

Elucidation of a Role for the Aqueous Extract of Borage in Mammary Gland Growth and Development

Wasan Al-Saidi *, Malak Al-Yawer **, Hana Mangalo *
, Salim R. Hammoudi ***

ABSTRACT:

BACK GROUND :

Herbal remedies are used in induction of lactation . In view of that ,*Borage Officinalis* was employed to ensure an abundant milk supply or rectify milk insufficiency .However ,this remedy has not been scientifically tested .

METHODS:

The animals were treated with the aqueous extract of *Borage Officinalis* flowers at a daily concentration of 100 mg / kg body weight /ml for each rat through Oro-gastric tube for 14 days . Animals were subdivided into subgroups according to their physiological status . Mammary glands of these animals were processed for histological , histochemical and immunohistochemical studies .

RESULTS:

The results of all parameters indicated that the aqueous extract of *Borage Officinalis* flowers induced lactogenesis in the mammary glands of virgin and pregnant rats and promoted lactation when had given to lactating rats .

CONCLUSION : *Borage Officinalis* is a lactogenic herb.

KEY WORDS : *Borage Officinalis* , Mammary glands , Alkaline Phosphatase , Estrogen and Progesterone receptors .

INTRODUCTION:

Borage Officinalis , in folk medicine , is used as a mucilaginous agent , an anti inflammatory agent , an astringent and an adrenal tonic and gland balancer agent ^(1,2) .It was also used as a galactagogue for lactating women ⁽¹⁾ and it is also used for treatment of the disorders of the immune system ⁽³⁾ . Today the interest of induced lactation stems from a desire of some adopting mothers to nurse the adopted child ^(4, 5,6) .Moreover , lactation has been induced for scientific and commercial purposes in non-parturient animals ⁽⁷⁾ .

Some galactagogues have been used in the preparation of the breast for lactation e.g. chlorpromazine ⁽⁸⁾ , metoclopramide ⁽⁹⁾ , oxytocin ⁽¹⁰⁾ and theophylline ⁽¹¹⁾ . Herbal remedies are used in induction as well by eastern cultures ⁽¹²⁾ .

In view of that , *Borage Officinalis* was employed particularly in rural areas to ensure an abundant milk supply or rectify milk insufficiency . However , this remedy has not been scientifically tested but women swear by it .

We designed this study to investigate the effect of *Borage Officinalis* flowers on the mammary glands of virgin rats and compare this effect with that on the mammary glands of pregnant and lactating ones, making use of the available histological, histochemical and immunohistochemical means.

MATERIALS AND METHODS:

Sixty ,female, albino rats (*Rattus Norvegicus*) were employed in this study . Animals were grouped according to their physiological status into three groups (Table I) The experimental group was treated daily with aqueous extract of *Borage Officinalis* at a concentration of 100 mg / kg body weight / ml (13) The aqueous extract was given through nasogastric tubes and the duration of treatment was 2 weeks. The control group received 1 ml of distilled water as a placebo under similar conditions. From each ether –anesthetized rat, three pieces of the mammary glands were excised together with one piece of the liver.

Two of these pieces were immediately fixed in 10 % formalin for 24 hours. These specimens were processed for routine haematoxylin and eosin and for immunohistochemistry (4).The third specimen of the mammary gland and the small piece of the liver were immediately fixed in formal calcium at 4 C for 18 hours , rinsed in tap water and finally placed in gum sucrose at 4 C for 18 hours .

* Department of Biology , College of Science , University of Al- Mustansiriyah.

** Department of Anatomy , College of Medicine , University of Baghdad .

***Department of Pathology , College of Medicine , University of Baghdad .

Then , the tissues were quenched in liquid nitrogen and sectioned to 6 microns thickness (at -22, using SLEE cryostat) . These sections were processed for demonstration of alkaline phosphatase activity (15). Via cardiac puncture, blood samples were obtained from each ether anesthetized rat to measure the level of estrogen , progesterone and prolactin in their serum. Morphometrical study was done using an eye piece micrometer fitted to a light microscope at 10x 40 magnification making use of mammary gland sections stained with haematoxylin and eosin . The diameter of the alveoli and the number of nuclei per one alveolus were studied morphometrically .

RESULTS:

Histological study:

Haematoxylin – eosin stained sections of control virgin mammary glands exhibited small lobules scattered among huge amount of adipose tissue (Fig. 1 –A) . Mammary tissue of virgin rats treated with borage (Fig. 1-B) showed an increase in the size of lobules which were packed by alveoli . These alveoli were dilated and their lumen were filled with pink homogenous material (i.e. milk secretion) . Mammary tissue of control pregnant rats showed lobules plentiful with alveoli (Fig. 2- A) .Pregnant rats treated with borage demonstrated in their mammary tissue dilated alveoli. Some of these alveoli were filled with pink homogenous material i.e. milk secretion (Fig.2-B). Mammary tissue of control lactating rats showed an increase in the lobular size with a corresponding decrease in the adipose tissue. The alveoli and ducts were dilated and filled with milk secretion (Fig. 3 –A) . Mammary tissue of lactating rats treated with borage showed greatly dilated alveoli which were filled with milk secretion. Pouring of milk from adjacent alveoli was frequently seen (Fig. 3-B).

Histochemical study :

Mammary tissue of virgin rats treated with borage(Fig. 4-B) exhibited positive alkaline phosphatase activity around the basal part of the secretory epithelium (black rings) . No such black rings were demonstrated in control virgin mammary glands (Fig.4-A). Positive alkaline phosphatase activity(black rings) were noticed around the basal part of the secretory epithelium of control pregnant rats (Fig.5-A) . Similar black rings were reported in the mammary tissue of pregnant rats treated with borage but these rings were thinner (Fig.5-B) .

Mammary tissue of control rats showed thin discontinuous black rings around the basal part of the secretory epithelium (Fig.6-A).

On the other hand, mammary tissue of lactating rats treated with borage , showed more dilatation of the alveoli with more discontinuity of the black rings (Fig. 6-B) .

Immunohistochemical study :

Mammary tissue of control virgin rats (Fig.7-A) showed strong (+++) expression of both estrogen (nuclear staining) and progesterone (cytoplasmic staining) receptors .Mammary tissue of virgin rats treated with borage exhibited moderate (++) expression of both receptors (Fig. 7-B). Mammary tissue of control pregnant rats(Fig. 8-A) showed nearly strong (+++) expression of both receptors while the estrogen and progesterone receptors were weakly (+) expressed in the mammary tissues of prgnant rats treated with borage (Fig. 8- B). Mammary tissues of control lactating rats showed weak (+) expression of both receptors (Fig.9-A) . The mammary tissues of lactating rats treated with borage showed nearly –ve expression of both estrogen and progesterone receptors i.e. no brownish staining were noticed in the cytoplasm and nuclei of their alveoli (Fig. 9-B).

Hormonal study:

Radioimmunoassay for estradiol , progesterone and prolactin were assessed using mean +/- SD (Table–II). Progesterone and prolactin were significantly increased in virgin rats treated with borage. Statistically, no significant difference in estradiol between control and experimental of virgin group. Estradiol , progesterone and prolactin were significantly increased in the experimental pregnant group and in the experimental lactating group than their controls .

Morphometrical study : Diameters of alveoli were significantly ($P < 0.05$) increased in virgin, pregnant and lactating rats treated with borage than their controls (Table- III).Number of nuclei per one alveolus was significantly increased in virgin, pregnant and lactating rats treated with borage than their controls (Table– III).

DISCUSSION:

The aqueous extract of *Borage Officinalis* flowers induced lactogenesis in the mammary glands of virgin and pregnant rats . This mean that this herb had a prolactin like action in addition to estrogen and progesterone. Granner (16) concluded that in addition to estrogen and progesterone, complete differentiation of the rat mammary gland requires the additional action of prolactin , glucocorticoids, insulin or growth peptides and undifferentiated serum factors . Moreover, the terminal stage of mammary gland development, lobuloalveolar growth is regulated by prolactin (17).

On the other hand, the aqueous extract of Borage promoted lactation when had given to lactating rats. The action of such herb may be similar to other stimulators of prolactin release like chlorpromazine, metochlorpramides and theophyline. Brown (12) and Speroff *et al.*, (18) had reported that such drugs increase prolactin secretion via reducing levels of prolactin inhibitory factors. Mammary glands of virgin, pregnant and lactating rats treated with Borage exhibited positive alkaline phosphatase activity around the basal part of the secretory epithelium. However, these black rings were thin and discontinuous in pregnant rats treated with Borage. This may indicate that the basement membrane and myoepithelial cells will be compressed by expanding alveoli as it has been found by Al-Yawer (19) that acute expansion of alveoli occurs at a faster rate than proliferation of myoepithelial cells resulting in creating a discontinuous cellular layer between epithelium and basement membrane. More discontinuity of these black rings were noticed in lactating rats treated with Borage. This finding may indicate more expansion of alveoli in such groups. This observation coincided with previous study of Al-Yawer (19) and (20). Expressions of both estrogen and progesterone receptors were low in all experimental groups when compared with their controls. Moreover, the expressions of these receptors were high in virgin group. This was followed by pregnant group while lactating group ranked third. This finding may indicate that Borage induced more proliferation and more differentiation in the

mammary glands of all experimental groups when compared with their controls.

This may indicate that a reduction in the expressions of both progesterone and estrogen receptors coincided with functional differentiation. The foregoing studies (21), (22) and (23) showed that estrogen and progesterone receptors present in the mammary gland of non pregnant female mice is reduced during pregnancy and is virtually undetectable during established lactation.

Serum progesterone and prolactin were significantly increased in all experimental groups when compared with their controls while serum estradiol was significantly increased in the experimental groups of pregnant and lactating rats only. These findings may indicate that progesterone and prolactin were necessary for the development of the mammary glands but estradiol has been considered essential for the early but not for the late development of the mammary gland. These results coincided with the observations of other workers (24), (25), (26) and (27).

Our morphometrical studies showed that in all experimental groups, there is significant increase in the diameter of alveoli and the number of nuclei per one alveolus when compared with their controls. This finding may indicate that Borage may induce more proliferation and more differentiation in mammary glands of all experimental groups when compared with their controls. These findings coincided with the results obtained by other workers: Al-Khateeb using FennGREEK (28), Al-Ssaidu using Aniseed (29) and Al-Yawer using Garden cress (20).

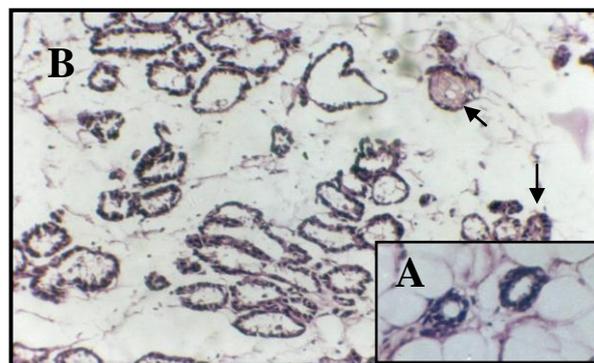


Fig1: A & B: Mammary glands of virgin rats . (A) : Control virgin rats , (B) : Virgin rats treated with Borage showed large size lobules packed by large size alveoli . Some of them filled with milk secretion (arrows) . Haematoxylin & Eosin (X200) .

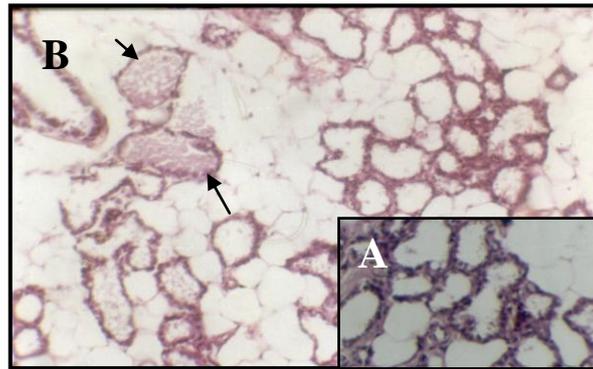


Fig2: A & B: Mammary glands of pregnant rats. (A): control pregnant rats, (B): Pregnant rats treated with Borage exhibited more dilation of alveoli . Some of these alveoli were filled with milk secretion (arrows) . Haematoxylin & Eosin stain (X 200) .

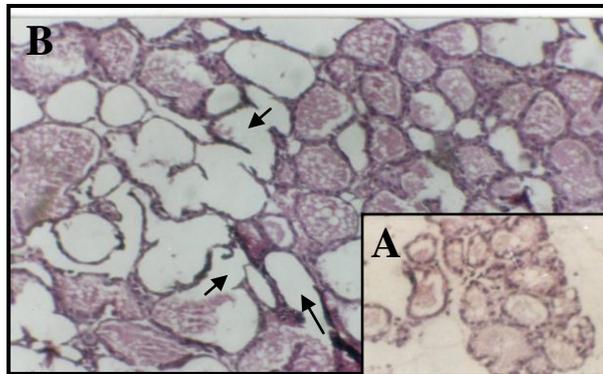


Fig3: A & B Mammary glands of lactating rats . (A): Control lactating rats . (B) Lactating rats treated with Borage demonstrated greatly dilated alveoli which were filled with milk secretion . Pouring of milk from the adjacent alveoli were noticed (arrows) .Haematoxylin & Eosin stain (X200)

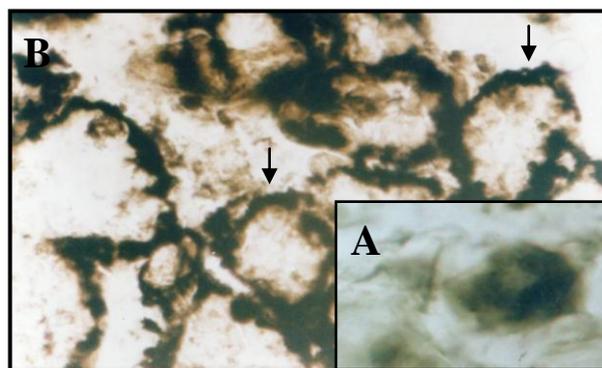


Fig4: A & B: Mammary tissue of virgin rats . (A): control virgin rats , (B): Virgin rats treated with Borage showed the presence of positive alkaline phosphatase activity around the basal part of the secretory epithelium (arrows) No such activity were noticed in control virgin group . Alkaline phosphatase activity (X200)

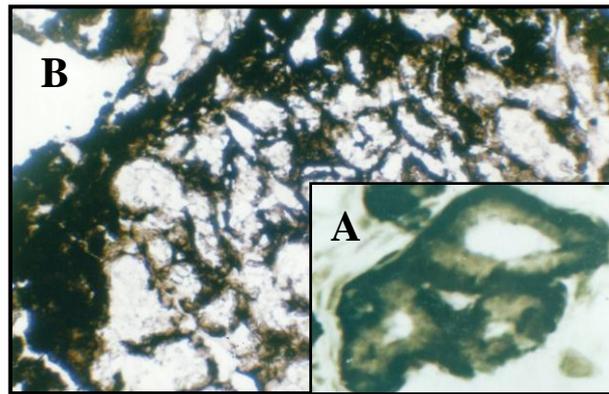


Fig 5: A & B : Mammary tissues of pregnant rats . (A) Control pregnant group (B) Pregnant group treated with Borage . Both groups demonstrated the presence of black rings around the basal part of the secretory epithelium but these black rings were thinner in group (B) . Alkaline phosphatase activity (X200).

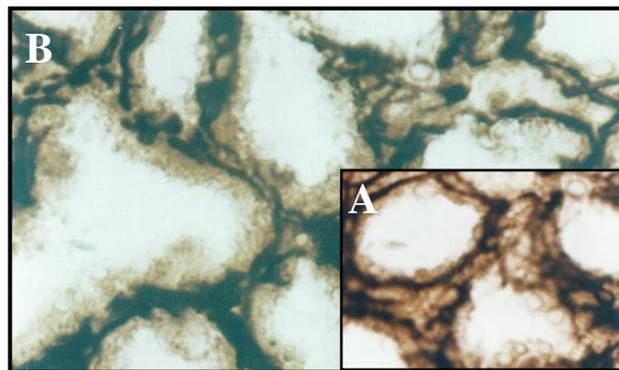


Fig. 6 : Mammary tissues of lactating rats (A) control lactating group (B) Lactating rats treated with Borage showed more dilatation of alveoli with more discontinuity of the black rings . Alkaline phosphatase activity (X400) .

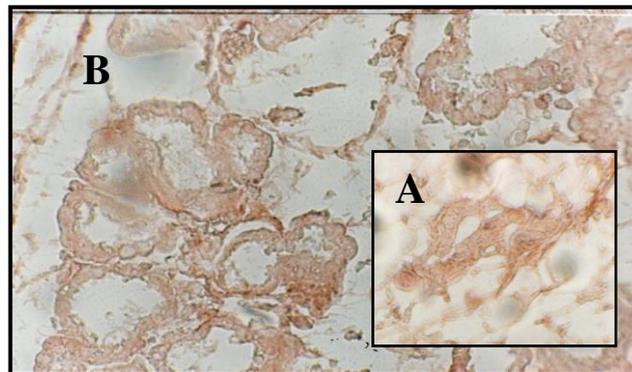


Fig 7: A & B: Mammary tissues of virgin rats . (A) Control virgin group showed strong (+++) expression of both estrogen and progesterone receptors while (B) virgin group treated with Borage demonstrated moderate (++) expression of such receptors . Estrogen & Progesterone receptors (X200)

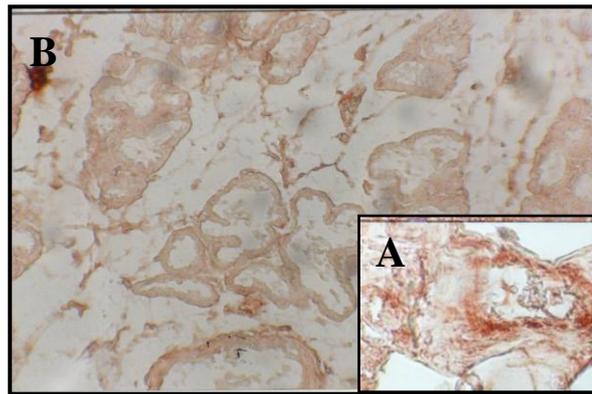


Fig. 8: A & B Mammary tissue of pregnant rats. (A) Control pregnant rats showed strong (+++) expressions of both estrogen and progesterone receptors while (B) Pregnant rats treated with Borage demonstrated weak (+) expression of these receptors . Estrogen & progesterone receptors (X200)

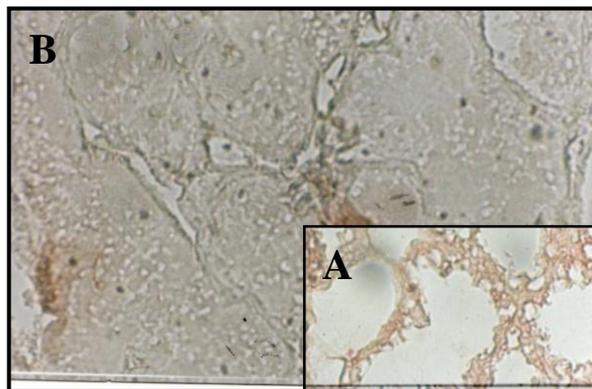


Fig 9: A & B: Mammary tissues of lactating rats. (A) Control lactating rats showed weak (+) expression of both estrogen & progesterone receptors while (B) Lactating rats treated with Borage demonstrated nearly -ve expression of such receptors. Estrogen & progesterone receptors (X400).

Table 1: Showing the animal groups.

Groups	Number of Rats		Description
	Control	Experimental	
Virgin	10	10	Normal, two months, old virgin
Pregnants	10	10	Seven days, pregnant
Lactators	10	10	1 st day of lactation

Table 2: Serum Progesterone Estradiol and Prolactin in control and experimental groups

Groups	Control			Groups Treated with Borage		
	(Virgin) Mean ± SD	Pregnant Mean ± SD	Lactating Mean ± SD	(Virgin) Mean ± SD	Pregnant Mean ± SD	Lactating Mean ± SD
Estradiol ng/ml	72.9 ± 0.2	120.76±1.89	96.363±0.3	77.6±3.46	*1.36±3.6	*110.5±1.5
Progesterone ng/ml	7.103±1002	11.36±0.73	15±0.2	*9.2±0.4	*15.6±0.2	*25±0.2
Prolactin ng/ml	4.5± 1.0	9.4±0.4	10.4±0.4	*9.467±0.3	*10.56±0.3	*13.13±0.11

P* < 0.05

Table 3: Showing mean of alveolar diameter (μm) and number of nuclei of the epithelium lining the alveolus in control and experimental groups

Groups	(μm) mean of alveolar diameter			Number of nuclei of the epithelium lining the alveous		
	(Virgin) Mean (μm) \pm SD	Pregnant Mean (μm) \pm SD	Lactating Mean (μm) \pm SD	(Virgin) Mean (μm) \pm SD	Pregnant Mean (μm) \pm SD	Lactating Mean(μm) \pm SD
Control	23.98 \pm 2.026	32.87 \pm 2.1667	38.393 \pm 0.879	11 \pm 1.53	17 \pm 0.58	21.0 \pm 1.0
Borage	*39.22 \pm 1.045	*46.35 \pm 1.1304	*54.38 \pm 4.285	*17.33 \pm 1.53	*20.0 \pm 1.15	*25.33 \pm 0.58

P* < 0.05

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