

## Morphometric and Hormonal Study of the Effect of *Urtica diocia* Extract on Mammary Glands in Rats

### Estudio Morfométrico y Hormonal del Efecto de Extracto de *Urtica diocia* en Glándulas Mamarias en Ratas

Jalili, C.\*; Salahshoor, M. R.\*; Yousefi, D.\*; Khazaei, M.\*; Shabanizadeh Darehdori, A.\*\* & Mokhtari, T.\*\*\*

---

JALILI, C.; SALAHSHOOR, M. R.; YOUSEFI, D.; KHAZAEI, M.; DAREHDORI, A. S. & MOKHTARI, T. Morphometric and hormonal study of the effect of *Urtica diocia* extract on mammary glands in rats. *Int. J. Morphol.*, 33(3):983-987, 2015.

**SUMMARY:** *Urtica diocia* is a multipurpose herb in traditional medicine. Its hydroalcoholic extract (20, 50 and 100 mg/kg) administered interaperitoneally to Wistar female rats for 21 consequent days resulted in significant increase in the number of alveoli of mammary glands in doses of 20 and 50 mg/kg. Changes in serum prolactin and alveolar diameter were not significant in comparison with control group. Also, there was an increase in serum prolactin and alveolar diameter in doses of 20 and 50 mg/kg. *Urtica diocia* extract has positive effects on mammary glands.

**KEY WORDS:** *Urtica diocia*; Morphometric; Hormonal; Mammary glands; Rat.

---

### INTRODUCTION

*Urtica diocia*, in folk medicine, is used as an agent causing excessive secretion of urine, setting the operating cycle, as well as an astringent, an adrenal tonic, and gland balancer agent (Di Lorenzo *et al.*, 2013). Previous studies have shown that *Urtica diocia* infusion exhibits antioxidant capacity against oxidation of phospholipids and acid linoleic resulting from iron (Gülcin *et al.*, 2004). *Urtica diocia* is a plant containing essential amino acids, vitamins and numerous nutrients (Mishra *et al.*, 2006). It has also been used as a galactagogue for lactating women (Ang-Lee *et al.*, 2001). Today the interest in induced lactation stems from a desire of some adopting mothers to nurse the adopted child (Lawrence, 1985). In view of that, *Urtica diocia* was employed particularly in rural areas to ensure an abundant milk supply or to rectify milk insufficiency. However, this remedy has not been scientifically tested but women swear by it. Exclusive breastfeeding is considered as the best nutrition for the child up to the age of 6, which, in addition to providing physical and physiological needs, is a supplement to psychological needs of the mother and child (Harden & Crosby, 2000). Only a small number of women are able to breastfeed during the time their children need milk and in most cases they face early cessation of breastfeeding (Adhami *et al.*, 2005). The significance of

breastfeeding in the first two years after birth and the problems taking place for mothers necessitate new research in order to achieve milk producing medications (Rosenbaum *et al.*, 2005). However, according to the World Health Organization (WHO), 1.5 million infants die annually due to deprivation from or lack of breast milk (Perrine & Scanlon, 2013). The level of prolactin secretion increases physiologically during pregnancy and after delivery while breastfeeding so that the prolactin concentration during pregnancy is 10 to 20 times higher than the normal state (Oakes *et al.*, 2008). Numerous functions have been recognized for prolactin, including stable secretory activity of the mammary glands, accompaniment of its activity with androgens and effect on the metabolism of androgens (Akinloye & Oke, 2013). Various studies have been carried out on chemical drugs as breast milk producing medications, but these drugs have not been used much due to their side effects (Knoppert *et al.*, 2013; Paten *et al.*, 2013). It seems that using medicinal plants is one of the methods of increasing breast milk. We designed this study to investigate the effect of *Urtica diocia* leaves on the mammary glands of Wistar rats and compare this effect with that on the mammary glands of lactating ones, making use of the available morphometrical and histological analysis.

\* Fertility and Infertility Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran.

\*\* Department of Anatomical Sciences and Molecular Biology, Medical School, Rafsanjan University of Medical Sciences Rafsanjan, Iran.

\*\*\* Department of Anatomical Sciences and Molecular Biology, Medical School, Kermanshah University of Medical Sciences Kermanshah, Iran.

## MATERIAL AND METHOD

Preparation of plant extract. *Urtica dioica* was purchased from a traditional medicine center and identified and authenticated by a botanist. Extracting method was described previously (Lotfi *et al.*, 2013). In this method, *Urtica dioica* leaves (200 g) were powdered and added to 400 cc of 70% ethanol and were left to macerate at room temperature for 4 hours. The soaked leaves were extracted by percolation method and the obtained extract was concentrated in the vacuum and dried in the flat surface. The weight of the obtained extract was 8.5 g. The extract was dissolved in distilled water and immediately administered interaperitoneally (IP) to rats, expressed as mg of extract per kg of body weight.

Animals. Twenty four Wistar female rats with a weight range of 180-220 g were used. Animals were kept at a temperature of  $22\pm 2$  °C, under controlled environmental conditions, 12/12 h light/dark cycle and free access to water and food ad libitum. The rats were randomly assigned to four groups (n= 6). The control group received distilled water and the experimental groups 1, 2, and 3 received *Urtica dioica* extract with doses 20, 50, and 100 mg/kg, respectively for 21 consequent days (Haller *et al.*, 2013).

Method of extract administration and prolactin level measurement. The animals were weighed and anesthetized 24 hours after the last injection. Blood was taken from the heart and preserved at a temperature of 37 °C for 30 minutes and was centrifuged (1000 g) for 15 minutes. Its serum was collected and preserved in -20 °C until measuring the prolactin hormone. Prolactin hormone measurement was performed by ELISA method. The mammary glands were separated and preserved in 10% neutral buffered formalin (Daniel *et al.*, 2013).

Preparing and staining tissue sections and measuring the diameter of mammary alveoli. After fixation of mammary glands, tissue processing, including dehydration, clearing, and embedding were performed. Microscopic sections (5  $\mu$ m) were prepared and stained using H-E method. Twenty full linear sections were prepared from each tissue block and sections numbered 5, 10, 15, and 20 were selected and photographed separately from three random scopes. The diameter of alveolars was measured by Motic camera and software (Moticam 2000, Spain). The mean of alveolar tubule diameter in micrometers was determined for mammary glands (Fig. 1).

Alveolar number. Via cardiac puncture, blood samples were obtained from the rats anesthetized with ether

to measure the level of prolactin in their serum. Morphometrical study was done using an eyepiece micrometer fitted to a light microscope at 10x 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 (Al- Saidi *et al.*, 2006).

**Statistical Analysis.** All the quantitative data were presented as the Mean $\pm$ SD. One-way analysis of variance (ANOVA) and LSD post-hoc test were applied on the data to determine the statistical significance among different groups using SPSS software package 16.0.  $P<0.05$  was considered significant.

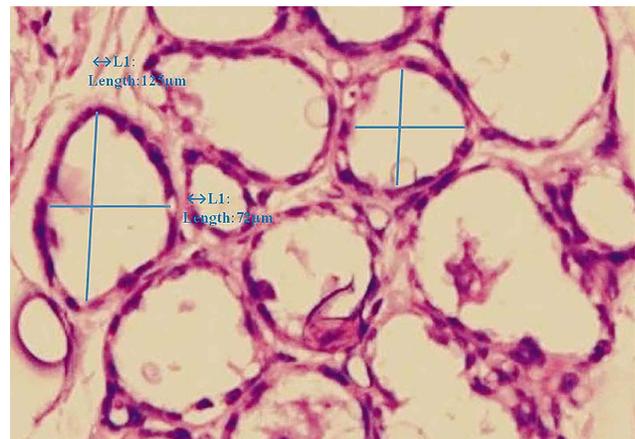


Fig. 1. Effect of *Urtica dioica* extract on serum prolactin in control and experimental groups.

## RESULTS

Hormonal study. ELISA assay for prolactin was assessed using Mean $\pm$ SD (Table I). Prolactin increased in Wistar rats treated with *Urtica dioica*. Statistically, no significant difference in prolactin was observed between control and experimental groups ( $P>0.05$ ) (Fig. 2).

Morphometrical study. The number of alveolus significantly ( $P<0.05$ ) increased in 50 mg/kg group in comparison with control group in Wistar lactating rats treated with *Urtica dioica* than control groups (Table I and Fig. 3). Diameters of alveoli increased in Wistar lactating rats treated with *Urtica dioica* than their controls. Also, no significant difference was observed in control and experimental groups ( $P>0.05$ ) (Table I and Fig. 4).

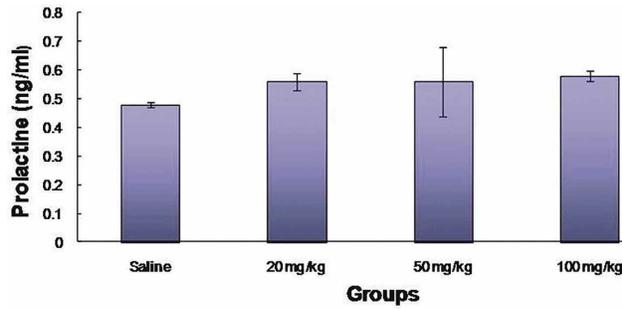


Fig. 2. Effect of *Urtica dioica* extract on the number of mammary glands' alveoli in control and experimental groups. \*= $P$ -value  $< 0.05$  was considered significant.

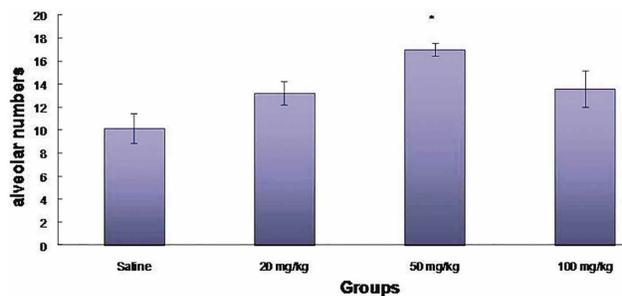


Fig. 3. Effect of *Urtica dioica* extract on the diameter of alveoli in control and experimental groups.

Histological study. Haematoxylin eosin stained sections of control Wistar mammary glands exhibited small lobules scattered among huge amount of adipose tissue (Fig. 5). The mammary tissue of Wistar rats treated with *Urtica dioica* showed an increase in the size of lobules which were packed by alveoli. The mammary tissue of control lactating rats showed an increase in the lobular size with a corresponding decrease in the adipose tissue (Fig. 5).

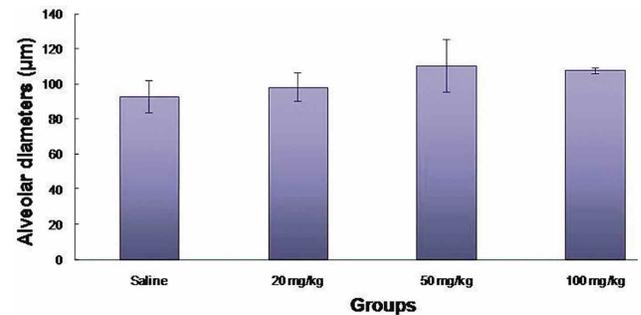


Fig. 4. Photomicrograph of mammary tissue (100 mg/kg *Urtica dioica* extract).

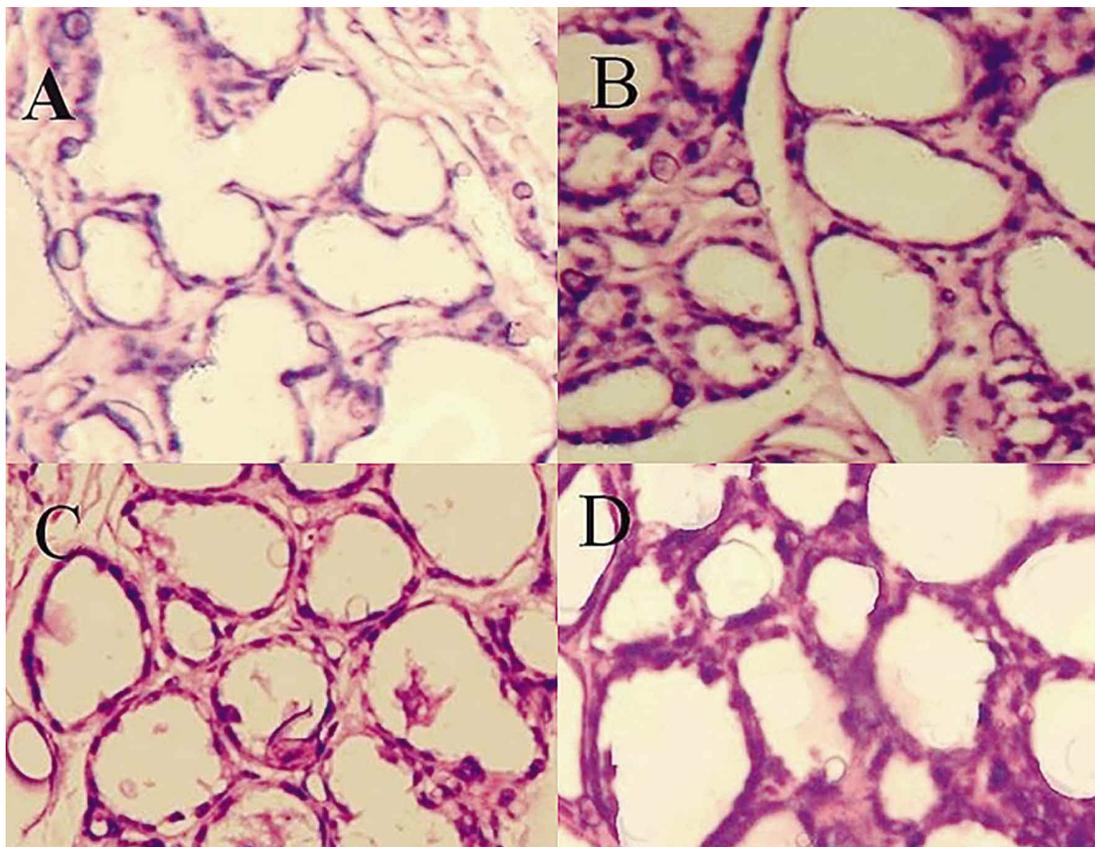


Fig. 5. A. Mammary gland in saline group. B. Mammary gland in 20 mg/kg group. C. Mammary gland in 50 mg/kg group. D. Mammary gland in 100 mg/kg group (All groups of H&E staining and 100X magnification).

Table I. The results of prolactin hormone, alveolar number and alveolar diameter in control and experimental groups (results are indicated as Mean±SE). \*P-value < 0.05 was considered significant (One-Way ANOVA).

	Control	20 mg/kg	50 mg/kg	100 mg/kg
Prolactin (ng/ml)	0.48±0.007	0.56± 0.031	0.7±0.121	0.66± 0.017
Alveolar diameters (μm)	93.21±9.2	98.38±8.04	111.44±14.77	107.47±1.5
Alveolar number	10.2±1.28	13.2±1.01	17± 0.54	13.6± 1.5

## DISCUSSION

In the present study, *Urtica dioica* extract increased the number of alveoli, but it had no significant impact on serum prolactin and alveolar diameter. Nowadays, plant extracts have been largely taken into consideration and their positive and negative effects on various organs and tissues of the body have been identified. One of the target tissues of plant extracts is the tissue of reproduction system organs such as mammary glands parameters. In this study, *Urtica dioica* increased serum prolactin in doses of 20, and 50 mg/kg, whereas this increase did not indicate significant difference with saline group. It seems that the effect of *Urtica dioica* on increasing the breast milk indicated in the previous studies is the result of essential nutrients, which are received by mother through this plant, and is not due to its effects on prolactin hormone production.

The findings of this study confirm the results of studies conducted by (Gülcin *et al.*), in which they introduced *Urtica dioica* as a substance containing essential amino acids and argued that it supports lactation via supplying essential nutrients. On the other hand, these findings contradict the results of (Ganong, 1995; Knoppert *et al.*). They stated that in the terminal stage of mammary gland development, lobulo alveolar growth is regulated by prolactin. These findings may indicate that prolactin has been considered essential for the early but not for the late development of the mammary gland. The *Urtica dioica* hydroalcoholic extract promoted lactation when given to lactating rats. Such an herb may not act like other stimulators of prolactin release such as chlorpromazine, metochlorpramides and theophylline. Our

morphometrical studies showed that in all experimental groups, there is significant increase in the number of alveoli when compared with their controls. This finding may indicate that *Urtica dioica* 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 (Demiralp *et al.*, 2010). *Urtica dioica* contains numerous antioxidants as well as phenolic and flavonoid compounds (Inic & Kujundzic, 2012), also flavonoids belong to such compounds called phytoestrogens (Papiez, 2004), and phytoestrogens are natural compounds derived from the plants that are structurally similar to estrogen (Panjeshahin *et al.*, 2005). Accordingly, one reason for the increase in the number of lobuloalveolars can be associated with estrogen mechanisms that affect mammary gland and increase the number of lobuloalveolars by inducing slight increase in prolactin. These results confirm the findings of the study carried out by (Al-Saidi *et al.*, 2006). The present study can provide new evidence on the dose-dependent role of hydroalcoholic extract of *Urtica dioica* in increasing lactation.

## ACKNOWLEDGEMENTS

This study was approved and financially supported by the Fertility and Infertility Research Center, Kermanshah University of Medical Sciences. There is no conflict of interest in this study.

---

JALILI, C.; SALAHSHOOR, M. R.; YOUSEFI, D.; KHAZAEI, M.; DAREHDORI, A. S. & MOKHTARI, T. Estudio morfométrico y hormonal del efecto de extracto de *Urtica dioica* en glándulas mamarias en ratas. *Int. J. Morphol.*, 33(3):983-987, 2015.

**RESUMEN:** *Urtica dioica* es una hierba de usos múltiples en la medicina tradicional. Su extracto hidroalcohólico (20, 50 y 100 mg/kg) administrado por vía intraperitoneal en ratas hembras Wistar de 21 días resultaron en un aumento significativo en el número de alvéolos de las glándulas mamarias en dosis de 20 y 50 mg/kg. Los cambios en la prolactina sérica y el diámetro alveolar no fueron significativos en comparación con el grupo control. Además, hubo un aumento en la prolactina sérica y en el diámetro alveolar en dosis de 20 y 50 mg/kg. El extracto de *Urtica dioica* tiene efectos positivos sobre las glándulas mamarias.

**PALABRAS CLAVE:** *Urtica dioica*; Morfométrico; Hormonal; Glándulas mamarias; Rata.

## REFERENCES

- Adhami, A.; Reihani, H.; Fattahi, Z.; Nakhaei, N. & Fasihi Harandi, T. Comparison of student assessment of educational performance of the faculty with the teacher's self assessment in Kerman University of Medical Sciences. *S. D. M. E.*, 2(1):25-32, 2005.
- Akinloye, A. K. & Oke, B. O. Sex hormonal pattern of the female african giant rat (*Cricetomys gambianus*, Waterhouse) at different stages of the oestrous cycle. *Niger. Vet. J.*, 33(3):586-91, 2013.
- Ang-Lee, M. K.; Moss, J. & Yuan, C. S. Herbal medicines and perioperative care. *JAMA*, 286(2):208-16, 2001.
- Al-Saidi, W.; Al-Yawer, M.; Mangalo, H. & Hammoudi, S. R. Elucidation of a role for the aqueous extract of borage in mammary gland growth and development. *Iraqi Postgrad. Med. J.*, 5(1):54-61, 2006.
- Daniel, E. E.; Hamman, W. O.; Olorunshola, K. V. & Malgwi, I. S. The study of effect of aqueous Cucurbita pepo linn seed extract on serum prolactine level of lactating female albinorats. *Sci. J. Med. Sci.*, 2(3):6-11, 2013.
- Demiralp, D. Ö.; Haznedaroglu, I. C. & Akar, N. Functional proteomic analysis of Ankaferd® Blood Stopper. *Turk. J. Hematol.*, 27(2):70-7, 2010.
- Di Lorenzo, C.; Dell'Agli, M.; Badea, M.; Dima, L.; Colombo, E.; Sangiovanni, E.; Restani, P. & Bosisio, E. Plant food supplements with anti-inflammatory properties: a systematic review (II). *Crit. Rev. Food Sci. Nutr.*, 53(5):507-16, 2013.
- Ganong, F. W. *The female reproductive system*. In: Ganong, F. W. (Ed.). *Review of medical physiology*. 17th ed. Toronto, Prentice Hall International Inc., 1995. pp.379-417.
- Gülcin, I.; Küfrevioglu, O. I.; Oktay, M. & Büyükkokuroglu, M. E. Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.). *J. Ethnopharmacol.*, 90(2-3):205-15, 2004.
- Haller, J.; Freund, T. F.; Pelczer, K. G.; Füredi, J.; Krecsak, L. & Zámbari, J. The anxiolytic potential and psychotropic side effects of an echinacea preparation in laboratory animals and healthy volunteers. *Phytother. Res.*, 27(1):54-61, 2013.
- Harden, R. M. & Crosby, J. The good teacher is more than a lecturer: the twelve roles of the teacher. *Med. Teach.*, 22(4):334-47, 2000.
- Inic, S. & Kujundzic, N. The original Croatian pharmacopoeia from 1901. *Pharmazie*, 67(7):652-7, 2012.
- Knoppert, D. C.; Page, A.; Warren, J.; Seabrook, J. A.; Carr, M.; Angelini, M.; Killick, D. & Dasilva, O. P. The effect of two different domperidone doses on maternal milk production. *J. Hum. Lact.*, 29(1):38-44, 2013.
- Lawrence, R. A. *Induced lactation and relactation (including nursing the adopted baby)*. In: Lawrence, R. A. (Ed.). *Breast feeding: A guide for the medical profession*. 2nd ed. Baltimore, Mosby Co., 1985. p.43-62.
- Lotfi, N.; Khazaei, M.; Shariatzadeh, S. M. A.; Soleimani-Mehranjani, S. & Ghanbari, A. The effect of Cannabis sativa hydroalcoholic extract on sperm parameters and testis histology in rats. *Int. J. Morphol.*, 31(1):82-6, 2013.
- Mishra, U. K.; Kanesh, J. S.; Mandal, A. K.; Das, R. K.; Rayaguru, K. & Parija, S. C. Potentials of herbal galactogogues in milk production in ruminants. *Indian Cow*, 2(9):44-52, 2006.
- Oakes, S. R.; Rogers, R. L.; Naylor, M. J. & Ormandy, C. J. Prolactin regulation of mammary gland development. *J. Mammary Gland Biol. Neoplasia*, 13(1):13-28, 2008.
- Panjehshahin, M.; Panahi, Z. & Dehghani, F. The effects of hydroalcoholic extract of *Actinidia chinensis* on sperm count and motility, and on the blood levels of estradiol and testosterone in male rats. *Arch. Iran. Med.*, 8(3):211-6, 2005.
- Papiez, M. A. Influence of naringenin on the activity of enzymes participating in steroidogenesis in male rats. *Rocz. Akad. Med. Bialymst.*, 49(Suppl. 1):120-2, 2004.
- Paten, A. M.; Kenyon, P. R.; Lopez-Villalobos, N.; Peterson, S. W.; Jenkinson, C. M.; Pain, S. J. & Blair, H. T. Lactation Biology Symposium: maternal nutrition during early and mid-to-late pregnancy: Comparative effects on milk production of twin-born ewe progeny during their first lactation. *J. Anim. Sci.*, 91(2):676-84, 2013.
- Perrine, C. G. & Scanlon, K. S. Prevalence of use of human milk in US advanced care neonatal units. *Pediatrics*, 131(6):1066-71, 2013.
- Rosenbaum, M. E.; Lench, S. & Ferguson, K. J. Outcomes of a teaching scholars program to promote leadership in faculty development. *Teach. Learn. Med.*, 17(3):247-52, 2005.

Correspondence to:  
Dr. Mohammad reza.Salahshoor  
Fertility and Infertility Research Center  
Medical School  
Kermanshah University of Medical Sciences  
Paraster Street  
Kermanshah  
IRAN

Received: 20-07-2013  
Accepted: 25-06-2015

Email: Reza.salahshoor@yahoo.com