

## Observations on the ultrastructure of a rat mammary gland treated with harmal and borage

Malak A. Al- Yawer \*\*Ph.D  
Wasan Al- Saidi \*  
Shatha M. Al-Faisaly\*\*\*  
Salim R. Hamoudi \*\*

### Summary:

**Background :** It had been identified by histological, histochemical and morphometrical studies that *peganum harmala* is a mammogenic herb and *borage officinalis* is a lactogenic one . To complete our investigation about these two herbs , we performed electron microscopical study .

**Materials and methods :** Rats were grouped according to their physiological status into three groups . Each group was subdivided in to three subgroups : one control and two experimental . The two experimental group were treated daily; the 1<sup>st</sup> one with an aqueous extract of *peganum harmala* seeds and the 2<sup>nd</sup> with an aqueous extract of *borage officinalis* flowers . After two weeks of treatment , mammary glands were employed for electron microscopical study .

**Results :** In virgin rats , the epithelial and myoepithelial cells were partially differentiated when harmal was given and completely differentiated when borage was given . In pregnant rats , harmal and borage optimize mammary parenchymal growth and induce lactation when these herbs were given . In lactating rats ,these herbs exhibited a picture similar to control lactating group but the budding of lipid droplets and the swelling of secretory vesicles were markedly increased .

**Conclusion:** Both harmal and borage stimulate the release of prolactin and induce galactogenesis during pregnancy and promote it during lactation .

**Key Words :** Mammary gland , Electron microscope , Harmal , Borage

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### Introduction :

*Peganum harmala* and *borage officinalis* , in folk medicine , were used in treating various disease and disorders (1,2) . They were also used as galactagogue for lactating women (3,4) .

Using histological , histochemical and morphometrical studies we have identified that harmal is a mammogenic herb and borage is a lactogenic one . The former induced mammogenesis in the mammary glands of virgin rats , initiated lactogenesis in a well prepared mammary gland ( i.e. during pregnancy) and promoted galactogenesis when it administered during lactation (5) . On the other hand , borage induced lactogenesis in the mammary glands of virgin and pregnant rats and promoted lactation when had given to lactating rats (6) .

The ultrastructure of mammary gland has been described in virgin (7) , pregnancy (7,8,9) and during lactation (7,8,10) but no reports have been made of any alteration in it's ultrastructure after

treating with the above mentioned herbs ( midline 2006-1965) . In this study , material was taken from the mammary glands of virgin , pregnant and lactating rats in order to determine whether any structural changes occurs in the mammary epithelium ,at subcellular level , after treatment with harmal and borage .

### Materials and methods :

Fifty –four ,female ,albino , Norway rats ( *Rattus norvegicus* ) were employed in this study . Animals were grouped according to their physiological status in to three groups ( Table I )

The 1<sup>st</sup> experimental group was treated daily with an aqueous extract of *peganum harmala* seeds at a concentration o 125 µg/ ml (11) . The 2<sup>nd</sup> experimental group was treated daily with an aqueous extract of *borage officinalis* flowers at a concentration of 100 mg/ml (12) . The aqueous extract was given through orogastric tubes and the duration of treatment was 2 weeks . The control group received 1ml of distilled water as a placebo under similar conditions .

Small pieces (2-4 mm) of mammary glands were cut out and fixed in 2.5% gluteraldehyde for two hours and posfixed in osmium tetroxide for overnight . Both fixatives were phosphate buffered (pH 7.4) .Samples were dehydrated in a graded series of ethanol , cleared in propylene oxide and embedded in aralidite (13, 14) . Thick sections (1-

\* University of Al- Mustansiriyah / College of Science

\*\* University of Baghdad / College of Medicine

\*\*\* Al-Nahrain University / College of Medicine

2µm) were stained with toluidine blue . Selected areas were trimmed and ultrathinned silver sections were cut on a Reichert OM ultramicrotome with a diamond knife . Sections were stained with uranyl acetate lead citrate and then examined with electron microscopy ( Philips transmission electron microscope ) .

## Results :

### 1. Virgin rats :

**a. Control virgin rats :** The secretory tubules which were identified in histological sections were easily recognizable at the ultrastructural level (Fig.1) . Their main characteristic features were an irregular cell shape and an indented nucleus containing clumped peripheral heterochromatin and prominent nucleoli . Apart from this they were of undifferentiated appearance since neither junctional complexes and arrays of microvilli typical of epithelial cells nor extensive myofilaments typical of myoepithelial cells were seen .

**b. Virgin rats treated with harmal ( Fig.2 ) :** The cells often formed connected chains that conveyed on the lumina . Numerous microvilli were displayed on the cellular surface exposed to lumen . Cells located near lumina sometimes contained vacuoles which varied in size and contain small vesicles and dark granules in varying amounts . These cells frequently contained large numbers of polyribosomes, strands of rough endoplasmic reticulum and Golgi apparatus within their cytoplasm . Dark membrane bound granules were markedly increased in amount .

Some cells were seen stretched by the enlarged epithelial cells . These myoepithelial cells were differentiated with pronounced cell processes full of myofilaments .

**c. Virgin rats treated with borage (Fig.3) :** The cytoplasm of the epithelial cells were crowded by the presence of vesicles containing secretory product and lipid droplets . These membrane – bounded compartments extend from the deeply infolded basal plasma membrane to the tips of apical microvilli . The nuclei of epithelial cells were more or less rounded , basally located and surrounded by parallel membrane arrays of rough endoplasmic reticulum . The Golgi complex was well developed and occupied a supranuclear position .

### 2. Pregnant rats :

**a. Control pregnant rats ( Fig.4 ) :** The changes that occur at subcellular level in this group was identified and described in ultrastructural studies carried out many years ago (7,8,9) . The epithelial cells exhibited many ribosomes and polysomes . The mitochondria increase in size and number .

**b. Pregnant rats treated with harmal ( Fig.5 ) :** The cytoplasm of epithelial cells showed marked increase in the number of ribosomes , mitochondria and rough endoplasmic reticulum . The latter were

organized in parallel arrays . The Golgi complex became prominent . Lipid droplets were observed and the luminal surface possessed microvilli and secretory vesicles .

**c. Pregnant rats treated with borage ( Fig.6 ) :** The epithelial cells characterized by an abundance of cytoplasmic organelles , hypertrophy of Golgi complex and increment of lipid droplets and protein secretory vesicles .

### 3. Lactating rats :

**a. Control lactating rats :** The stunningly beautiful organization of mammary epithelial cells during lactation was evident from earliest electron micrographs (7,8,10) . The cytoplasm of epithelial cells (Fig.7) is characterized by abundance of organelles extending from the lumen to the basal lamina . Golgi complex is well developed , rough endoplasmic reticulum assumed a parallel organization and mitochondria were numerous and elongated . These cells contain abundance of fat globules and protein secretory vesicles .

**b. Lactating rats treated with harmal ( Fig.8 ) :** The same ultrastructural organelles were observed as in the control group but there was an increase in the number of fat globules and protein secretory vesicles .

**c. Lactating rats treated with borage ( Fig.9 ) :** The budding of lipid droplets and swelling of secretory vesicles were markedly increased both in number and size .

## Discussion :

The undifferentiated appearance of cells of the secretory tubule, in virgin rats , became partially differentiated when harmal was given and fully differentiated when borage was given . It has been found from previous researches in this field that harmal is a mammogenic herb (5) induced mammatogenesis in the mammary glands of virgin rats and borage is a lactogenic one (6) induced lactogenesis in the mammary glands of virgin rats . As rat mammary gland is known to undergo rapid proliferation at the onset of sexual maturity (15) and full development during pregnancy and lactation (16) , it was necessary to determine the effects of harmal and borage on the mammary glands at various physiological status . Previous hormonal study revealed that the levels of progesterone and prolactin were significantly increased in both virgin rats treated with harmal and borage but their levels were more in virgin rats treated with borage (5,6) . This may explain why harmal induced partial differentiation of the mammary epithelial cells and borage induced full differentiation of them . From this we may hypothesize that harmal and borage increase the levels of serum prolactin by either inhibiting the release of prolactin inhibitory factor from the hypothalamus or stimulating the release of prolactin from several extrapituitary sites including mammary epithelial cells . It is claimed by

Lkhider (17), Escalada *et. al.* (18) and Jwasaka *et. al.* (19) that prolactin is also synthesized in several extrapituitary sites including mammary epithelial cells raising the possibility that prolactin may regulate mammapoiesis via autocrine or paracrine mechanism.

Based on electron microscopical study, control pregnant group and experimental pregnant ones exhibited differences. The latter contained polarized epithelial cells with microvilli. In addition, secretory vesicles containing milk proteins and lipid droplets were prominent. In contrast, control pregnant group did not exhibit secretory activity and the gland contained very little fat and secretory vesicles. From this we may conclude that harmal and borage induced activation of stat 5 pathway by prolactin hormones. Miyoshik *et. al.* (20) propose that activation of stat 5 pathway by prolactin, growth hormone and epidermal growth factor transduces signals that instruct cells at the branch points to proliferate and adopt alveolar characteristics. Stat 5 determines cell fate through the establishment of cell – cell adhesion. The intracellular organelles (endoplasmic reticulum, Golgi apparatus and casein containing secretory vesicles) involved in the secretory pathway in mammary epithelial cells became evident when pregnant rats treated with harmal and borage and Golgi cisternal progression and maturation were more pronounced in pregnant rats treated with borage than those treated with harmal. This resulted in an increase in the secretory activity of the epithelial cells and an increase in the synthesis of lactose. The pathway for the synthesis, processing, transport and secretion of caseins,  $\alpha$ -lactalbumin and other milk proteins synthesized in the gland was showed to follow the rough endoplasmic reticulum / Golgi route (21,22). Keenan (23) and Linzell and peaker (24) showed that the major milk sugar, lactose was synthesized in the Golgi complex by the action of galactosyl transferase. The epithelial cells in pregnant rats treated with harmal and borage became crowded with arrays of rough endoplasmic reticulum. This in turn resulted in an increase in the number of lipid

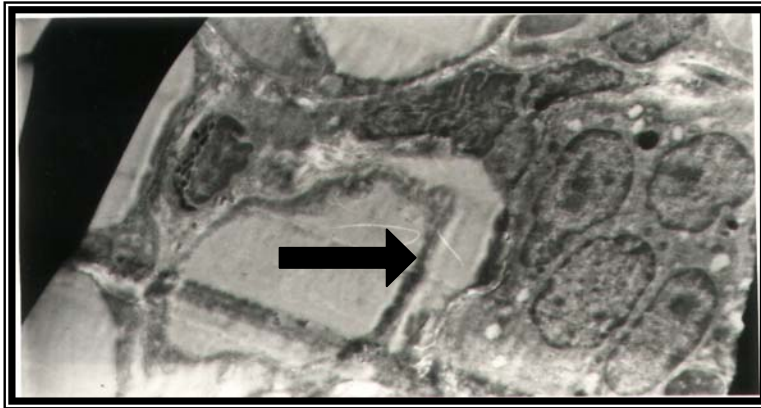
droplets and caseins. Stein and Stein (25) demonstrated by autoradiography that lipid precursors are assembled in to droplets in the endoplasmic reticulum and are then transported through the cytoplasm to the apical surface. Expression of caseins reached it's peak during pregnancy when pregnant rats were treated with harmal and borage. This unlike the results obtained by Nakhasi and Qasba (26) who found that initial expression of caseins occurs in the mid-late pregnant gland and peaks during lactation.

The expression of caseins in the mammary epithelial cells of control and experimental lactating rats was evident. Their expression was more pronounced in lactating rats treated with borage than those treated with harmal. This again may be due to the different concentrations of prolactin hormone in these two groups. It is stated by Hennighausen *et. al.* (27) that prolactin is a lactogenic hormone that stimulates  $\beta$ - casein transcription. The expression of milk protein was dose-dependent on prolactin (28).

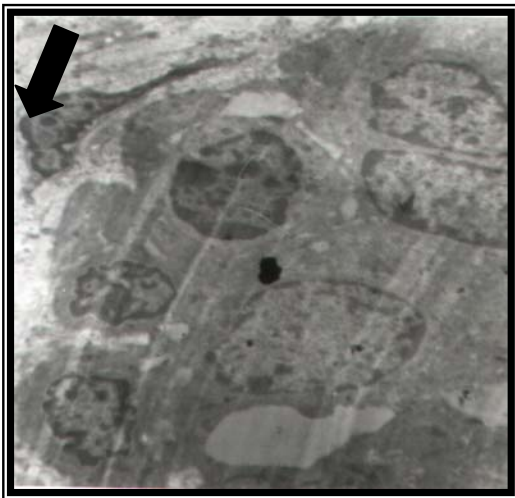
It is well known that prolactin is a lactogenic hormone in various species and is also involved in stimulation of mammary growth (29). Horseman (30) reported that prolactin regulates mammapoiesis at three stages of reproduction in females more specifically during organogenesis that occurs around puberty, during pregnancy and for lactational differentiation and maintenance of milk secretion after parturition. The present results showed that both harmal and borage stimulate the release of prolactin and induce galactogenesis during pregnancy and promote it during lactation. Therefore, during these periods, attempts should be made to increase prolactin concentrations of pregnant and lactating rats in order to optimize mammary parenchymal growth and subsequent lactation performance. This is consistent with the results obtained by Farmer *et. al.* (31) and Petitclerc (32) that there is a specific period in late gestation during which prolactin plays an essential role for mammary parenchymal development and prolactin concentrations must be maintained through out lactation to support galactopoiesis.

**Table I: Showing the animal groups**

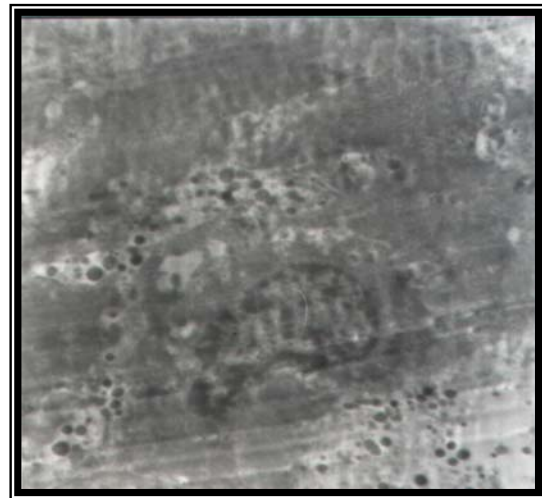
Groups	Number of Rats			Description
	Control	Harmal-treated	Borage-treated	
Adult	6	6	6	Normal, two months old, virgin
Pregnant	6	6	6	Seven days pregnant
Lactating rat	6	6	6	1 <sup>st</sup> day of lactation



**Fig.1: Control virgin rat . The secretory tubule (arrow) composed of a heterogeneous collection of undifferentiated cells . (X2400).**



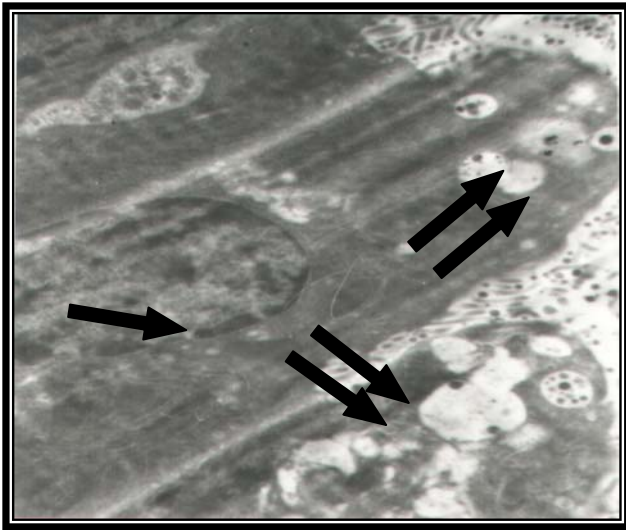
**Fig.2: Virgin rat treated with harmal . The cells formed connected chains These cells contained large number of polyribosomes ,strands of rough endoplasmic reticulum and Golgi complex within their cytoplasm in addition to dark granules and fat droplets . Myoepithelial cell (arrow), stretched by the enlarged epithelial cells,contained myofilaments in their cell processes (X4400)**



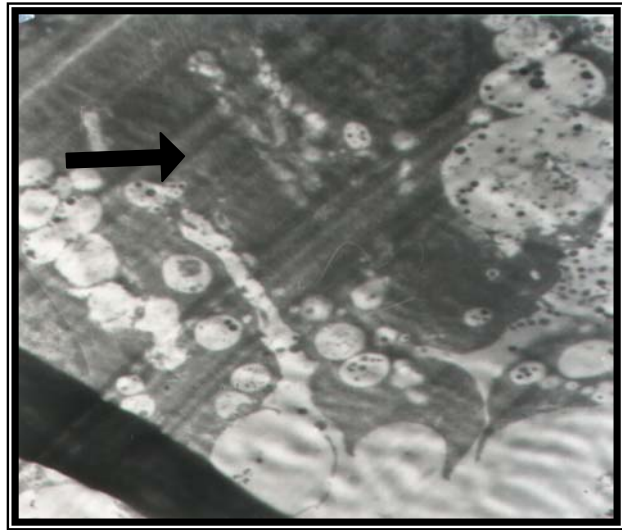
**Fig.3: Virgin rat treated with borage . Their cells crowded by membrane bounded compartments which extend from the deeply infolded basal plasma membrane to the tips of apical microvilli . The Golgi complex was well developed . (X6200)**



**Fig.4: Control pregnant rat . The epithelial cells exhibited many ribosomes and polysomes ,mitochondria increased in number . fat droplets and dark granules were also seen .(X1650)**



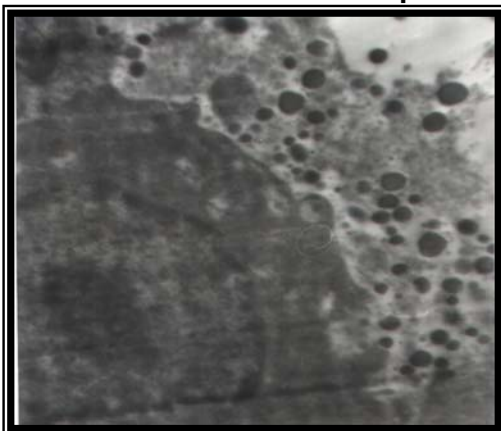
**Fig.5:** Pregnant rat treated with harmal . All organelles were markedly increased in the cytoplasm of epithelial cells . The Golgi complex (arrow) became prominent . Luminal surface (double arrows) possessed microvilli and secretory vesicles . (X4400)



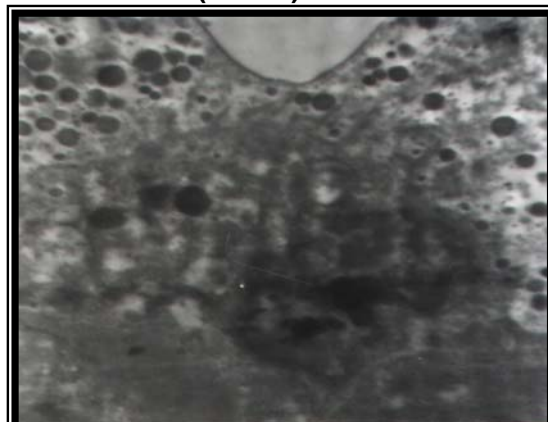
**Fig.6:** Pregnant rat treated with borage . Golgi complex (arrow) was hypertrophied. Lipid droplets and secretory vesicles were markedly increased . (X8700)



**Fig.7:** Control lactating rat . Typical ultrastructure of lactating epithelial cells was noticed . (X3400).



**Fig.8:** Lactating rat treated with harmal . The number of fat globules and protein vesicles were increased . (X6200)



**Fig.9:** Lactating rat treated with borage . The size and the number of secretory vesicles were markedly increased . (X6200)

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