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

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


SUMMARY: *Utrica 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. *Utrica diocia* extract has positive effects on mammary glands.

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

INTRODUCTION

*Utrica 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 *Utrica diocia* infusion exhibits antioxidant capacity against oxidation of phospholipids and acid linoleic resulting from iron (Gülcin *et al*., 2004). *Utrica 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, *Utrica 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 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 prolactine secretion increases physiologically during pregnancy and after delivery while breastfeeding so that the prolactin concentration during pregnancy is 10 to 20 times higher than thenormal 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 *Utrica 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.
MATERIAL AND METHOD

Preparation of plant extract. *Utrica 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, *Utrica 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 ± 2 ºC, 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 *Utrica 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 ºC for 30 minutes and was centrifuged (1000 g) for 15 minutes. Its serum was collected and preserved in -20 ºC 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 µm) 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 Moticam 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±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.

RESULTS

Hormonal study. ELISA assay for prolactin was assessed using Mean±SD (Table I). Prolactin increased in Wistar rats treated with *Utrica 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 *Utrica dioica* than control groups (Table I and Fig. 3). Diameters of alveoli increased in Wistar lactating rats treated with *Utrica dioica* than their controls. Also, no significant difference was observed in control and experimental groups (P>0.05) (Table I and Fig. 4).
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. 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.

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).
DISCUSSION

In the present study, *Utrica diocia* 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, *Utrica diocia* 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 *Utrica diocia* 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 (Gülçin et al., 2010). Inic & Kujundžic, 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 lobulalveolars can be associated with estrogen mechanisms that affect mammary gland and increase the number of lobulalveolars 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 *Utrica diocia* 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.

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

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).
REFERENCES


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

Email: Reza.salahshoor@yahoo.com