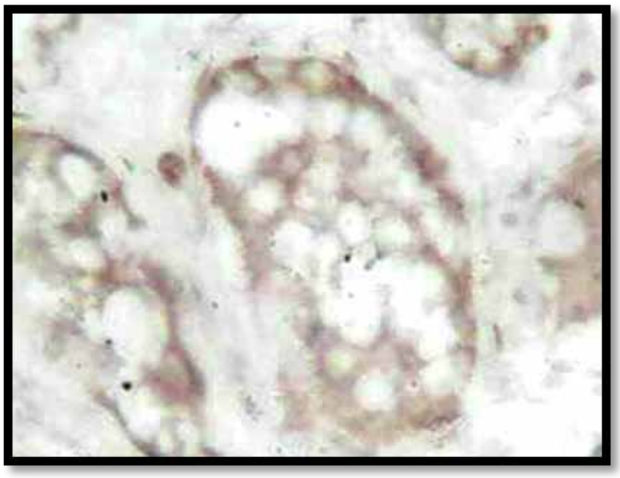

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Figure 6: Lactating groups. Sham group (A), OXV group (B) and Spearmint group (C) showed positive reaction of myoepithelial to the ALP (head of arrow) (400X).

Discussion

Histological results showed a decrease in the size of the lobules and alveoli of mammary gland with less dilation in OVX virgin rabbits when compared to the sham group, this suggested that synthesize estrogen by the ovaries was very low in concentration of OVX animals due to the development of the mammary gland during the adult period[19]. Ovariectomized lactating rabbits showed decreased in the mammary parenchyma with an increase in the connective tissue. In contrast, the alveoli were rudimentary in ovariectomized animals when compared with the Control group, that might be because the estrogen produced by ovaries is very low in levels of ovariectomy rabbits[20]. Ovariectomy showed an impact on epithelial cell and induced a deep morphological disorganization of the mammary tissue[19]. The current study revealed that the intubation of female rabbits of all groups with 5.25 ml/kg b.w of hot aqueous extract of spearmint caused expansion in the size of lobules and number of alveoli, especially during lactation stages when compared with OXV group. These results revealed that the spearmint treated group had the ability to induce mammogenesis in virgin and lactating rabbits. Some studies concluded that in addition to estrogen and progesterone hormones, entire differentiation of the rabbit mammary gland development requires additional action of prolactin. The terminal stage of mammary gland development, lobuloalveolar growth is regulated by the production of prolactin hormone[21,22]. During early pregnancythe prolactin regulation the stimulation of ovarian progesterone in addition to assist in maintaining the required levels of progesterone [23,24]. Prolactin and progesterone up-regulation of PR expression suggest that those hormones can interact synergistically to regulate the alveolar growth. The prolactin stimulation compounds may raise the prolactin secretion via dropping levels of prolactin inhibitory factors[25,26], such compounds are found in spearmint. Carvon is one of the compounds that resembles the chemical structure of dopamine (prolactin inhibitory compound) and competes with dopamine receptors which leads to prolactin secretion[27,28]. The results also revealed that spearmint had estrogen like effects. The estrogen hormone play important role in Proliferation of luminal epithelium, their differentiation and survivalin mammary glandduring pregnancy, lactation and virgin stages through its receptor ERα[29]. In addition, progesterone receptor and cycline D1 are the downstream estrogen receptors. The progesterone receptor regulates the cyclin D_1 that has positive

feedback on is required for transcriptional activity of ERα in mammary gland development. On other hand, the progesterone receptor required for epithelial differentiation and morphogenesis[29,30].

Moreover, the mammary stem cell might be ER positive[31]. This cell has the capability to produce whole mammary gland tissues; where the primary mammary stem cell leads by different processes to produce different cells found in the mammary parnchyma[32,33]. Accordingly, when estrogen hormones binded to estrogen receptor in stem cells of mammary gland that will induction the growth in mammary tissue constituent[34.]. The proliferation effect of spearmint in mammary gland tissue may result from the ability of spearmint to decline the level of androgen hormone that causes suppression in growth of mammary gland with increase in estrogen hormone level[35]. Additionally, the hyperactivity of the experimental mammary gland in this study proposed to be due to biotransformation of saponin compound into sex hormones, where spearmint is capable of inducing[36]. The ALP enzyme plays an important role in the transport mechanism of phosphate, cell division, phosphorylation and dephosphorylation of other substances[37],the mammary gland tissue of rabbits treated with spearmint hot aqueous extract seems to be strongly activated by the ALP enzymes in all physiological stages. This finding supported by the growth and development of mammary glands which attributed to an increase in the proliferation of mammary gland cells in rabbits[22].

Conclusion

The hot aqueous extracts of spearmint Mentha spicata leaves have Mammogenesis effect of mammary glands during virgin and lactating stages in ovariectomized rabbits.

Conflict of Interest: The authors declare that there was no conflict of interest

Ethical Clearance: Taken from Helsinki Declaration of 1975, as revised in 2000 committee.

Source of Funding: Self

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