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Effect of Silitidil, a Standardized Extract of Milk Thistle, on the Serum Prolactin Levels in Female Rats

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The aim of this study was to assess the effect of Silitidil, a standardized extract of milk thistle, on the serum levels of prolactin in female rats. A 14-day treatment with Silitidil (25-200 mg/kg, *per os*), a standardized extract of *Silybum marianum* fruits (milk thistle), increased, in a dose dependent manner, the serum prolactin levels in female rats. Galega (200 mg/kg, *per os*) given alone neither modified the basal levels of prolactin nor increased further serum prolactin levels when associated with Silitidil. Bromocriptine (1 mg/kg, *per os*) significantly reduced the high serum prolactin levels induced by Silitidil (200 mg/kg, *per os*). The results show that the extract of *S. marianum* fruits significantly increases prolactin levels in female rats; this effect is not potentiated by galega and seems to involve, at least in part, dopamine D_2 receptors.

Keywords: Silitidil, Prolactin, *Silybum marianum*.

Breast milk is considered to be the optimal nutrition for infants from birth to 1 year by several Academies of Pediatrics and by the World Health Organization [1a,b,2a]. Because breastfeeding is widely acknowledged to have important health benefits for infants and mothers, a variety of herbal and phytopharmaceutical products have been recommended as galactogogues, substances that promote lactation. Among herbal drugs, milk thistle is traditionally used to stimulate milk production [2b,3].

Milk thistle is the dried ripe fruit of *Silybum marianum* (L.) Gaertneter, a biennial herb native to Europe. Milk thistle contains flavonolignans silybinin, silydianin, and silychristin, with silybin being the most biologically active and the most abundant (60-70%); other constituents include taxifolin and other flavonoids [4]. Traditionally, milk thistle has been used by nursing mothers as a galactogogue and some studies have demonstrated that the drug increases lactation in cows [5a] and women [5b]. Prolactin is a lactogenic hormone and recently Capasso *et al*. [5c] have demonstrated that an extract of milk thistle (Silimarin BIO-C®) increases prolactin levels in female rats. The aim of this study was to investigate the effect of a new extract of milk thistle (Silitidil) on prolactin levels in female rats. Galega, another herb used in traditional medicine to increase milk production [5d,6,7a,b], will be administered with Silitidil with the intention to increase further the serum prolactin levels. Treatment of animals for 14 days with Silitidil (25-200 mg/kg) significantly increased, in a dose dependent manner, the serum levels of prolactin (Figure 1A).

Serum prolactin levels were measured also in rats treated for 14 days with galega (200 mg/kg) and with Silitidil/galega (50-100 mg/kg) (Figure 1B). Galega given alone did not modify the basal levels of prolactin in the serum of female rats, while in association with Silitidil serum levels of prolactin were raised. However, no significant difference was observed between the prolactin levels of animals treated with galega and those treated with Silitidil/galega.

Bromocriptine (1 mg/kg, *per os*), a dopamine D_2 receptor agonist, significantly reduced the high serum prolactin levels induced by Silitidil (Figure 1C). Bromocriptine, *per se* did not modify the serum prolactin levels in the rats (prolactin ng/mL: control 21.0±0.6, BR 1 mg/kg 21.3±1; n=6).

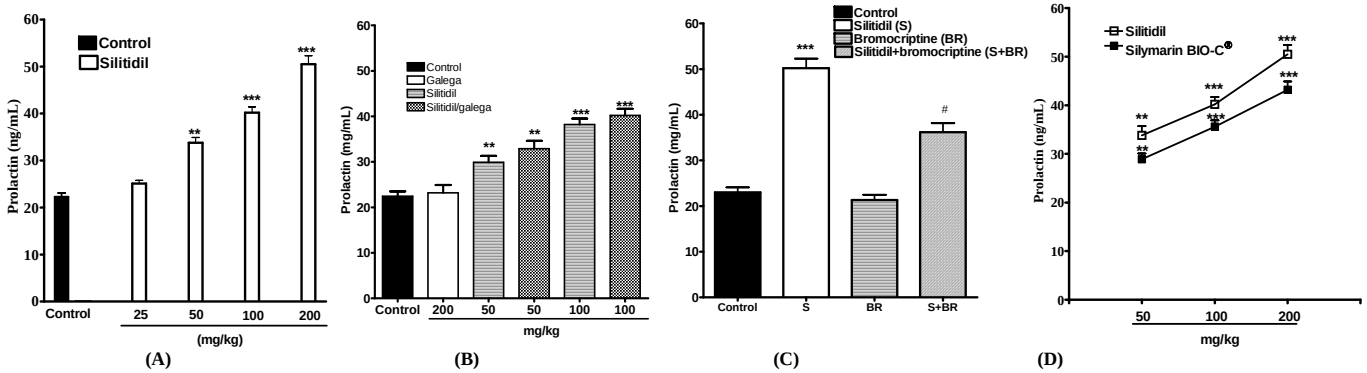

Figure 1: (A) Prolactin serum concentrations in control female rats and in rats receiving Silitidil (25-200 mg/kg, *per os*) for 14 days. Results are expressed as mean±sem of 8-10 animals. ***p*<0.01, ****p*<0.001 vs control; (B) Prolactin serum concentrations in control female rats and in rats receiving Silitidil (50-100 mg/kg, *per os*), Silitidil/galega (50-100 mg/kg, *per os*) and galega (200 mg/kg, *per os*) for 14 days. Results are expressed as mean±sem of 8-10 animals. ***p*<0.01, ****p*<0.001 vs control; (C) Prolactin serum concentrations in control female rats and in rats receiving Silitidil (S, 200 mg/kg, *per os*), bromocriptine (BR, 1 mg/kg, *per os*) and Silitidil (200 mg/kg, *per os*) plus bromocriptine (BR, 1 mg/kg, *per os*) for 14 days. Results are expressed as mean±sem of 6-8 animals. ***p<0.001 vs control; $^{\#}p$<0.01 vs S alone; (D) Prolactin serum concentrations in female rats and in rats receiving either Silitidil (50-200 mg/kg, *per os*) or Silimarin BIO-C® (50-200 mg/kg, *per os*) for 14 days. Results are expressed as mean±sem of 8-10 animals. ***p*<0.01, ****p*<0.001 vs control (22.5±1.1 ng/mL).

The major application of milk thistle is to act as a liver protector [7c,d]. However, in recent years, this herb has been reported to have a galactogogue effect [2c,3,5b,d,7e,8]. The active compound in milk thistle is silymarin, a mixture of four flavonolignans: silybin (60-70%), silycrisin (20%), silydianin (10%) isosilybin (5%) and their isomers. The flavonolignans possess a steroid-like structure which might explain their ability to protect the liver through a stimulation of prolactin synthesis. They also could act on estrogen receptors by limiting the endogenous receptors antagonism of milk production. The present results allow us to theorize that milk thistle galactogogue effects may be secondary to an increase in prolactin levels, as seen in female rats. However, the effect of milk thistle found in this study is consistent with a previously published report [5c], although the effect of the novel formula (Silitidil) is greater than that of the previous one (Silimarin Bio-C®) (Figure 1D). Galega is another herb widely used as a galactogogue [5d,6], but, in our studies, galega was unable to increase prolactin levels and to potentiate the effect of Silitidil.

Experimental

Drug and animals: Silitidil, silitidil plus galega and galega were kindly supplied by Milte Italia SpA (Milan, Italy). Silitidil is a standardized extract (consisted of 33% silymarin, 33% lecithin and 33% phosphatidylserine; galega is a dried aqueous extract of *Galega officinalis* [flowering tops s.e. E/D: 1/4 (maltodextrin). Bromocriptine mesylate salt was purchased from Sigma (Milan, Italy). Silitidil, silitidil plus galega and galega were dissolved in water; bromocriptine was suspended in 0.5% sodium carboxymethylcellulose. Wistar female four-days cycling rats (12–15 weeks old) weighting 220–240 g were used (Harlan, Italy). They were caged individually, isolated from males, and maintained in a room with a light/dark period of 12L: 12D, at a temperature of 23±2°C and a relative humidity of 50±2%. Animals had free access to standard food, purchased from Mucedola Mangimi (Settimo Milanese, Italy) and water *ad libitum*. Rats were used after 1 week of acclimation and all experiments complied with the Italian D.L. no. 116 of 27 January 1992 and associated guidelines in the European communities Council Directive of 24th November 1986 (86/609/ECC).

Estrous cycle induction and evaluation: The synchronization of the estrous cycle in sexually mature rats and the stage of the cycle were determined as previously described [5c].

Drug administration and serum preparation: After estrous-guided selection, female rats were treated with Silitidil at doses of 25–200 mg/kg (orally). Vehicles (water) and Silitidil were administered daily for 14 days. In some experiments, animals were randomly divided into 4 groups as follows: group 1 was treated with vehicles; group 2 with white galega 200 mg/kg; group 3 with Silitidil at doses of 50 and 100 mg/kg; and group 4 with Silitidil/galega at doses of 50 and 100 mg/kg. In another set of experiments the animals were randomly divided into 3 groups as follows: group 1 was treated with vehicles; group 2 with Silitidil (200 mg/kg, *os* for 14 days); group 3 with bromocriptine (1 mg/kg, *os*, on day 14); and group 4 with Silitidil (200 mg/kg, *os* for 14 days) plus bromocriptine (1 mg/kg, *os*, on day 14, 3 h before the collection of blood). After nitrous oxide/isoflurane anesthesia, blood samples were collected from rats at the beginning and end of treatment (corresponding to days 1 and 14). For the experiment with bromocriptine alone, the blood samples were collected on day 1 (before treatment with bomocriptine) and on day 2 (3 h after the administration of bromocriptine). Briefly, through intracardiac injection, blood (about 500 μL) was transferred into sterile 2 mL plain centrifuge tubes and allowed to clot at 4°C for 1 h. Subsequently, blood samples were centrifuged at 1200 *g* for 5 min at 4°C. Supernatants still containing platelets and white/red blood cells, were transferred into new sterile 2 mL plain centrifuge tubes and subjected to a second centrifugation at 1200 *g* for 5 min a 4°C. The supernatants (serum) were collected and stored at −80°C until use.

Serum rat prolactin determination: Serum prolactin levels were measured using an EIA kit (Spi-Bio Bertin Group, France). This colorimetric assay is based on the competition between serum rat prolactin and acetylcholine linked to rat prolactin for limited specific rabbit anti-rat prolactin antiserum sites [7b]. Results are expressed as ng of prolactin *per* mL of rat serum.

Statistical analysis: Results are expressed as mean±S.E.M. of *n* experiments. The analyses of results were carried out using GraphPad Prism Software (San Diego, USA). Comparisons between two sets of data were made by Student's test for paired data. When multiple comparisons against a single control were made, one-way analyses of variance (ANOVA) was used, followed by Turkey–Kramer multiple comparisons test. A *p* value less than 0.05 was considered significant.

References

[1] (a) Hoddinott P, Tappin D, Wright C. (**2008**) Breast feeding. *British Medical Journal*, ***336***, 881-887; (b) Eidelman AI. (**2012**) Breastfeeding and the use of human milk: an analysis of the American Academy of Pediatrics 2012 Breastfeeding Policy Statement. *Breastfeeding Medicine*, ***7***, 323-324.

[2] (a) World Health Organization, (**2003**) *Global Strategy for Infant and Young Child Feeding.* Geneva, Switzerland; (b) Forinash AB, Yancey AM, Barnes KN, Myles TD. (**2012**) The use of galactogogues in the breastfeeding mother. *Annals of Pharmacotherapy*, ***46***, 1392-1404.

[3] Zapantis A, Steinberg JG, Schilit L. (**2012**) Use of herbals as galactagogues. *Journal of Pharmacy Practice*, ***25***, 222-231.

[4] Abenavoli L, Capasso R, Milic N, Capasso F. (**2010**) Milk thistle in liver diseases: past, present, future. *Phytotherapy Research*, ***24***, 1423-1432.

[5] (a) Tedesco D, Tava A, Galletti S, Tameni M, Varisco G, Costa A, Steidler S. (**2004**) Effects of silymarin, a natural hepatoprotector, in periparturient dairy cows. *Journal Dairy Science*, ***87***, 2239-2247; (b) Carotenuto D, Di Pierro F. (**2005**) Studio sulla tollerabilità ed efficacia della silimarina BIO-C® (Più latte®) micronizzata come galattagogo. *Acta Neonatology Pediatric*, ***4***, 393–400; (c) Capasso R, Aviello G, Capasso F, Savino F, Izzo AA, Lembo F, Borrelli F. (**2009**) Silymarin BIO-C®, an extract from *Silybum marianum* fruits, induces hyperprolactinemia in intact female rats. *Phytomedicine*, ***16***, 839-844; (d) Zuppa AA, Sindico P, Orchi C, Carducci C, Cardiello V, Romagnoli C. (**2010**) Safety and efficacy of galactogogues: substances that induce, maintain and increase breast milk production. *Journal of Pharmacy and Pharmaceutical Sciences*, ***13***, 162-174.

[6] Mortel M, Mehta SD. (**2013**) Systematic review of the efficacy of herbal galactogogues. *Journal of Human Lactation*, ***29***, 154-162.

[7] (a) Sim TF, Sherriff J, Hattingh HL, Parsons R, Tee LB. (**2013**) The use of herbal medicines during breastfeeding: a population-based survey in Western Australia. *BMC Complementary and Alternative Medicine*, ***13***, 317; (b) Duhau L, Grassi J, Grouselle D, Enjalbert A, Grognet JM. (**1991**) An enzyme immunoassay for rat prolactin: application to the determination of plasma levels. *Journal of Immunoassay*, ***12***, 233–250; (c) Giese LA. (**2001**) Milk thistle and the treatment of hepatitis. *Gastroenterology Nursing*, ***24***, 95–97; (d) Capasso F, Gaginella TS, Izzo AA, Grandolini G. (**2003**) *Phytotherapy. A quick reference to herbal medicine*. Springer, Berlin and Heidelberg; (e) Vojtísek B, Hronová B, Hamrík J, Janková B. (**1991**) [Milk thistle (*Silybum marianum*, L., Gaertn.) in the feed of ketotic cows]. *Veterinary Medicine* (Praha), ***36***, 321-330.

[8] Tedesco D, Domeneghini C, Sciannimanico D, Tameni M, Steidler S, Galletti S. (**2004**) Silymarin, a possible hepatoprotector in dairy cows: biochemical and histological observations. *Journal of Veterinary Medicine. A, Physiology, Pathology, Clinical Medicine*, ***51***, 85-89.