



Evaluation of the Lactogenic activity of *Erythrina indica* Lam. leaves

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Erythrina indica Lam. is used as a galactagogue in folklore medicine. But no report is available to validate its herbal galactagogue activity in literature. So, this *in vivo* study was designed to assess the galactagogue activity of juice of *Erythrina indica* tender leaves. The milk production was assessed by parameters including the milk yield, weight gain of mother rats and pups during the experimental period, serum cortisol and prolactin level, glycogen and protein content of mammary gland also were measured and compared with experimental group and respective control group. To estimate the milk yield, the weights of the pups were taken before and after 60 min of drug administration. On oral administration of the *Erythrina indica* tender leaf juice, milk yield and prolactin level were statistically significant. The other parameters like body weight of mother and pups, glycogen, and protein level were also found to be clinically significant and statistically not significant and the cortisol level is not significantly decreased. This study proves the galactagogue activity of *Erythrina indica*, by increasing the milk yield, and serum prolactin level in the lactating rats.

Keywords: *Erythrina indica*, Galactagogue activity, Lactogenic, Milk production, Paribhadra

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Milk is the primary source of nutrition for newborns. The milk produced or secreted just after the child's birth is Colostrum, which is also known as 'liquid gold', not only due to its yellow orangey color but also due to its importance in nourishing and protecting the newborn. Colostrum has a very high concentration of IgG, lacto albumin, lactoferrin, growth factor and lacto protein which conform to passive immunity to the newborn. Colostrum has a laxative effect which helps the infants to pass the early stools aiding in the excretion of excess bilirubin¹. After the 3rd or 4th day of childbirth, breast milk is secreted and is the most valuable gift given by a mother to her offspring, as it imparts positive benefits in terms of physical and psychological health aspects. Breast milk is considered to be healthier than any other milk that is obtained from animals. Feeding the baby with breast milk is recommended since it provides the required nutritional benefits and, the natural antibodies in it help the infant to fight against infections and boost up their immune system. Galactagogues are medications or substances that assist the initiation, maintenance and augmentation of

maternal milk production. Many galactagogues like metoclopramide, domperidone, chlorpromazine, sulphiride, etc. are available in the modern system of medicine. Among these domperidone is used widely since it does not cross the blood-brain barrier². But based on the side effect and toxicity of these drugs, people are now turning towards alternative herbal galactagogues for overcoming the situation.

According to our Acharyas, *stanyakṣaya* occurs due to psychological factors of the mother like *Krōdha*, *Śōka*, *Bhaya*, etc., diet regimen of mother and physiological condition. *Stanyakṣaya* is a major problem with increasing prevalence ranging from 23-63% in Western countries and 45-53% in some parts of India³. It is a great irony that such an important problem that is directly associated with the health of mother and child is gradually neglected.

In our classics, a large number of drugs, diet and behavior regimens are described as *stanyajanana* and are useful in the management of *stanyakṣaya*. *Stanya Janaka* or *stanyaśōdhaka* drugs like *Vīrana*, *Śāli*, *Śaṣṭika*, etc. possesses properties like *madhura rasa*, *Snigdha*guna, *sītavīrya*, *madhuravipāka* and are used in different formulations. In folklore practice, the plants like *Moringa oleifera* Linn, *Hibiscus sabdariffa*

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L., *Pimpinella anisum* L., and *Erythrina indica* Lam. are given to mothers after childbirth and is found to be beneficial in producing milk. In Kerala and Karnataka, the stem bark and leaf paste of *Erythrina indica* is used to increase milk production⁴. In Kerala, the tender leaves of *Erythrina indica* were cooked with rice gruel and given to postpartum women for facilitating lactation⁵. In Karnataka, folklore practitioners use the paste of tender leaf and bark of *Erythrina indica* as a galactagogue⁶. Although researchers are going on to prove the galactagogue action of herbal drugs, no scientific study has been done to evaluate the galactagogue action of *Erythrina indica*, which is known by the name Paribhadra in Sanskrit. Here, an *in vivo* study is designed to prove the galactagogue activity of tender leaves of *Erythrina indica* Lam to use it as a drug of choice to increase breast milk production.

Materials and Methods

Method of sample collection

Taxonomically identified fresh leaves of *Erythrina indica* (500 g approximately) were collected in April 2018 from S D M Ayurveda College Udupi, Herbal Garden. The required amount of the collected leaf was cleaned thoroughly and made into *swarasa* for phytochemical evaluation and the remaining portion was dried and made into fine powder form (80 mesh sieve) for physicochemical evaluation. For the experimental study, the drug was collected freshly on the day itself.

Preparation of medicine- Juice of *Erythrina indica* leaf

Erythrina indica leaf *swarasa* is prepared based on classical reference. Fresh leaves (100 g) were triturated to a fine paste with a stone motor. It was then taken in a Cora cloth and squeezed by hand to get the juice.

Animal selection criteria & rat maintenance

Healthy female Wistar albino rats (30 in number) of approximately 250-300 g were selected from the animal house attached to the department of pharmacology, SDM Centre for Research in *Āyurvēda* and Allied Sciences, Udupi. The animals were housed in standard laboratory conditions of light and dark cycle of 7 am to 7 pm, temperature of 25°C and 30%-60% relative humidity in well-ventilated polypropylene cages. Animals were provided with normal mouse chow (Sai Durga food and feeds, Bangalore, India) and water and libitum. The animals

were randomly selected, marked with the help of picric acid to permit individual identification as head, neck, body, forelimb, hind limb and tail and kept for breeding for 21 days. The doses started from the 5th day of parturition. The experiment was conducted after obtaining permission from the institutional ethics committee by the guideline formulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals. (Approval number –SDMCRA/IAEC/AM-DG-21)

Milk production effect by oral intake of *Erythrina indica*

Thirty healthy female rats were selected from animal house, were housed, and kept breeding for 21 days, From the first day of breeding the weight of all the female rats were noted. The weight was noted to confirm the pregnancy. After 21 days, the female rats were separated from males and kept in a single cage for delivery⁷. The delivery day was considered the first day of lactation. All the lactating rats were randomly divided into five groups containing six rats in each group (n=6). Each mother was adjusted to have 6 pups. All the three trial groups were treated with test drug *Erythrina indica* tender leaf juice orally at 2.16, 4.32 & 6.48mL/kg body weight for 10 days of lactation starting from the 5th day of lactation. Group four, which served as normal control, received regular rat food and drinking water ad libitum for 10 days. Group five was kept as standard and was administered with domperidone (2.5 mg/kg body wt.) for 10 days. *Erythrina indica* and domperidone were suspended in a suitable vehicle and administered daily using an animal gavage needle to lactating mothers at 8.00 am starting from the 5th to 14th day of the lactation period. The milk yield was measured daily after 12 h. of drug administration. The weights of the pups were noted just before the drug administration and also after 60 min of drug administration to calculate milk yield. The weight changes of the pups were considered as the amount of yield on that day. The milk yield and weight changes of littermates as well as mother rats during the medication period were measured and compared with the experimental group and their respective non-treated control group. All the measurement of weight was read with an accuracy of 0.1 g using an electronic balance⁷.

Evaluation of serum prolactin and cortisol level

On the 10th day of medication, the blood samples of all the rats were collected. Blood was collected

from retro-orbital plexuses of the rat, after giving anesthesia using diethyl ether. The blood was collected by retro-orbital sinus puncture into a sterilized plane tube with clot activator and kept for centrifuge at 2000 rpm for 8 min. After centrifugation, the serum was separated and collected for analyzing parameters like prolactin and cortisol. Using enzyme immunoassay, the estimation was done⁷.

Evaluation of glycogen and protein content of mammary gland tissue

On 10th day of medication, the blood was collected from the orbital plexus and the mother rats were euthanized for the excision of whole mammary glands. About 0.1 g of mammary tissue was homogenized in distilled water using a tissue homogenizer and 30% saturated potassium hydroxide. Then the mixture was incubated for half an hour at 65°C. The resulting homogenate was sent to the lab for quantitative estimation of protein and glycogen. For glycogen assessment, 2 mL of 95% ethanol was added to mammary homogenate and centrifuged. The accelerated glycogen was collected from the alkaline digest, dissolved in distilled water, and evaluated by the phenol sulfuric acid method. Total protein assessment was performed using a total protein kit⁷.

Statistical analysis

All the values were expressed as MEAN±SEM. Analysis of the data was done using one-way ANOVA followed by Dunnet's multiple 't' test as post HOC test is performed using GraphPad In Stat version 3 software and p<0.05 was considered as statistically significant.

Results

Effect of *Erythrina indica* on milk yield

The non-treated control group, standard, and all the three *Erythrina indica* trial groups were measured daily to estimate milk production (Table 1). The observation shows that there was a significant increase (p<0.001) of milk production in *Erythrina indica* trial group as compared to the non-treated control. *Erythrina indica* treated groups also showed dose-dependent changes of total milk yield between trial period. During this lactation period, as compared to the non-treated control group, total milk yield was the highest in *Erythrina indica* juice trial 3 (6.48 mL/kg) group. The juice of *Erythrina indica* at 2.16, 4.32 and 6.48 mL/kg and domperidone (2.5 mg/kg) as a standard group produced

303.93%, 349.34%, 403.93% and 60.04% augmentation of milk yield, respectively, as compared to non-treated control. The percentage of milk production during the trial period was found significant at p<0.01 in *Erythrina indica* juice trial groups.

Effect on weight gain of pups during the lactation period

The difference in weight gain of 5 groups were observed (Table 2 and 3). By comparison with the control group, there was increased weight gain in the domperidone-induced standard group and all three trial groups. And the observed change was found to be clinically significant but not statistically significant.

Effect of *Erythrina indica* on body weight of mother rat

As compared to the control group, *Erythrina indica* trial group has not shown any significant increase in weight gain of a lactating mother (Table 4-5). The observation shows that there was a slight enlargement in the size of the mammary gland in *Erythrina indica* trial group as compared to the control group.

Table 1 — Total milk yield during experimental period

Group	Total Milk yield during study period (g)	% change
Control	9.16±1.55	
Standard	14.66±2.51	60.04 ↑
Trial 1	37±6.26**	303.93 ↑↑
Trial 2	41.66±3.997**	349.34 ↑
Trial 3	46.16±3.84**	403.93 ↑

Data= MEAN±SEM

p<0.01, *p<0.01, ***p<0.01 compared with untreated control group

Table 2 — Effect of *Erythrina indica* on body weight of pups on 5th day

Group	Pups body wt.(g) on 5 th day	% change
Control	16.09±1.16	
Standard	19.01±0.81	18.14 ↑↑
Trial 1	15.99±1.229	0.62 ↓↓↓
Trial 2	16.1±0.72	0.06 ↑
Trial 3	16.19±0.41	0.62 ↑

Table 3 — Effect of *Erythrina indica* on body weight of pups on 15th day

Group	pups body wt.(g) on 15 th day	%change
Control	22.42±2.05	
Standard	26.46±2.62	18.01 ↑
Trial 1	22.77±1.64	1.56 ↑
Trial 2	24.08±0.89	7.40 ↑
Trial 3	23.37±0.71	4.23 ↑

Effect on cortisol level and serum prolactin

The experimental study showed that the prolactin level increased more than two fold in the trial 2 groups (4.32 mL/kg). There were no significant decreases in the standard group, trial 1 and trial 3 group, with the comparison of untreated control. The percentage of prolactin level on the 15th day of lactation was found significant at $p < 0.01$ in *Erythrina indica* juice trial 2 groups, but the cortisol level was not significantly decreased in all three trial groups and standard groups (Table 6-7).

Effect of *Erythrina indica* leaf juice on protein and glycogen level

The result of protein and glycogen content of mammary gland shows no significant increase in all three trial groups and standard group with the comparison of non-treated control groups Table 8 and 9. The total protein content was increased in all three trial groups and found high in the trial 3 group (6.48 mL/g). Similarly, glycogen results also showed high in medium dose (4.32 mL/kg) as compared to control groups

Table 4 — Effect of *Erythrina indica* on body weight of mothers on 5th day

Group	On 5 th day (g)	% change
Control	229.33±11.27	
Standard	225.33±10.14	1.74 ↓
Trial 1	226.16±7.12	1.38 ↓
Trial 2	237.35±9.690	3.49 ↑
Trial 3	247±11.16	7.70 ↑

Table 5 — Effect of *Erythrina indica* on body weight of mother's on 15th day

Group	on 15 th day (g)	% change
Control	228.33±10.37	
Standard	222.33±11.83	2.77 ↓
Trial 1	233.5±7.46	2.26 ↑
Trial 2	238.83±8.031	4.59 ↑
Trial 3	249±11.08	9.05 ↑

Table 6 — The Effect of *Erythrina indica* on prolactin level on 15th day of lactation

Group	Prolactin level on 15 th day (ng/mL)	% change
Control	0.045±0.014	
Standard	0.033±0.0071	25.00 ↓
Trial 1	0.03±0.0044	25.00 ↓
Trial 2	0.148±0.045**	250.00 ↓
Trial 3	0.02±7.57	50.00 ↓

Data —MEAN ±SEMns $p > 0.05$, p-value is 0.0017 considered very significant compared to untreated control /domperidone standard group

Discussion

Lactogenesis is a neuro-endocrine event of the complex neuro-physiological process that involves the interaction of several physical and emotional factors along with the action of multiple hormones, mainly prolactin and oxytocin. Lactogenesis occurs in two stages, the release of prolactin which acts on the glandular cells of the breast to stimulate milk secretion and the release of oxytocin which act on the myoepithelial cells of the breast to induce the milk ejection reflex⁸. Although the two mechanisms are similar it gets activated by suckling by the baby. The key event in lactogenesis is suckling and the sensitivity of the breast accommodates itself to this activity, dopamine agonists and antagonists regulate prolactin synthesis and secretion and thereby control milk production.

Table 7 — Effect of *Erythrina indica* on cortisol level on 15th day of lactation

Group	Cortisol level on 15 th day (ng/mL)	% change
control	0.46±0.056	
standard	0.286±0.082	39.13 ↓
Trial 1	0.40±0.064	13.04 ↓
Trial 2	0.4±0.051	13.04 ↓
Trial 3	0.45±0.058	2.17 ↓

Data MEAN±SEM ns $p > 0.05$, p-value is 0.3396 considered not significant compared to untreated control /domperidone standard group

Table 8 — Glycogen content of mammary gland (15th day) of lactation

Group	Glycogen level on 15 th day µg/mL	% change
Control	1.65±0.07	
Standard	1.78±0.25	7.87 ↑
Trial 1	1.71±0.13	3.63 ↑
Trial 2	1.92±0.13	16.36 ↑
Trial 3	1.84±0.14	11.51 ↑

Data- Mean±SEMns $p > 0.05$, p-value is 0.7725 considered not significant compared to untreated control /domperidone standard group

Table 9 — Protein content of mammary gland

Group	Protein level on 15 th day (mg/100mg)	% change
Control	719.6±62.685	
Standard	907±112.29	26.08 ↑
Trial 1	900.33±98.9	25.11 ↑
Trial 2	992.3±149.18	37.89 ↑
Trial 3	1002.6±116.12	39.32 ↑

Data -MEAN ±SEMns $p > 0.05$, p-value is 0.4074 considered not significant

Erythrina indica belongs to the Fabaceae family and is a tree with prickly stems. It is distributed throughout India. In Kerala, the tender leaves of *Erythrina indica* cooked with rice gruel and given to postpartum women for facilitating lactation. In Karnataka folklore practice, paste of *Pāribhadra* (*Erythrina indica* Lam.) tender leaves and bark were administered internally and externally and found to be effective in stimulating and increasing the breast milk secretion. In Karnataka, the plant is called as Hāluvāna by the local people, in Kannada, hālu means milk⁹. *Pāribhadra* is also known by the name *Sūthavriksha* and is *Dugdha Vardakas* in action. The textbook “Keralathile Vana Sasyangal’ by Dr. P.N. Nair had mentioned that *Pāribhadra* leaves cooked with coconut milk are used to increase milk production¹⁰. The present study was designed to investigate the galactagogue activity of tender leaves of *Pāribhadra* in an animal model.

The lactogenic activities depend upon the chemical constituents present in herbs. Previous studies reported that the presence of alkaloids, saponins, cardiac glycosides, flavonoids, tannins and steroidal ring increases the level of serum prolactin, the hormone that helps in milk secretion⁷. The presence of alkaloids, flavonoids, steroids, tannins, triterpenoids carbohydrates, saponins, phenols and resin in the tender leaves of *Erythrina indica* shows that these compounds may have influenced the growth and metabolism of the individual there by promoting the secretion of breast milk. The study on aqueous leaf extract of *Euphorbia hirta* showed that the consumption of plants rich in antioxidants, phenolic compounds, and flavonoids may have a beneficial effect on slowing the aging of alveolar cells of the mammary gland. It will help to increase the number of alveolar cells and the production of milk¹¹. The present study also shows that the presence of flavonoids and phenolic compounds. A clinical study of *Asparagus racemosus* wild showed that the presence of steroidal saponins and sapogenins is responsible for milk production¹². Phytochemical constituents of *Erythrina indica* also show the presence of saponins. Among the phytochemical constituents of *Erythrina indica*, the active principle which is responsible for the lactogenic activity is still unknown. The evaluation of phytochemical constituents of mature leaves showed the presence of alkaloids, phenols and flavonoids only.

In *Āyūrvēda*, *Stanya*, considered as *upadhāthu* of *Rasa dhāthu*, is a complete food that promotes the growth of the child. The affliction of *stanya* is

described by *Acharyas* as *stanyavikṛuti*, *stanyanāśa*, etc. The treatment modalities of these comprise the use of *Stanyajanana*, *Stanyavardhaka*, *Stanyōpaga* and *Stanyaśōdhaka* drugs. The drug *pāribhadra* has *tiktha*, *katurasa*, *laghu saraguna*, *ushnavīrya*, *katuvipāka*, due to *tiktha rasa* it pacifies pitta, helps in *rakthaprasādana* and it acts as *rōchaka*. Due to *katu rasa* and *vipāka*, it pacifies *Kapha*, it is *dīpanapāchana* in action, thereby preventing the production of *sāmarasa*. *Laghuguna* helps in easy digestion and *Saraguna* helps in *vātānulōmana* by preventing the *srōtāvarōdha*. Due to *ushnavīrya*, it acts as *vātakaphasāmaka* and helps in *pāchana karma stanyakṣhaya* is also caused by the *vātavidhi*, *apatarpana* and dhātu *kṣhayajavikāras*. The drug having *karma* like *agnidīpana*, *vātahara*, *rakthaprasādana* can be used in such cases to prevent *sthanyanaśa* due to *vātavidhi*.

In this study, the milk yield of trial groups was significantly increased than the untreated control. It was observed that there was an increased proliferation of cells of the mammary gland in all the trial drugs and has been considered as an indicator of their lactogenic action. The quantity of milk available during the suckling time is one of the most important factors for the growth development of pups. The result shows a significant increase in body weight of pups and mother rats as compared to control, indicating that the drugs are good health promoters too.

Prolactin is a protein hormone of the anterior pituitary gland that was originally named for its ability to promote lactation in response to the suckling stimulus¹³. The roots of *Triumfetta rhomboidea* L. also caused a very significant increase in serum prolactin which stimulates the development of the mammary gland⁷. In the present study, the Prolactin level is significantly increased in a medium dose of *Erythrina indica* by direct stimulation of the synthesis of milk protein in the epithelial cells and indirect stimulation of the proliferation of secretory cells¹⁴. Level of prolactin increase in the serum reflects the increase of glycogen in the mammary tissue, shows that the drugs have a positive effect in the development of the mammary gland and milk production. After parturition, the initiation and maintenance of lactation¹¹ are controlled by the prolactin secretion. Cortisol is a stress hormone, the present study showed that the cortisol level is decreased in the three trial groups and the standard group as compared to the control group and it indicates the drug has an influence in decreasing the stress factor and thereby increasing milk production.

The increase of glycogen content may be due to an increase in milk production during lactation, which results in an increase of bodyweight in pups as well as in lactating rats. Glycogen stimulates the lactogenic hormone Prolactin, which is increased by activation of the enzymes involved in glycogenesis.

Conclusion

In the experimental observation on Wistar albino rats, it has been observed that leaf juice of trial drug *Erythrina indica* Lam. affects milk production. The drug *Erythrina indica* is having extensive use in folklore practice and it was mentioned in Samhitas, contemporary text, and journals. *Stanyavardakas* drugs generally need to have *vātahara*, *agnidīpaka*, *rasa prasadaka* and *dhātuvardakas* properties. *Pāribhadra* appears to be more potent in *dīpana* and it is having *vātahara* property. The study shows a significant increase in milk yield and serum prolactin level as compared with control and a significant increase in body weight of mother and pups and protein and glycogen level in mammary tissue as compared with standard. The increase in the body weight of the mother shows that the drug also helps to promote the health of mother rats during the lactation period.

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Conflict of Interest

We declare that there is no conflict of interest.

Authors' Contributions

All authors conceived and designed the study. Data collection done by SC. SC conducted the experiments, analyzed the data, and wrote the paper. PPR has done the critical revision of the article. SP has done the grammatical and technical corrections. All authors approved the final version of the manuscript and agree to be held accountable for the content therein.

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