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Improvement of *Phaseolus vulgaris* on breastfeeding in female ratsShiva Roshankhah<sup>1</sup>, Cyrus Jalili<sup>2</sup>, Mohammad Reza Salahshoor<sup>1✉</sup><sup>1</sup>Department of Anatomical Sciences, Medical School, Kermanshah University of Medical Sciences, Kermanshah, Iran<sup>2</sup>Medical Biology Research Center, Department of Anatomical Sciences, Kermanshah University of Medical Sciences, Kermanshah, Iran

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## ABSTRACT

**Objective:** To evaluate effect of *Phaseolus vulgaris* (*P. vulgaris*) on the breastfeeding in female rats.**Methods:** This experimental study was done from May 2018 to December 2018 in the Anatomical Department of Medical School in Kermanshah University of Medical Sciences in Iran. In this study, after one-week adaptation and fertilization by male, 40 female rats within 20 days of pregnancy (on average, every mother had 10 newborns) were equally separated into four groups (animals were administrated after delivery of offspring). Group 1 was control group receiving normal saline interaperitoneally, and groups 2, 3, 4 were treatment groups receiving the dose of 20, 50, 100 mg/kg of *P. vulgaris* interaperitoneally respectively once a day for 60 days. The prolactin hormone was measured by radio immune assay, number and diameter of alveoli *via* histological and morphometrical examinations, and receptor prolactin gene expression by real-time polymerase chain reaction method.**Results:** *P. vulgaris* significantly improved alveoli's number and diameter, prolactin hormone and receptor prolactin expression when compared to the control group ( $P < 0.001$ ).**Conclusions:** *P. vulgaris* is helpful to improve the breastfeeding parameters of rats' mammary glands.

## 1. Introduction

Herbal medicine is one of the methods to raise breastfeeding. *Phaseolus vulgaris* (*P. vulgaris*) plant, with the local name of green bean, is a herbaceous annual plant grown worldwide for its edible dry seeds or unripe fruit[1]. This vegetable has usually been used to recover gastric ulcer, liver disease, and kidney and bladder stones in the west of the Iran. Phytochemical studies of the vegetable have revealed the existence of tannin and saponin[2]. In customary medicine, this vegetable exhibits antihyperglycemic activity[3]. It similarly contains vitamin D, protein and starchy materials and is used to treat skin sores, like many antibiotics[4]. The consequences

of higher formance liquid chromatography displayed that the antioxidant and anti-microbial compounds of *P. vulgaris* contain the highest concentrations of carvacrol and fumaric acid[5]. The foremost rudiments of diverse slices of the vegetable have shown antioxidant properties[6].

Breast milk can avoid some illnesses in the initial year of lifetime[7]. However, less than 60% mothers are capable to remain breastfeeding throughout lactation period, and mothers encounter early breastfeeding discontinuation in most cases[8]. Conversely, according to the World Health Organization, two million newborn deaths occur each year in arrears to absence of breastfeeding[9].

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Mammary epithelial cells manufacture and secrete milk. Information of the molecular proceedings in mammary epithelium cells throughout lactation would provide new knowledge for breeding of dairy cattle[10]. Several studies on gene expression in the mammary gland have been carried out, which is invasive and costly and disturbs the normal lactation process[11].

The equal of prolactin excretion rises physiologically throughout gravidity and post-partum during lactation[12]. Prolactin has influence on the androgens metabolism, the secretion of the mammary gland and its associated act with androgens[13]. Drugs are often used in treatments of reducing breastfeeding. but they are not broadly used due to their side effects[14].

Considering that no studies have been carried out on the effects of *P. vulgaris* on breastfeeding, we designed this study to examine the effect of *P. vulgaris* on breastfeeding parameters of female rats.

## 2. Materials and methods

### 2.1. Animals

The present experimental study was carried out in the Anatomy Department of the Medical School of Kermanshah University of Medical Sciences in Iran from May 2018 to December 2018. This study was done on 40 female Wistar rats (weighing 220-250 g) at Kermanshah University of Medical Sciences. After fertilization of female rats by male, within 20 days of pregnancy, every mother had 10 newborns on average. The rats were administrated after delivery of offspring. All animals were treated in accordance with guidelines of National Institute of Health for the Care and Use of Laboratory Animals approved by Research Deputy at Kermanshah University of Medical Sciences (ethic approval No. IR.KUMS.REC.1397.564; approval date: January 31, 2018) based on World Medical Association Declaration Ethic of Helsinki. The rats were maintained on a regular diet and water *ad libitum* with a 12:12 h light/dark cycle at (23±2) °C by considering 1-week adaptation prior to the experiments[15].

### 2.2. Extract preparation

*P. vulgaris* vegetable was attained from a local store (the time of picking and buying this plant was in spring and in western Iran). After confirmation by a botanist from Department of Pharmacognosy, Faculty of Pharmacy, Kermanshah University of Medical Sciences (voucher specimen NO. PMP- 919), the vegetable was cleaned. The leaves were dehydrated in shadow for 7 days and ground to power. And 100 g power was added to ethanol. The solution was kept in hot water bath. Then, the solution was progressively poured on Buchner funnel filter paper and cleaned by vacuum pump. It was then moved to rotary device to change into the additional solvent. The extract was dissolved in distilled water and administered interaperitoneally (*i.p.*) per a kilogram of animal's weight[16].

### 2.3. Experimental protocol

A total of 40 female rats were randomly divided into 4 groups, with 10 rats in each group. Group 1 served as control group receiving normal saline (*i.p.* injection), and groups 2, 3, 4 were treatment groups receiving 20, 50 and 100 mg/kg of *P. vulgaris i.p.* for 60 days at 10:00 am, respectively[16-18].

### 2.4. Radio immune assay

Twenty-four hours after the last injection, the animals were anesthetized and blood was collected from their hearts. Blood was taken from the heart and preserved at 37 °C for 30 min and was centrifuged (1 000 g) for 15 min. Samples were transferred to the Eppendorf tubes, and transferred to the freezer at -20 °C. Prolactin was measured by radio immune assay method. The kit used monoclonal antibodies against 2 dissimilar prolactin epitopes. Sample was enclosed with the initially monoclonal antibody in the attendance of a secondary antibody. The quantity of radioactivity was proportional to the prolactin concentration of the sample[8].

### 2.5. Reverse transcription and real-time polymerase chain reaction (PCR) analysis

Real time-PCR method was used to measure expression of receptor prolactin (*PRLR*) gene in the mammillary gland. On the last day of treatment, the animals were sacrificed. The mammillary gland was removed, frozen in liquid nitrogen and stored in freezer at -80 °C. RNA was extracted from the mammillary tissue using RNeasy mini kit (Qiagen Co.) according to the manufacturer's instructions. DNA samples were treated by DNase set kit (Qiagen). cDNA version was synthesized from the RNA extracted by RevertAid™ First Strand cDNA Synthesis Kit (Fermentas). The expression level of the given gene was measured with glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) primer as endogenous control by Maxima SYBR Green/Rox qPCR master mix (Fermentas Co.) through comparative Ct (<sup>ΔΔ</sup>Ct) technique. The sequence of primers was listed in Table 1[19,20].

**Table 1.** Primers in real-time PCR.

Primer ID	Primer sequences
<i>GAPDH</i> -F	AAGCTCATTTCTGGTATG
<i>GAPDH</i> -R	CTTCCTCTGTGCTCTTG
<i>PRLR</i> -F	TCCTATTGAGTCTGCAGCTTCAGTAGTCA
<i>PRLR</i> -R	CTCCGTGACCAGAGTCACTGTCGGGATCT

*GAPDH*: Glyceraldehyde-3-phosphatedehydrogenase, as endogenous control; *PRLR*: receptor prolactin.

### 2.6. Histological and morphometrical examinations

The non-parenchymal tissues (fat, fascia and vessels) were removed. Mammary glands were fixed, dehydrated, embedded and dissected using Automatic Tissue Processor (Leica, Germany). The steps of this process consequently included: fixation with 10%

formal saline (for 72 h), washing thoroughly under running water, dehydration by raising doses of ethanol (50%, 60%, 70%, 80%, 90% and 100%, 3 min for each step and 100% ethanol step was repeated for three times), clearing by xylene (3 times and 10 min in each), and embedment in soft paraffin (3 times and 15 min in each). At this stage, 5- $\mu$ m coronal histological thin sections were cut from paraffin-embedded blocks by a Leica Microtome (Leica RM 2125, Leica Microsystems Nussloch GmbH, Germany), and 5 sections per animal were chosen. For the unification of the section selection, 4 section intervals were selected, and finally the routine protocol for hematoxylin and eosin staining was implemented. At the end of tissue processing, the stained sections were mounted by entalan glue and assessed under a Olympus BX-51T-32E01 research microscope connected to a DP12 Camera with 3.34-million pixel resolution and Olysia Bio software (Olympus Optical Co. LTD, Tokyo, Japan). For each alveolus the complete cellular area was measured. Outline of alveolus was measured subsequently taking an image with a  $\times$  40 objective. The largest and smallest axes were measured in the drawing of each alveolus in order to estimate the mean axis[9].

## 2.7. Statistical analysis

The Kruskal–Wallis test was used to examine data normality and the homogeneity of variance at a significance level of 0.05. Statistical differences between groups were analysed by one-way analysis of variance (ANOVA), followed by the Toukey test. SPSS software (Statistical Package for the Social Sciences, version 16 SPSS Inc., Chicago, IL, USA) was used.  $P < 0.05$  was considered as significant.

## 3. Results

### 3.1. Hormonal study

Prolactin hormone levels were significantly enhanced in the treatment groups compared to the control group ( $P < 0.001$ ) (Table 2).

### 3.2. mRNA expression of PRLR gene in mammary gland

Effect of *P. vulgaris* on the mRNA expression of *PRLR* gene in mammary gland was evaluated. *P. vulgaris* significantly up-regulated *PRLR* gene expression compared to the control group ( $P < 0.001$ ) (Table 2).

### 3.3. Mean diameter of alveolus

*P. vulgaris* significantly increased the diameter of alveolus in the treatment groups compared to the control group ( $P < 0.001$ ) (Table 3).

### 3.4. Alveolus number

*P. vulgaris* significantly improved the mean number of alveoli of the mammary glands in the treatment groups when compared to the control group ( $P < 0.001$ ) (Table 3).

## 3.5. Histological study

In control group, mammary glands demonstrated minor lobules distributed among massive volume of adipose tissue. The gland tissue treated with *P. vulgaris* showed growth in the dimension of lobules and volume of alveolus which were packed through alveoli. The mammary tissue of treatment groups showed increase in the lobular size with a corresponding lessening in the adipose tissue (Figure 1).

**Table 2.** Results of prolactin hormone and mRNA expression of *PRLR* gene in control and experimental groups (mean  $\pm$  SEM).

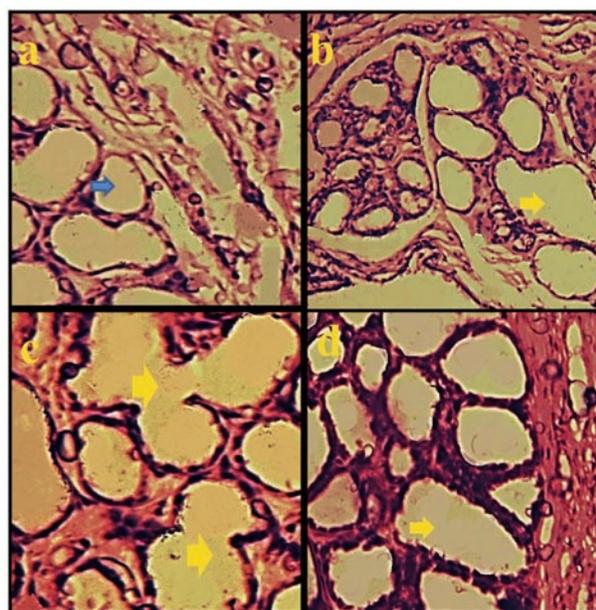
Groups	Prolactin (ng/mL)	mRNA expression
Control	14.23 $\pm$ 0.60	1.00 $\pm$ 0.10
Group 2	17.83 $\pm$ 1.24*	1.30 $\pm$ 0.10*
Group 3	20.53 $\pm$ 2.11*	1.50 $\pm$ 0.10*
Group 4	20.43 $\pm$ 1.06*	1.52 $\pm$ 0.03*

\* $P < 0.05$  compared to control group. Control group received normal saline (*i.p.*), and groups 2, 3, 4 were treatment groups receiving 20, 50 and 100 mg/kg of *P. vulgaris* (*i.p.*).

**Table 3.** Results of alveolar number and diameter in control and experimental groups (mean  $\pm$  SEM).

Groups	Alveolar diameter ( $\mu$ m)	Alveolar number
Control	80.83 $\pm$ 3.60	10.00 $\pm$ 0.78
Group 2	107.83 $\pm$ 5.60*	16.00 $\pm$ 1.31*
Group 3	115.50 $\pm$ 10.01*	20.00 $\pm$ 2.09*
Group 4	118.52 $\pm$ 5.90*	21.00 $\pm$ 0.60*

\* $P < 0.05$  compared to control group. Control group received normal saline (*i.p.*), and groups 2, 3, 4 were treatment groups receiving 20, 50 and 100 mg/kg of *P. vulgaris* (*i.p.*).



**Figure 1.** Effect of *P. vulgaris* extract on mammary gland tissue.

a) Control group: alveolus was seen (blue arrow); (b, c, d) Treatment groups with 20, 50, 100 mg/kg *P. vulgaris* respectively. Growth in the size of lobules (yellow arrow) and alveolar amount were seen in the treatment groups. All groups were used by hematoxylin-eosin staining with magnification  $\times$  100.

#### 4. Discussion

Herbal medicine has diverse properties on different organs[9], such as endocrine and exocrine glands[8]. The study examined the effect of *P. vulgaris* on mammary tissue. The outcomes displayed that the expression of *PRLR* gene was significantly improved in the *P. vulgaris* groups. The *PRLR* gene is a cytokine receptor and has been found in alveoli of the mammary glands. The *PRLR* determined through a gene on chromosome 7p12-17 is related with prolactin by a transmembrane receptor[21]. Raised expression of *PRLR* increases the serum levels of prolactin and also reduces the size of interstitial lipid tissue in breast tissue[22]. Gass et al[23] suggested that antioxidant compounds can raise the expression of the *PRLR* gene. Similarly, *P. vulgaris*, due to antioxidants, improves the *PRLR* expression in the current study. Furthermore, the consequences of Gass et al[23] are consistent with the outcomes of the contemporary study. Saponin can up-regulate *PRLR* and increase the volume of milk production. In view of saponin content of *P. vulgaris*, the improvement of *PRLR* expression and prolactin level in the current study was attributed to treatment with *P. vulgaris* and the existence of saponin in this vegetable[1,2]. *P. vulgaris* augmented the number and diameter of alveoli in the *P. vulgaris* groups. This proposes that *P. vulgaris* has positive influence on the mammary gland. The expansion of alveoli in mammary lobules is related with serum prolactin levels during breastfeeding[24]. It appears that this increase in alveoli was moreover linked with an increase in prolactin levels, considering that *P. vulgaris* contains numerous antioxidants and flavonoid compounds[3]. Flavonoids are portion of phytoestrogens[25]. Phytoestrogens are natural compounds resulting from plants, which in fact have the similar organization as estrogen[26]. Increase of alveoli of the lobules may be attributed to estrogen mechanisms.

The results of Jalili et al[8] displayed that *Utrica dioica*, containing the flavonoids, increased the diameter and number of alveoli in the mammillary tissue, which approves the outcomes of the current research. The existing study displayed that *P. vulgaris* improved the amount of prolactin of the mother rats in this study. The equal of prolactin upsurges physiologically through pregnancy and postpartum, and plays diverse roles such as stabilising the secretory action of the milk production. So, aggregated prolactin levels can be linked with a rise in milk production[27]. The effect of *P. vulgaris* on prolactin hormone in the current study may be due to sufficient vital nutrients and minerals provided by this plant[28]. It similarly seems that this vegetable is rich in tanins, ascorbic acid, saponins and many antioxidants; these compounds increase the prolactin hormone *via* affecting on the pituitary gland[29].

The outcomes of AL-Shemary et al[30] showed that tannin in *Ocimum gratissimum* can increase the prolactin concentration which confirms the results of the present study. The results of Daniel et al[31] displayed that *Cucurbita pepolinn* extract significantly increased serum prolactin levels, which emphasizes the effect of herbal medicine on prolactin levels and is consistent with the results of

the present study. According to the study results of Bolzán et al[32], which showed that antioxidants can increase the prolactin blood level, it seems that the high antioxidant property of *P. vulgaris* extract is another factor leading to elevated prolactin level of animals. However, further research is needed to clarify the issues and ambiguities in this regard. However, the present study provides new evidence of the role of *P. vulgaris* extract in improving milk production. Determining role of molecular factors and more precise mechanisms involved in this regard will require more detailed studies.

In conclusion, the current research explores the role of *P. vulgaris* in improving breastfeeding. Administration of *P. vulgaris* leads to increased *PRLR* gene expression, raised serum prolactin levels, and positive variations in mammary gland tissue in favor of milk production. Examining the action of the *P. vulgaris* extract on breastfeeding needs more additional detailed experimentations in this regard.

#### Conflict of interest statement

The authors declare that there is no conflict of interest.

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#### References

- [1] Toubart P, Desiderio A, Salvi G, Cervone F, Daroda L, De Lorenzo G, et al. Cloning and characterization of the gene encoding the endo polygalacturonase-inhibiting protein (PGIP) of *Phaseolus vulgaris* L. *Plant J* 1992; **2**(3): 367-373.
- [2] Li PB, Jing CC, Wang ZY, Ma MG, Li MJ, Wu GT, et al. First report of tobacco curly shoot virus infecting *Phaseolus vulgaris* in China. *Plant Dis* 2019; **103**(1): 165-165.
- [3] Nuñez-Aragón PN, Segura-Campos M, Negrete-León E, Acevedo-Fernández JJ, Betancur-Ancona D, Chel-Guerrero L, et al. Protein hydrolysates and ultrafiltered < 1 KDa fractions from *Phaseolus lunatus*, *Phaseolus vulgaris* and *Mucuna pruriens* exhibit antihyperglycemic

- activity, intestinal glucose absorption and  $\alpha$ -glucosidase inhibition with no acute toxicity in rodents. *J Sci Food Agric* 2019; **99**(2): 587-595.
- [4] Türkan I, Bor M, Özdemir F, Koca H. Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P. acutifolius* Gray and drought-sensitive *P. vulgaris* L. subjected to polyethylene glycol mediated water stress. *Plant Sci* 2005; **168**(1): 223-231.
- [5] Barmantlo SH, Meirmans PG, Luijten SH, Triest L, Oostermeijer JG. Outbreeding depression and breeding system evolution in small, remnant populations of *Primula vulgaris*: Consequences for genetic rescue. *Conserv Genet* 2018; **19**(3): 545-554.
- [6] Li J, Cocker JM, Wright J, Webster MA, McMullan M, Dyer S, et al. Genetic architecture and evolution of the S locus supergene in *Primula vulgaris*. *Nat Plants* 2016; **2**(12): 1-7.
- [7] Tombs E, Sridaromont K, Moore A. Continuum of care for mother and child during breastfeeding. *Nurs Made Incred Easy* 2018; **16**(4): 16-20.
- [8] Jalili C, Salahshoor MR, Yousefi D, Khazaei M, Shabanizadeh Darehdori A, Mokhtari T. Morphometric and hormonal study of the effect of *Urica dioica* extract on mammary glands in rats. *Int J Morphol* 2015; **33**(3): 983-987
- [9] Zhou W, Yu Y, Qian Z. Challenges of breastfeeding in China under its universal two-child policy. *J Matern Fetal Neonatal Med* 2018; **4**: 1-2.
- [10] Walker AR, Adam FI. Breast-feeding in sub-Saharan Africa: Outlook for 2000. *Public Health Nutr* 2000; **3**(3): 285-292.
- [11] Velle-Forbord V, Underdal MO, Vanky E. Breastfeeding and future maternal health—No causal evidence. *JAMA Intern Med* 2018; **178**(6): 870-871.
- [12] Rosenbaum ME, Lench S, Ferguson KJ. Outcomes of a teaching scholars program to promote leadership in faculty development. *Teach Learn Med* 2005; **17**(3): 247-252.
- [13] Akinloye AK, Oke BO. Sex hormonal pattern of the female African giant rat (*Cricetomys gambianus*, Waterhouse) at different stages of the oestrous cycle. *Niger Vet J* 2013; **33**(3): 586-591.
- [14] Hum J. The effect of two different domperidone doses on maternal milk production. *J Hum Lact* 2013; **29**: 38-44.
- [15] Salahshoor MR, Roshankhah S, Hosseni P, Jalili C. Genistein improves liver damage in male mice exposed to morphine; genistein improves liver damage in male mice exposed to morphine. *Chin Med J* 2018; **131**: 1598-1604.
- [16] Khazaei M, Salehi H. Protective effect of *Falcaria vulgaris* extract on ethanol induced gastric ulcer in rat. *Iran J Pharmacol Exp Ther* 2006; **5**: 43-46.
- [17] Rafiey Z, Jalili F, Sohrabi M, Jalili C. Effects of hydro-alcoholic extract of *Falcaria vulgaris* on pancreas tissue in streptozotocin-induced diabetic rats. *Iran J Endocrinol Metab* 2017; **19**: 91-98.
- [18] Salahshoor MR, Jalili P, Roshankhah Sh, Makalani F, Jalili C. The effect of *F. vulgaris* extract on streptozotocin induced diabetic rats. *Pharmacophore* 2017; **8**: e-1173298.
- [19] Nikbakht M, Salahshoor MR, Mardani M, Rabbani M, Hashemibeni B, Gharagozloo M, et al. The apoptotic effects of sirtuin1 inhibitor on MCF-7 and MRC-5 cell lines. *Res Pharm Sci* 2013; **8**(2): 79-89.
- [20] Nagano M, Kelly PA. Tissue distribution and regulation of rat prolactin receptor gene expression. Quantitative analysis by polymerase chain reaction. *J Biol Chem* 1994; **269**(18): 13337-13345.
- [21] Adler S, Waterman ML, He X, Rosenfeld MG. Steroid receptor-mediated inhibition of rat prolactin gene expression does not require the receptor DNA-binding domain. *Cell* 1988; **52**(5): 685-695.
- [22] Lan H, Hong P, Li R, Suo L, Anshan S, Li S, et al. Internal image anti-idiotypic antibody: A new strategy for the development a new category of prolactin receptor (PRLR) antagonist. *Mol Immunol* 2017; **87**: 86-93.
- [23] Gass S, Harris J, Ormandy C, Brisken C. Using gene expression arrays to elucidate transcriptional profiles underlying prolactin function. *J Mamm Gland Biol Neopl* 2003; **8**(3): 269-285.
- [24] Zhou G, Tang L, Wang T, Zhou X, Kou Z, Wu J, et al. Phytochemistry and pharmacological activities of *Vaccaria hispanica* (Miller) Rauschert: A review. *Phytochem Rev* 2016; **15**(5): 813-827.
- [25] Oakes SR, Rogers RL, Naylor MJ, Ormandy CJ. Prolactin regulation of mammary gland development. *J Mamm Gland Biol Neopl* 2008; **13**(1): 13-23.
- [26] Bialymstoku W. Influence of nargenin on the activating of enzymes participating in steroidogenesis in male rats. *Exp Gerontol* 2004; **49**: 37-46.
- [27] Panjeshahin M, Dehghani F, Tahei T, Panahi Z. The effects of hydroalcoholic extract of *Actinidia chinensis* sperm count and motility and on the blood levels of estradiol and testosterone in male rats. *Arch Iran Med* 2005; **8**(3): 211-216.
- [28] Dill R, Walker AM. Role of prolactin in promotion of immune cell migration into the mammary gland. *J Mamm Gland Biol Neopl* 2017; **22**(1): 13-26.
- [29] Wirawati CU, Sudarwanto MB, Lukman DW, Wientarsih I. Local plants as feed supplementation to improve ruminant milk production and quality. *WARTAZOA* 2017; **27**(3): 145-157.
- [30] AL-Shemary NN, Mousa SA, Muslim ZZ. Histological and hormonal study about the effect of aqueous extract of *Ocimum gratissimum* on female reproductive system in albino mice. *Int J Pharm Sci Res* 2018; **10**(4): 765-767.
- [31] Danie EE, Hamman WO, Olorunshola KV, Malgwi IS. The study of effect of aqueous *Cucurbita pepo* Linn seed extract on serum prolactin level of lactating female albinorats. *Sci J Med Sci* 2013; **2**(3): 6-11.
- [32] Bolzán AD, Bianchi MS, Cónsole GM, Goya RG. Relationship between pituitary hormones, antioxidant enzymes, and histopathological changes in the mammary gland of senescent rats. *Exp Gerontol* 1997; **32**(3): 297-304.