

Evaluation of galactagogue activity of lactovedic: A polyherbal formulation

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Lactovedic is a lactogenic polyherbal formulation containing Jivanti, Shatavari, Vidarikanda, Yashtimadhu and Shatapushpa, and is processed with swarasas of Brahmi, Mandukaparni, Matsyakshi, Shatavari and Kokilaksha. The aim of this study was to evaluate the galactagogue activity of lactovedic. Rats (175–200 g) suckling eight to nine pups were divided into four groups ($n=6$). Control group rats were treated with vehicle (2 ml of 1% carboxymethyl cellulose sodium in normal saline) orally, Group II and Group III rats were orally administered 270 and 540 mg/kg body weight, respectively, of lactovedic suspended in vehicle, and Group IV animals were treated orally with 2.7 mg/kg body weight of domperidone suspended in vehicle from 3rd day of parturition to 15th day of parturition. Milk yield at 18 hours, the weight of pups at 18 and 23 hours and the daily weight of the mother rat were estimated. On 16th day, blood samples were collected and mother rats were sacrificed. Glycogen and total protein content in mammary gland and serum prolactin and cortisol were estimated. Results were statistically analysed using analysis of variance (ANOVA), followed by Tukey-Kramer *post hoc* test. Histopathology of mammary gland was performed. Lactovedic increases the milk yield, pups' body weight, weight of the mother rat, glycogen and protein content of mammary gland tissue, and serum prolactin and cortisol, compared to the control animals. Transverse section of mammary gland of lactovedic treated rats showed proliferation of acini and marked increase in milk secretion in the ducts. It can be concluded that lactovedic possesses significant galactagogue activity.

Key words: Cortisol, galactagogue, histopathology of mammary gland, lactovedic, milk yield, prolactin, pups weight

INTRODUCTION

Mother's milk is important for survival, proper development and growth of the neonate. Milk is the only source of water, organic nutrients and minerals, to which the neonates have access. Colostrum (the first milk taken from the mammary gland after parturition) and mature milk contain non-nutrient substances (such as antibodies and bioactive factors) that are important for growth, development and survival of the neonate. Low supply of milk is one of the most common reasons given for discontinuing breast feeding. Galactagogues are medications or substances to assist initiation, maintenance and augmentation of maternal milk production.^[1] Ayurvedic medicines based on ancient scripts like Sushruta Samhitha, Charaka Samhitha, etc., are herbal formulations. Some of the herbal drugs having galactagogue activity are *Leptadenia reticulata*,^[2] *Asparagus racemosus*,^[3,4] *Ipomoea digitata*,^[5] *Glycyrrhiza glabra*,^[5,6] *Centella asiatica*,^[6]

Bacopa monnieri,^[7] *Anethum sowa*,^[7] etc. Vedic Bio Labs, Bangalore, has developed lactovedic 500 mg capsule, a natural milk enhancer which contains extracts of Jivanti, Shatavari, Vidarikanda, Yashtimadhu and Shatapushpa. The chemical constituents and medicinal uses of these plants are given in Table 1. The present study was taken up to generate preclinical data to support the clinical use and hence standardisation of lactovedic as a galactagogue. The galactagogue activity of lactovedic was evaluated by estimation of milk yield, pups' body weight, weight of the mother rat, glycogen and protein content in mammary gland tissue, and estimation of serum prolactin and cortisol.

MATERIALS AND METHODS

Animals

Wistar female rats weighing 175–200 g, procured from Drug Testing Laboratory, Bangalore, were maintained under standard laboratory conditions (25±2°C and RH 60±5%) with free access to food and water *ad libitum*. Our institute's animal house registration no. is 152/99/CPCSEA/1999. Rats were divided into four groups of six animals each. Control group rats were treated with vehicle (2 ml of 1% carboxymethyl cellulose sodium in normal saline) orally, Group II and Group III rats were orally administered 270 and 540 mg/kg body weight, respectively, of lactovedic suspended in vehicle, and Group IV animals were treated orally with 2.7 mg/

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Table 1: Chemical constituents and medicinal uses of plants in polyherbal formulation lactovedic

Name of constituent	Botanical name	Family	Part of plant used	Chemical constituents	Medicinal uses
Jivanti ^[2]	Leptadenia reticulata	Asclpiadaceae	Roots	Leptadenol, triacontane, cetyl alcohol, leptidin1, -sitosterol	Galactagogue, nutritive, aphrodisiac, stimulant, diuretic
Shatavari ^[3,4]	Asparagus acemosus	Liliaceae	Roots	Shatavarin I-IV, quercetin, rutin, hyperoside	Galactagogue, aphrodisiac, demulsant
Vidarikanda ^[5]	Ipomoea digitata	Convolvulaceae	Tuberous root	Pterocarpanone, hydroxytuberosone, pterocarpene-anhydrotuberosine, 3-O methyltuberosin	Galactagogue, antitubercular, in hepatomegaly and splenomegaly
Yastimadhu ^[5,6]	Glycyrrhiza glabra	Leguminosae	Radix	Glycyrrhizin, liquiritin, stigmasterol, β -sitosterol, glycythetic acid, anethole, eugenol, etc.	Expectorant, demulcent, anti-inflammatory, diuretic, hepatoprotective, anti-spasmodic
Shatapushpa ^[7]	Anethum sowa	Umbelliferae	Flower head	Anethole, estragole, fenchone, β -sitosterol, etc.	Carminative, galactagogue, aromatic, diuretic, anti-spasmodic, expectorant, anti-tussive

kg body weight of domperidone suspended in vehicle, from 3rd day of parturition to 15th day of parturition. The dose of lactovedic was calculated from human dose (as given in the ancient scripts). Institutional Animal Ethics Committee's permission was obtained before starting the experiments.

Milk Yield and Pups' Body Weight

Every day during the study period, 13 hours after the treatment of mother rat, the pups were weighed (w1) and subsequently isolated from their mother for 4 hours. At the end of 4 hours, the pups were weighed (w2), returned to their mother and allowed to feed for 1 hour and were weighed (w3) again. Subsequently, pups were isolated from their mother for 4 hours and then weighed (w4), following which they were reunited with their mother for 1 hour of feeding and, finally, they were weighed (w5). They were subsequently left with their mother during the night. Milk yield at 18 and 23 hours after the gavage was estimated as (w3 – w2) and (w5 – w4), respectively. Daily milk yield at 18 hours was corrected for weight loss due to metabolic processes in the pup (respiration, urination and defaecation) during suckling. The value used was (w2 – w1)/4. This value was multiplied by the number of suckling hours per day and added to the daily suckling gain. Daily weight gain of pups was calculated from the pup weight at w2.^[8]

Weight of the Mother Rat

Daily weight of mother rat was taken and the difference of weight between 3rd and 15th day after parturition was determined.

Estimation of the Glycogen and Protein Content Mammary Gland Tissue

On 16th day, blood samples were collected and mother rats were sacrificed. Mammary gland was isolated and

cleared from the other connective tissue and weighed accurately using electronic balance. About 100 mg of tissue was homogenised in 1.5 ml of distilled water using tissue homogeniser (Remi Motors RQ127A, Mumbai, India), mixed with 1.5 ml of 30% KOH saturated with Na₂SO₄ and heated for 30 minutes in boiling water bath, cooled and centrifuged (Remi C24) after adding 2 ml of 95% ethanol. The precipitated glycogen was collected from the alkaline digestate, dissolved in distilled water and estimated by phenol-sulphuric acid method.^[9]

Total protein content^[10] was estimated using total protein kit (Span Diagnostic Ltd., Bangalore, India).

Serum Prolactin and Cortisol Estimation

On 16th day after parturition, the blood was collected from retro-orbital sinus, serum was separated, the prolactin and cortisol were estimated by using electrochemiluminescence (Rat Prolactin ELISA kit, Catalog Number RPRL.96 of MD biosciences, USA) and enzyme immunoassay (Cortisol EIA kit, Cayman chemicals, Mumbai, India), respectively.^[11]

Histopathology of Mammary Gland

On 16th day, mother rats were sacrificed, mammary gland was isolated and cleared from the other connective tissue and processed for histopathological studies. The histological sections were projected on a white glazed paper through a projection microscope; the parenchyma area was counted against the mammary stroma. The microslides of mammary gland were examined using the projection microscope at 2500 \times magnification for scoring the secretory rating.

Statistics

The data were presented as mean \pm SEM. Results were statistically analysed using analysis of variance (ANOVA),

followed by Tukey-Kramer *post hoc* test, using Graph Pad InStat version 3.00, Graph Pad Software, CA, USA. The level of significance was set at $P < 0.05$

RESULTS AND DISCUSSION

As shown in Table 2, lactovedic significantly increases milk yield, pups' body weight, mother rat's weight, serum prolactin and cortisol compared to the control animals. During the lactating period, the mean milk yield at 18 and 23 hours was increased [Figure 1]. Lactovedic increases protein content, glycogen, parenchyma percentage and secretary rating of mammary gland tissue [Table 3].

As shown in Figure 2, transverse section (TS) of mammary gland of lactovedic treated rats showed proliferation of acini and marked increase in milk secretion in the ducts (back to back arrangement of cells, indicating increased secretory rate), as compared to normal control and standard

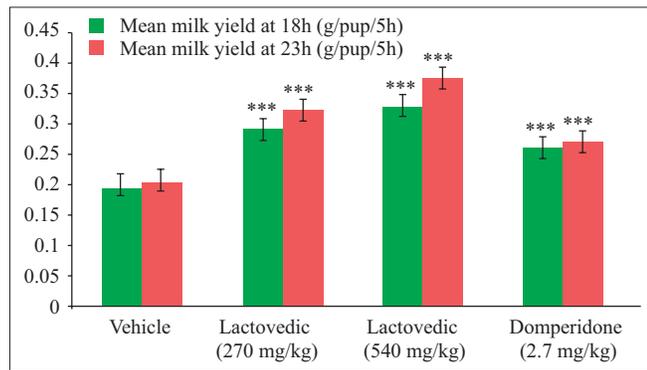


Figure 1: Effect of lactovedic on mean milk yield at 18 and 23 hours; values are expressed as mean±SEM (n=6); ANOVA; Tukey-Kramer *post hoc* test; *** $P < 0.001$

domperidone treated animals.

One of the many reasons for discontinuing breast feeding is low supply of milk in lactating mothers. Lactovedic is an Ayurvedic polyherbal galactagogue, which contains Jivanti, Shatavari, Vidarikanda, Yashtimadhu and Shatapushpa, and is processed with swarasas of Brahmi, Mandukaparni, Matsyakshi, Shatavari and Kokilaksha. Some of the medicinal plants identified as lactogenic stimulate the synthesis of lactogenic hormones (prolactin, growth hormone, cortisol) and/or β -endorphin and β -casein accumulation in the mammary gland.^[12] After parturition, prolactin stimulates the synthesis of milk proteins in the epithelial cells and proliferation of secretory cells.^[8,13] Our studies indicate that lactovedic increases serum prolactin, protein content and

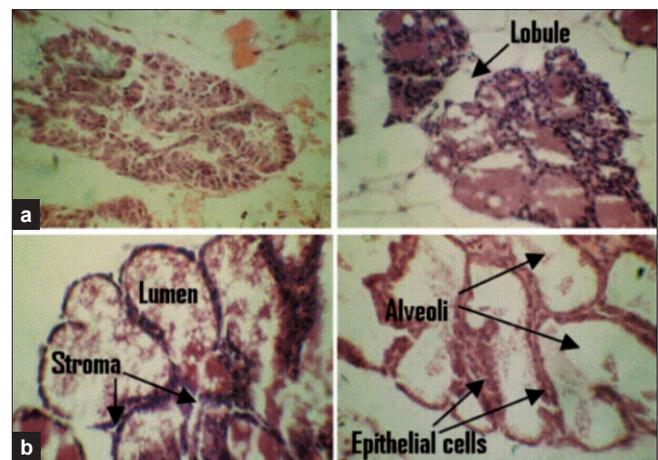


Figure 2: Effect of lactovedic on mammary gland; (a) TS of mammary gland of control rat, showing closely packed lobuloalveolar tissue; (b) TS of mammary gland of lactovedic treated rat, showing proliferation of parenchyma, acini and enhanced milk secretion in alveoli

Table 2: Effect of lactovedic on milk secretion in rat

Treatment	Milk yield (g/pup/day)	Pups body wt. (g/pup/day)	Serum prolactin (ng/ml)	Serum cortisol (ng/ml)	% Difference of mother rat wt (g)
Vehicle 1 ml	2.514±0.078	7.698±0.229	31.49±1.775	15.23±1.322	5.301±0.201
Lactovedic 270 mg/kg	4.993±0.101***	11.67±0.456***	68.77±2.791***	28.35±2.318**	8.521±0.155***
Lactovedic 540 mg/kg	5.520±0.098***	14.34±0.848***	84.54±3.765***	41.93±3.083***	10.18±0.185***
Domperidone 2.7 mg/kg	4.186±0.173***	10.68±0.430**	54.27±2.396***	23.27±1.849	7.521±0.098***
	F=122.84	F=25.849	F=72.374	F=25.084	F=153.37

Values are expressed as mean±SEM (n=6), ANOVA, Tukey-Kramer *post hoc* test; *** $P < 0.001$; ** $P < 0.01$

Table 3: Effect of lactovedic on mammary gland of rat

Treatment	Total protein in rat mammary gland (mg/100 mg)	Glycogen in rat mammary gland (µg/100 mg)	Parenchyma % of mammary gland tissue	Mammary tissue secretary rating
Vehicle 1 ml	8.533±0.194	7.283±0.568	40.36±1.341	1.917±0.16
Lactovedic 270 mg/kg	15.67±0.223***	13.38±0.916***	50.32±1.115***	3.033±0.271**
Lactovedic 540 mg/kg	17.78±0.473***	16.5±1.047***	62.73±1.651***	4.066±0.166***
Domperidone 2.7 mg/kg	11.38±0.351***	9.75±0.815	48.302±1.445**	2.916±0.256
	F=159.99	F=22.484	F=43.684	F=16.035

Values are expressed as mean±SEM (n=6), ANOVA, Tukey-Kramer *post hoc* test; *** $P < 0.001$; ** $P < 0.01$

glycogen of mammary gland. Histopathological studies reveal that lactovedic increases proliferation of acini, lobuloalveolar size, parenchyma percentage and milk secretion in the ducts. The measurement of milk yield in lactating rats is done indirectly by means of pup weight. The increase in milk yield in our studies is due to the increased serum prolactin, which stimulated the biosynthesis of milk.^[14,15]

CONCLUSION

To conclude, it can be said that lactovedic, a polyherbal formulation, possesses significant galactagogue activity.

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