The Effect of the Aqueous Extract of Peganum harmala Seeds on the Mammary Glands of Virgin, Pregnant, and Lactating Rats

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ABSTRACT:

BACKGROUND:

Penganum harmala is a well known herb that is used by different societies. It is used as a medical herb in treating various diseases and disorders .There was no earlier published work on the effects of the aqueous extract of Peganum harmala . Therefore, we designed this study to investigate the effect of the aqueous extract of Peganum harmala seeds on the mammary gland at the various physiological states (virgin, pregnancy and lactation) making use of the available histological, histochemical, and immunohistochemical means.

METHODS:

Aqueous extract of Peganum harmala was given for two weeks by an orogastric tube on single regular daily dosage to Norway albino female rats. Animals were subdivided into subgroups according to their physiological states. Mammary glands of these animals were routinely processed for histological, immunohistochemical and histochemical studies using formalin fixative ,paraffin embedded sections in the first two studies and formal calcium, frozen sections in the third study. Experimental specimens were compared with that of control subgroups.

RESULTS :

Harmal induced mammogenesis in the mammary glands of virgin rats. It's aqueous extract was able to initiate lactogenesis in a well-prepared mammary gland (i. e. during pregnancy) and finally this aqueous extract promotes lactogenesis when administrated during lactation.

CONCLUSION :

Peganum harmala is a mmammogenic herb .

KEYWORDS: Peganum harmala, mammary glands, alkaline phosphatase, estrogen and progesterone receptor's.

INTRODUCTION:

Peganum harmala is a well known herb that is used by different societies. It is used in treating various disease and disorders (1). It is also used as a galactagogoue for lactating women and has long been described as an abortifacient-emmenagouge. (1, 2). A number of herbs, known to be galactagouges have been investigated on a sound clinical pharmacological principle to determine their effect in promoting milk secretion. These herbs were fenugreek (3), fennel, cumin and garden cress (4). Based on the earlier mentioned reports and as there was no earlier published work on the effects of the aqueous extract of Peganum harmala seeds on the rat mammary glands (Medline and Extramed search 1965-2005); we designed this study to investigate the effect of the aqueous extract of Peganum harmala seeds on the mammary gland at the various physiological states (virgin, pregnancy and lactation) making use of the available histological, histochemical and immunohistochemical means .

MATERIAL AND METHODS:

Sixty, female, albino, Norway rats (Rattus norvegicus) were employed in this study. Animals were grouped according to their physiological states into three groups (table 1). The experimental group was treated daily with the aqueous extract of Peganum harmala seeds at a concentration of 125µg/gm body weight/day (5, 6). The aqueous extract was given through orogastric 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 pieces of the mammary glands were immediately fixed in 10% formalin for 24 h.

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Those specimens were processed for routine haematoxylin and eosin and for immunohistochemistry. (7). The 3rd specimen of the mammary gland and the small piece of the liver were immediately fixed in formal calcium at 4C° for 18h., rinsed in tap water and finally placed in gum sucrose at 4C° for 18h. (7). The tissues were quenched (in liquid nitrogen) and sectioned to 6microns thickness (at-22, using SLEE cryostat). These sections were processed for demonstration of alkaline phosphatase activity (7) .

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 10×40 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.

Table 1 Showing the animal groups

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

RESULTS:

1.Histological study: Mammary tissue from virgin rats treated with harmal demonstrated an increase in the size of the lobuler, alveoli and ducts when compared with their controls. Furthermore some alveoli revealed a pink homogenous material in their lumen. (Fig. 1 A & B). In pregnant rats treated with harmal, the mammary glands exhibited a remarkable increase in the size of their lobules when compared with the control group. Each lobule was packed by dilated alveoli which were lined by a single layer of cuboidal epithelium circumscribed by another layer of myoepithelial celles (Fig. 2 A & B). Alveoli and ducts, in the mammary tissue of lactating rats treated with harmal, were seen filled with milk secretion. Epithelial loss in the interlobular ducts were identified with a probable pouring of milk from the adjacent alveoli (Fig. 3 A & B).

2.Histochemical Study: Mammary tissue of virgin rats treated with harmal exhibited positive alkaline phosphatase activity (black rings) around the basal part of the secretory epithelium of the alveoli. However, no such black rings were observed in control virgin rats (Fig. 4 A & B). Positive alkaline phosphatase activity (black rings) were observed around the basal part of the secretory epithelium in control pregnant rats. Similar black rings were reported in the mammary tissue of pregnant rats treated with harmal but they were thinner (Fig. 5 A & B). Mammary tissue of control lactators showed thin, discontinuous black rings around the basal part of the secretory epithelium. The discontinuous black rings were more thinner in the mammary tissue of harmal treated lactators (Fig. 6 A & B).

3.Immunohistochemical study: Mammary tissue of virgin rats showed strong (+++) expression of both estrogen (nuclear staining) and progesterone (cytoplasmic staining) receptors. Virgin rats treated with harmal exhibited moderate (++) expression of both receptors. (Fig. 7 A & B). Moderate expression (++) of both estrogen and progesterone receptors were observed in the mammary tissue of control pregnant rats These receptors were stained weakly (+) in the mammary tissue of pregnant rats treated with harmal. (Fig. 8 A & B). Mammary tissue of control lactators showed weak expression (+) of both receptors. Nearly similar expressions of these receptors were observed in the lactators treated with harmal (fig. 9 A & B).

4.Morphometrical study : Diameters of the alveoli were significantly ($p<0.05$) increased in virgin, pregnant and lactating rats treated with harmal than their controls. (Table-2). The number of nuclei per one alveolus was significantly increased in virgin, pregnant and lactating rats treated with harmal than their controls. (Table-2).

5.Hormonal Study: Radio immunoassay for estradiol, progesterone and prolactin were observed using mean + SD. Progesterone and prolactin were significantly increased in virgin rats treated with harmal when compared with their controls (Table - 3). Estradiol, progesterone and prolactin were significantly increased in pregnant rats treated with harmal than their controls (Table-3). In lactators treated with harmal, estrdiol, progesterone and prolactin were significantly increased when compared with their controls (Table-3).

DISCUSSION :

The results of all parameters indicated that harmal is a mammogenic herb since it induced mammogenesis in the mammary glands of virgin rats. It's seeds were able to initiate lactogenesis in a well-prepared mammary gland (i. e. during pregnancy) and finally this herb promotes lactogenesis when administered during lactation. Alkaline phosphatase study showed the presence of black rings around the basal part of the secretory epithelium of alveoli in virgin, pregnant and lactators treated with harmal and these black rings were thinnest in lactators. It has been found by Al-Yawer and other workers (9,10) that alkaline phosphatase can be taken as a marker for the activity of both basement membrane and myoepithelial cells in the mammary gland and other structures. From this we can conclude that the basement membrane and myoepithelial cells were more developed in virgin, pregnant and lactating rats treated with harmal than their controls. Expressions of both estrogen and progesterone receptors were detected more in the mammary tissue of control virgin rats than that in harmal treated virgin group. Expression of these receptors decreases when pregnant rats treated with harmal and decreases more when lactating rats treated with harmal. These results indicate that a reduction in the expressions of progesterone and estrogen receptors coincident with functional differentiation and harmal may induce more differentiation in the mammary glands when administered.

It has been found by Shyamala (11), that progesterone receptor present in the mammary gland of non-pregnant female mice is reduced during pregnancy and is virtually undetectable during established lactation. Saji, et al. (12) had found that female mammary gland undergoes a surge of cell division during puberty and throughout adult life. In pregnancy, estrogen receptors expression is low and this percentage will become lower during lactation. 13, 14, 15. Morphometrical study showed that the diameters of alveoli and the number of nuclei per one alveolus were significantly increased in virgin, pregnant and lactating rats treated with harmal.

This may be due to the increase in the number and activity of the alveolar cells and these cells synthesize and secrete more milk when harmal was administered. Similar findings were observed when fennel, cumin and garden cress were given to virgin rats (4). Estradiol was significantly increased in pregnant and lactating rats treated with harmal but there is no such significant increase of estradiol in virgin rats treated with harmal.

On the other hand, progesterone and prolactin were significantly increased in virgin, pregnant and lactating rats treated with harmal. Taken together, these findings may indicate that progesterone and prolactin are necessary for the development of the mammary glands and estradiol may be necessary for the early but not the late development of the mammary glands. It has been demonstrated by Shyamala (11) that progesterone is essential for lobuloalveolar development and not for ductal morphogenesis. On the other hand, Bole, Feysot et al. agreed that prolactin is the hormone primarily responsible for the synthesis of milk proteins, lactose and lipids and the terminal stage of the mammary gland development, lobulo alveolar growth is directly regulated by prolactin.

Histological Study :

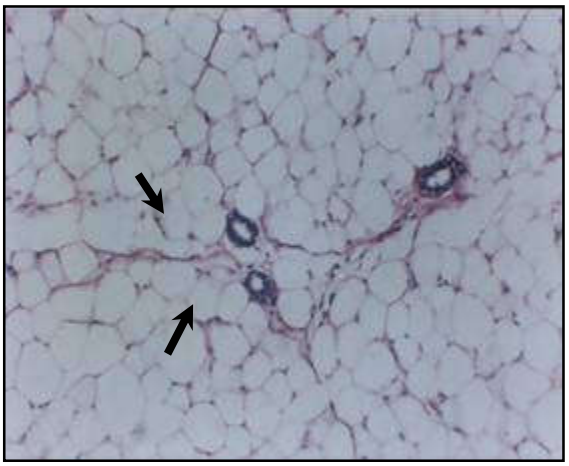
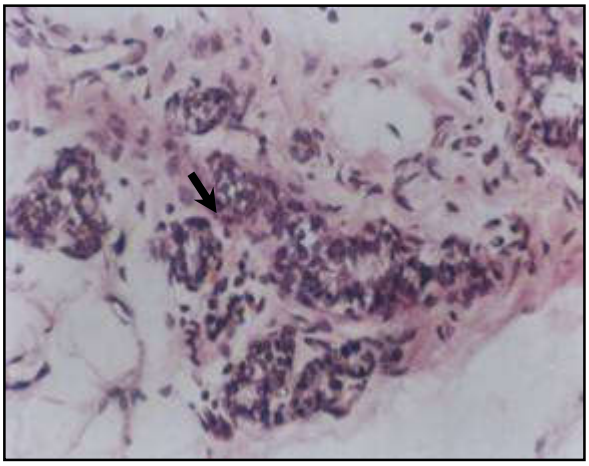


Fig. 1 (A) Control Virgin



(B) Virgin treated with Harmal

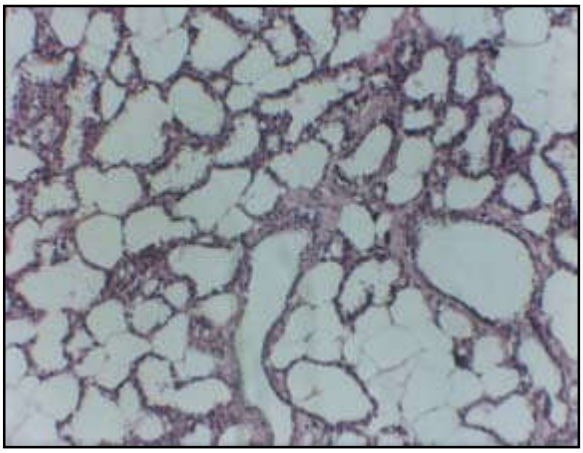
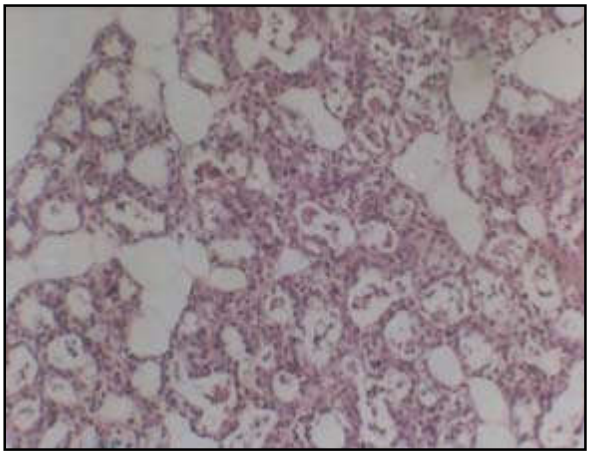


Fig. 2 (A) Control Pregnant



(B) Pregnant treated with Harmal Borage

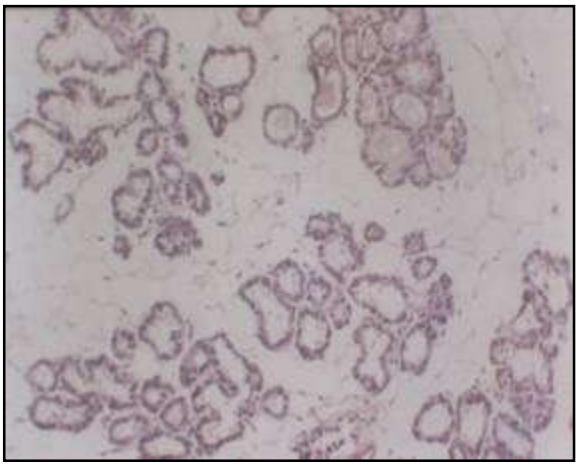
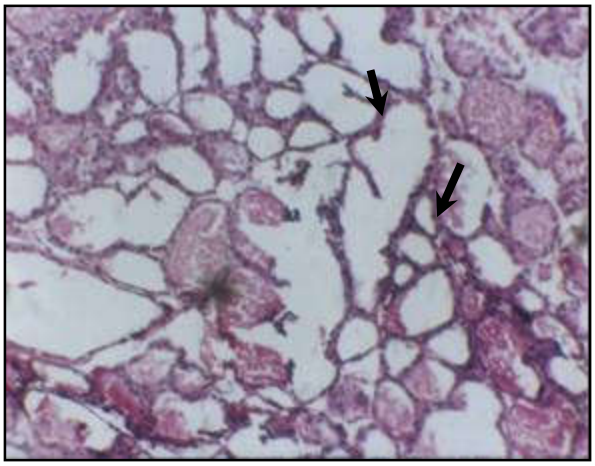
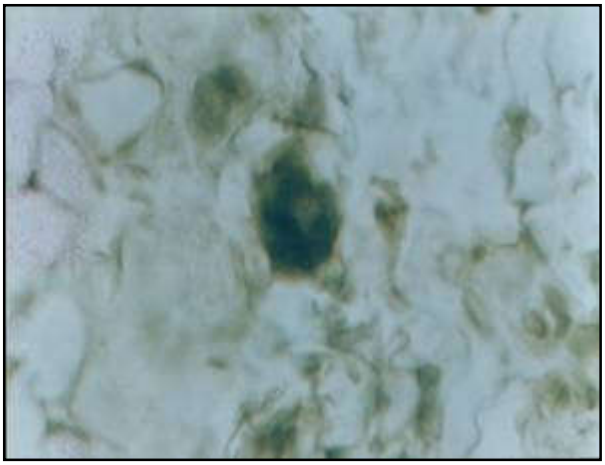


Fig. 3 (A) Control Lactating

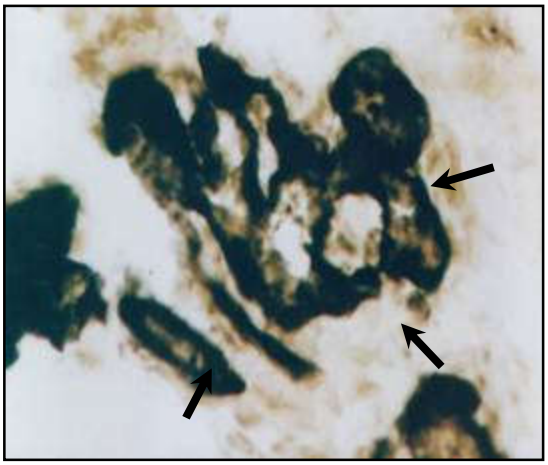


(B) Lactating treated with Harmal

Histochemical Study :

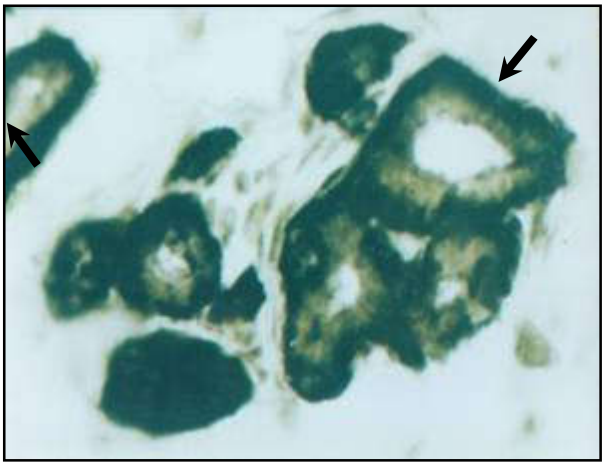


(A) Control Virgin

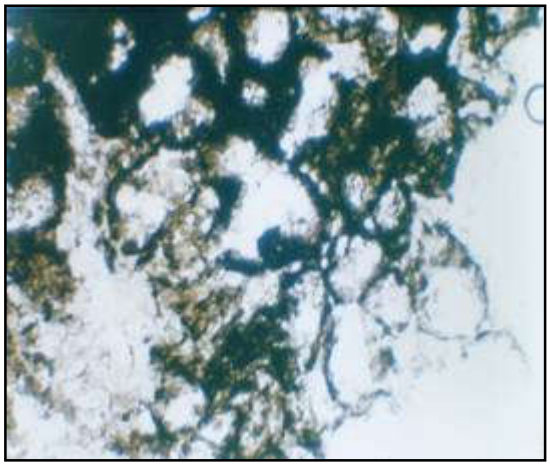


(B) Virgin treated with Harmal

Fig. 4

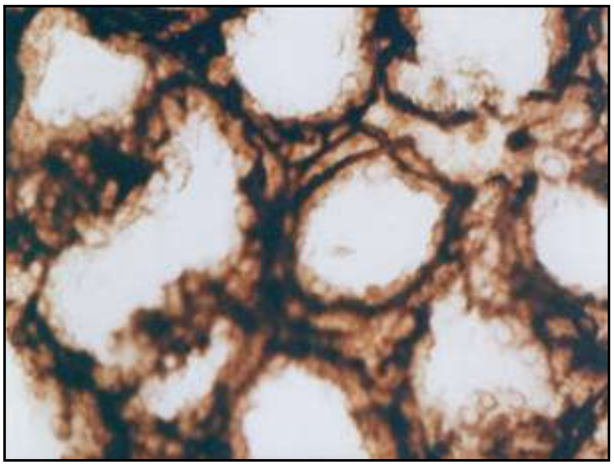


(A) Control Pregnant

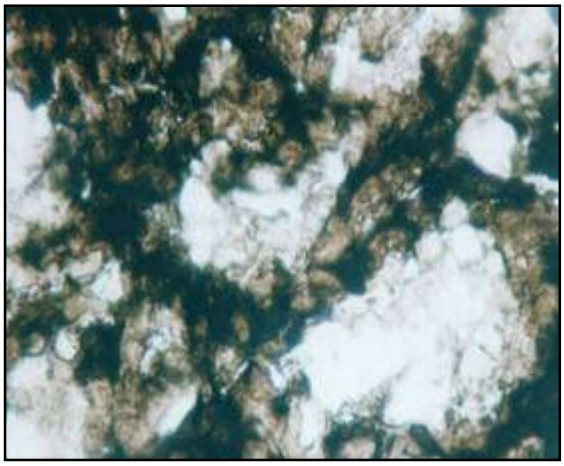


(B) Pregnant treated with Harmal

Fig. 5



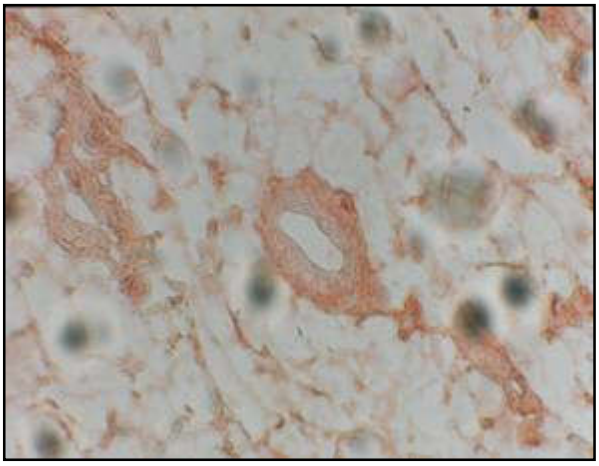
(A) Control Lactating



(B) Lactating treated with Harmal

Fig. 6

Immunohistochemical Study:



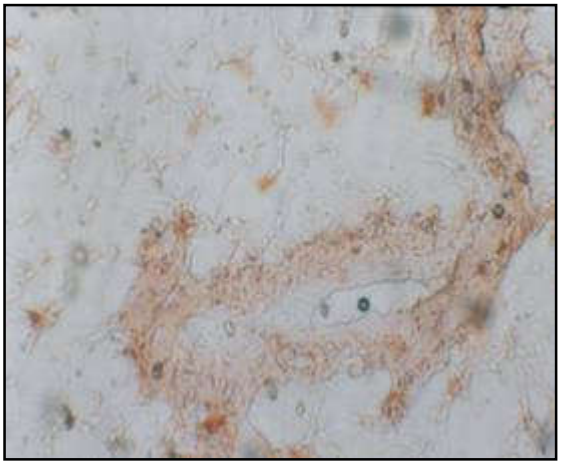
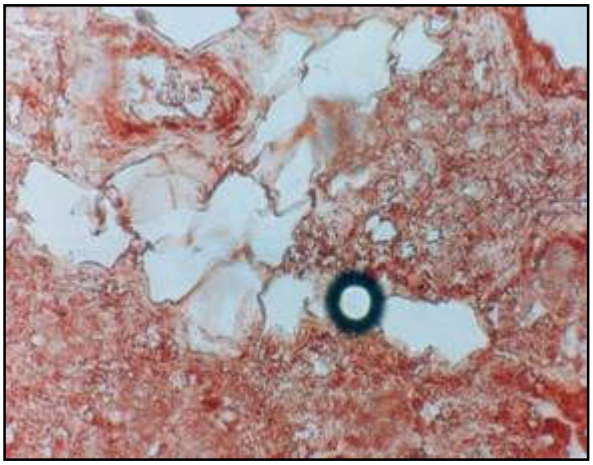


Fig. 7 **(A) Control Virgin** **(B) Virgin treated with Harmal**



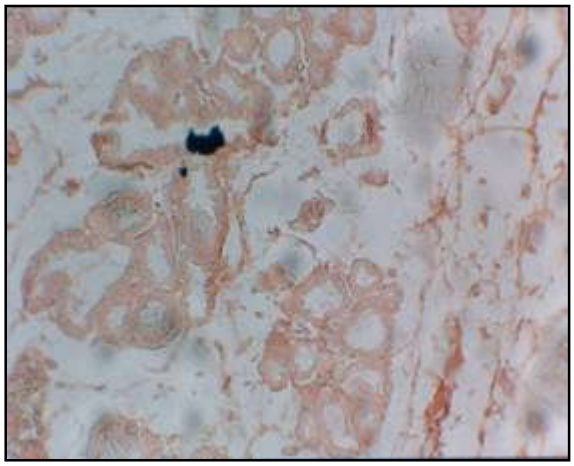


Fig. 8 **(A) Control Pregnant** **(B) Pregnant treated with Harmal**

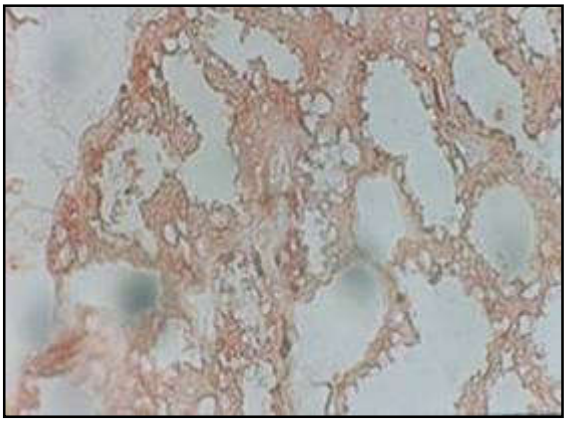


Fig. 9 **(A) Lactating Control** **(B) Lactating treated with Harmal**

Fig1: Mammary gland of virgin rat treated with aqueous extract of harmal (B) Dilated alveoli was seen. Each lined by a single layer of cuboidal cells (arrow) Control mammary gland of virgin rat (A) demonstrated few ill defined secretory tubules (arrow). (H&E) (X200).

Fig2: Mammary gland of pregnant rat treated with harmal exhibited a remarkable increase in the size of their lobules (B) when compared with their controls (A). (H&E)(X200).

Fig3: Mammary gland of lactating rat treated with harmal (B) demonstrated an increase in the size of their alveoli with pouring of milk from the adjacent alveoli (arrow). (A) Control lactating mammary gland (H&E)(X200).

Fig4: Mammary gland of virgin rat: (B) treated with harmal, positive alkaline phosphates activity (black rings) was noticed around the basal part of the secretory epithelium of the alveoli (arrows). No such black rings were demonstrated in control mammary gland (A). (Alkaline phosphates) (X200).

Fig5: Mammary gland of control pregnant rat (A) demonstrated thick black rings around the basal part of the secretory epithelium (arrows). Mammary gland of pregnant rats treated with harmal (B) demonstrated relatively thin black rings (Alkaline phosphates) (X200).

Fig6: Mammary glands of lactating rats of the experimental group (B) showed thinner, discontinuous black rings when compared with the control group (A) Alkaline phosphates (X400).

Fig7: Mammary glands of virgin rats of control group (A) showed strong (+++) expression of both estrogen and progesterone receptors, those treated with harmal (B) exhibited moderate (++) expression of both receptors (Estrogen and progesterone receptors) (X400).

Fig8: Mammary glands of control pregnant rats (A) showed moderate expression (++) of both estrogen and progesterone receptors. Weak expression (+) of these receptors were noticed in those treated with harmal (B) (X200).

Fig9: Mammary glands of lactating rats demonstrated weak expression (+) of both estrogen and progesterone receptors in both control (A) and experimental group (B) (Estrogen and progesterone receptors) (X200)

Table 2: Showing mean of alveoral diameter (µm) and number of nuclei of the epithelium lining the alveolus in control and experimental groups.

Groups	(Mm) mean of alveolar diameter			Number of nuclei of the epithelium lining the alveous		
	(virgin) mean (µm) ±SD	(pregnant) mean (µm) ±SD	lactating mean (µm) ±SD	(virgin) mean ±SD	(pregnant) mean ±SD	lactating mean ±SD
Control	23.98±2.026	32.87±2.1667	38.393±0.879	11±1.53	17±0.58	21.0±1.0
Harmal	*36.34±1.260	*42.17±1.0013	*50.58±1.478	14.0±1.0	17.0±1.0	23.3±1.53

P* < 0.05

Table 3: Serum progestrone, Estrogen and Prolactin in Control and Experimental Groups

Groups	Control Group			Groups treated with harmal		
	(virgin) mean ± SD	pregnant mean ±SD	lactating mean ±SD	Mean ±SD	mean ±SD	mean ±SD
Estradiol ng/ml	72.9±0.2	120.76±1.89	96.363±0.3	75.7±0.3	*15.5±4.3	*107.4±0.52
progesterone ng/ml	7.103±1002	11.36±0.37	15±0.2	*8.4±1.0	*14.4±0.4	*22.3±0.2
Prolactin ng/ml	4.5±1.0	9.4±0.4	10.4±0.4	*8.167±0.1	*10.3±0.2	*12.56±0.28

P* < 0.05

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