

Original Article

Comparative study of lactogenic activity reported on ethanolic extract of *Basella Alba* (Linn) leaf and root on lactating albino wistar rats

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ABSTRACT

In view of the traditional belief that the whole plant of *Basella alba* has been used to stimulate milk production in lactating women, experiments were performed to determine a comparative study on extract EBAR (Ethanolic *Basella alba* root) and EBAL (Ethanolic *Basella alba* leaves) on milk production in rats. Female lactating rats with suckling pups were orally administered with EBAL and EBAR extract and continued for 15th day of parturition. Milk yield, the body weight of pups and mother were measured daily. On 15th day, the total protein/glycogen contents from mammary tissue and serum prolactin/cortisol level from blood sample were measured and compared with control. Oral administration of EBAL and EBAR extract increases the milk yield, body weight of pups as well as mother rat. Along with glycogen and protein content as well as serum prolactin and cortisol level were also raised when compared to the control animal. In addition, the lactogenic effect of EBAL at dose (500 mg/kg, bw) was found to be higher as compared EBAR extract and control group. The present study revealed that the *Basella alba* leaf extract possesses significant lactogenic activity by enhancing milk production and prolactin concentration in nursing rats.

1. INTRODUCTION

Human breast milk is widely accepted to be the optimal source of nutrition for the new-born infant. Breastfeeding is very necessary for the survival of new-born's [1]. Many women with problems in milk production use traditional herbs to increase their milk production or yield. Some of the traditionally reported herbal galactagogue are *Foeniculum vulgare* Mill. *Urtica dioica* L., *Medicago sativa* L., *Anethum graveolens* L., *Galega officinalis* L., *Silybum marianum* (L.) Gaertn. *Withania somnifera* (L.) Dunal, *Asparagus racemosus* Willd. etc. [2- 4]. Breast milk production is a complex physiological process involving physical and emotional factors and the interaction of multiple hormones, the most important of which is believed to be prolactin [5]. Prolactin has important functions and roles in lactogenesis. Indeed, prolactin stimulates the production of milk proteins in

the epithelial cells and induces the proliferation of secretory tissue [6]. During interruption of lactation, galactagogues (or lactogogues) are used to assist initiation, maintenance or augmentation of milk production by induction of the lactogenic hormones (prolactin), growth hormone and casein accumulation in the mammary gland [7].

The main Indian traditional system of medicine namely Ayurveda and Siddha, are primarily plant based system and a rich source for various flora and fauna traditionally beneficial to human. In this regard, one such plant is *Basella alba* (Family: Basellaceae); is a perennial climber. It is also known as Malabar spinach, Indian spinach, Ceylon spinach and vine spinach. It is a fast-growing, soft-stemmed vine, reaching 10 m in length. Its thick, semi-succulent, heart-shaped leaves and green to reddish purple stem has a mild flavour and mucilaginous texture. The leaves

and stem part of the plant are used in cooking. It has been found to be a good source of calcium, iron, vitamin A and vitamin C [8]. It is used traditionally for treatment of hypertension by Nigerians in Lagos, and malaria in Cameroonian folk medicine [9]. In Ayurveda, it is used for haemorrhages, skin diseases, sexual weakness, ulcer and as laxative in children and pregnant women [10].

The pharmacological activity such as antibacterial and cytotoxic [11], analgesic and anti-inflammatory [12], anti-ulcer [13], antioxidant [14], nephroprotective [15], CNS depressant [16], androgenic [17] and wound healing [18] activities were reported on the various parts of the plant. Ethnobotanical studies on roots and leaves of *Basella alba* has been used for the removal of after birth stomach pains and increase milk production [19]. But no further studies have been reported on it. The present study is to reveal the galactagogue property by performing various experiments on experimental animal on the leaf and root extract of *Basella alba* plant.

2. EXPERIMENTAL

2.1 Preparation of plant material and extract

The leaves and roots of plant *Basella alba* were collected from the herbal garden of G. B Pant University, Pantnagar, Moradabad and was authenticated by Dr Atul Kumar, Head of Department, Plant Physiology, G. B Pant University, Moradabad (India). The fresh young leaves and roots of *B. alba* were collected; shade dried and then powdered using a mechanical grinder. The ethanol extract of the leaf and root of *B. alba* was obtained by soaking it separately in 500 ml of 95% ethanol solvent and left for overnight. After filtration, the residue obtained was again suspended in equal volume of 95% ethanol for 48h and filtered again. The above two filtrates were mixed separately and the solvent was evaporated in a rotavapour at 40-50°C, under reduced pressure. The percentage yield obtained from the ethanol extract from leaf and root after drying was 22.5% and 15.6% which were stored at 4 °C in air tight bottle until further use.

2.2 Animals

The study was performed on male and female albino mice, 25 ± 2 g for the acute toxicity study, and lactating rats, weighing 250 ± 25 g and suckling four to six pups each, for the evaluation of milk production. Animals were housed in ventilated room under a 12/12 hour light/dark cycle at 24 ± 2 °C. Animals were kept for 2 weeks to be acclimatized prior to the investigation. During this time they were given standard pellet diet (Lipton India Ltd., Mumbai, India) and water *ad libitum*. Animals were closely observed for any infection; those which showed signs of infection were separated and excluded from the study. The experimental procedures and research protocol used in this study were reviewed and approved by Institutional Animal Ethics Committee (1336/ac/10/CPCSEA) constituted as per the guidelines of Committee for Purpose of Control and Safety on Experiments on Animals.

2.3 Acute toxicity study

The albino mice weighing 25 ± 2 g were used for the study. The acute oral toxicity studies were carried out by using stair case method according to OECD guidelines [20]. They were divided into 5 groups each divided into six rats each with each group receiving graded dose of 200, 400, 800, 1200 and 1600 mg/kg of b.w of ethanol extract of leaf and root of *Basella alba*. The animals were allowed to access to food and water and behavioural changes were observed over a period of 72 h for sign of acute toxicity. The number of mortality caused by the compound within this period of time was observed in order to fix the lethal dose. LD₅₀ values and corresponding confidence limits were determined by the Litchfield and Wilcoxon method (PHARM/PCS Version 4) [6].

2.4 Experimental protocol for study of lactogenic activity in lactating Albino wistar rats

In the present study twenty four lactating dams weighing (250 ± 25 g) at the beginning of lactation and suckling four to six pups were used for this experiment. Females were divided into four groups of six animals each group. Group I treated as control and received distil water; Group II treated as standard and received domperidone (2.5 mg/kg body weight) and Group III, IV treated as test extract and were orally administered with EBAL and EBAR extract at 500 mg/kg and 250mg/kg body weight, respectively.

All animals were treated daily starting from the evening of day 2 of lactation. The EBAL and EBAR extract (500mg/kg, 250mg/kg b.w) was administered orally with a gavage syringe each day at 6.00 pm daily. Milk production was estimated 18 h after gavage. Milk production was measured from day 3 to day 15 of lactation. Milk yield and body weight of rats, and weight gain of pups were measured each day with an electronic balance accurate to 0.01 g [21].

2.5 Milk yield and pups body weight

Every day during the study period, the pups were weighed at 07:00 am (w1) and subsequently isolated from their mother for 4 h. At 11:00 am, the pups were weighed (w2), returned to their mother and allowed to feed for 1 h. At 12:00, they were weighed (w3). Milk yield 18 h after the gavage was estimated as w3–w2. Daily milk yield was corrected for weight loss due to metabolic processes in the pup (respiration, urination and defecation) during suckling. The value used was (w2 – w1)/4. This value was then multiplied by the number of suckling hours per day and added to the daily suckling gain [22]. Daily weight gain of pups was calculated from the pup weight at w2.

2.6 Estimation of serum prolactin and cortisol level

On 15th day of parturition, the blood samples collected from lactating rats through retroorbital plexus. The blood samples were centrifuged and allowed to separate the serum. From the serum sample, the prolactin and cortisol level were estimated using enzyme immunoassay [23].

2.7 Estimation of glycogen and tissue protein content of mammary gland tissue

On 15th day of parturition, the lactating mother rats were euthanized after the blood collection and whole mammary glands were excised. About 100 mg of mammary tissue was homogenized in distilled water using tissue homogenizer and 30% saturated potassium hydroxide. Then, the reaction mixture was incubated for 30 min at 65°C. The resulted homogenate was used further for quantitative estimation of glycogen and protein. For glycogen estimation, 2 ml of 95% ethanol was added to mammary homogenate and centrifuged. The precipitated glycogen was collected from the alkaline digestate, dissolved in distilled water, and estimated by phenolsulfuric acid method [24]. Total protein content was estimated using total protein kit [25].

2.8 Statistical analysis

The result was expressed as mean \pm standard deviation. The differences in mean value among the treatment groups were analysed by oneway ANOVA followed by Tukey–Kramer post hoc test (intra coefficient of variation). Values with $P \leq 0.01$ and $P \leq 0.05$ were considered statistically significant.

3. RESULT

3.1 Acute toxicity study

The acute toxicity study was performed on Albino Wistar rats. No mortality and the sign of toxicity were observed for EBAR and EBAL extract at the dose of 1600 mg/kg b.w when given orally.

3.2 Effect of ethanolic extract of *Basella alba* root and leaf on milk production of lactating rats and body weight of pups

The milk production of control, domperidone, and the groups of EBAR and EBAL was measured daily for 15 days. The result shows (Table 1) that there was a significant increase ($P < 0.001$) of milk yield and body weight of pup within 3rd, 5th, 11th and 15th day of lactation in EBAL and EBAR extract treated groups as compared to control and standard. A dose dependent increase of milk yield and pups body weight was observed during 13 days

of the lactation period. During this lactation period, total milk production on 15th day was highest in EBAL extract (500 mg/kg) 0.612 \pm 0.005 gm and (250 mg/kg) 0.456 \pm 0.050 gm. While the EBAR extract show less milk production 0.591 \pm 0.059 gm (500 mg/kg) and 0.348 \pm 0.050 gm (250 mg/kg) respectively when compared to standard and control group 0.300 \pm 0.001 gm and 3.290 \pm 0.010 gm of albino wistar rats. The daily changes of body weight of the suckling pups during lactation period was linearly increased over the period of 13 days of observation According to Table 1 the EBAL extract (500 mg/kg) treated groups, show highest body weight gain of pups (18.730 \pm 0.130 g) while less weight gain was seen in EBAR extract treated groups 17.950 \pm 0.183g (500 mg/kg b.w) and 16.990 \pm 0.003g (250mg/kg b.w) when compared with the control and standard group.

3.3 Effect of ethanolic extract of *Basella alba* root and leaf on the serum prolactin and cortisol level in lactating rats

The results obtained in this experiment showed that the EBAL and EBAR extract had increased serum prolactin and cortisol level significantly ($P < 0.001$) in lactating rats [Table 2, Figure 2]. The serum prolactin and cortisol level in EBAL extract at 500 and 250 mg/kg were found to be higher (16.50 \pm 0.671, 12.34 \pm 0.180 and 5.320 \pm 0.471, 3.090 \pm 0.120). While the EBAR extract show less prolactin and cortisol level in serum when compared with the control and standard group 11.59 \pm 0.321, 22.45 \pm 0.598 and 2.596 \pm 0.456, 8.270 \pm 0.569 respectively.

3.4 Effect of ethanolic extract of *Basella alba* root and leaf on total protein and glycogen content of mammary gland tissue

The status of milk protein and glycogen were significantly increased in mammary tissue of EBAR and EBAL extract treated mother rats as compared with control [Figure 2, Figure 1]. Total protein and glycogen content were increased in a dose dependent manner 9.20 \pm 0.481 (500 mg/kg), 7.670 \pm 0.160 (250 mg/kg) and 11.20 \pm 0.48 (500 mg/kg), 8.37 \pm 0.18 (250 mg/kg) found significant at $P < 0.01$ and $P < 0.05$ when compared to control and standard.

Table 1. Effect of ethanolic extract of leaf and root of *Basella alba* on milk yield in lactating mother and pups body weight

Groups	DAY 3		DAY 7		DAY 11		DAY 15	
	MY (g)	PBW (g)	MY (g)	PBW (g)	MY (g)	PBW (g)	MY (g)	PBW (g)
Control	0.057 \pm 0.002	5.210 \pm 0.030	0.127 \pm 0.008	8.230 \pm 0.020	0.223 \pm 0.010	10.290 \pm 0.030	0.300 \pm 0.001	16.970 \pm 0.108
Domperidone (2.5 mg/kg)	0.196 \pm 0.029	6.010 \pm 0.020	0.959 \pm 0.012**	12.390 \pm 0.070**	1.696 \pm 0.039**	16.690 \pm 0.060*	3.290 \pm 0.010*	22.370 \pm 0.186**
EBAL (mg/kg) 250	0.058 \pm 0.030	5.360 \pm 0.050	0.148 \pm 0.005*	8.560 \pm 0.130***	0.339 \pm 0.018*	11.520 \pm 0.040***	0.456 \pm 0.050***	17.004 \pm 0.060***
500	0.060 \pm 0.002	5.500 \pm 0.060	0.176 \pm 0.006*	8.750 \pm 0.060***	0.438 \pm 0.009***	12.170 \pm 0.040***	0.612 \pm 0.005***	18.730 \pm 0.130***

EBAR (mg/kg)	0.057 ± 0.010	5.280 ± 0.009	0.152 ± 0.003	8.290 ± 0.060	0.253 ± 0.023*	10.360 ± 0.060**	0.348 ± 0.050	16.990 ± 0.003
250								
500	0.059 ± 0.029	5.420 ± 0.006	0.160 ± 0.030*	8.560 ± 0.080***	0.390 ± 0.010***	11.170 ± 0.020***	0.591 ± 0.059***	17.950 ± 0.183***

Note: $n = 6$; results are Mean \pm SEM; MY= milk yield (g) and PBW= pups's body weight (g); body weight taken after 4 h isolation from their lactating mother; milk yield recorded between 18th and 19th hour after drug administration; all results are compared to control and statistically validated by analysis of variance (ANOVA) followed by Tukey-Kramer *post hoc* test; $P < 0.05$ is considered as significant; * indicate $P < 0.01$, ** means $P < 0.002$ and *** indicate $P < 0.001$.

Table 2. Effect of ethanolic extract of the roots and leaf of *Basella alba* in lactating wistar rats.

Groups	Glycogen content in mammary gland ($\mu\text{g}/100\text{mg}$)	Protein content in mammary gland ($\text{mg}/100\text{mg}$)	Serum Prolactin (ng/ml)	Serum Cortisol (ng/ml)
Control	6.034 \pm 0.678	6.596 \pm 0.456	11.59 \pm 0.321	2.596 \pm 0.456
Domperidone(2.5mg/kg)	12.75 \pm 0.815	11.75 \pm 0.980	22.45 \pm 0.598	8.270 \pm 0.569
EBAL 250 mg/kg	8.37 \pm 0.18*	7.670 \pm 0.160*	12.34 \pm 0.180* *	3.090 \pm 0.120* *
EBAL 500 mg/kg	11.20 \pm 0.48	9.20 \pm 0.481*	16.50 \pm 0.671*	5.320 \pm 0.471*
EBAR 250mg/kg	6.960 \pm 0.678*	6.990 \pm 0.234*	11.86 \pm 0.234*	2.940 \pm 0.252*
EBAR 500mg/kg	9.75 \pm 0.56* *	8.54 \pm 0.96* *	13.65 \pm 0.87* *	3.591 \pm 0.670* *

Note: $n = 6$; Results are Mean \pm SEM; body weight gain recorded every day in the morning from day 3 to 15 after parturition; all results are compared to control and statistically validated by analysis of variance (ANOVA) followed by Tukey-Kramer *post hoc* test; $P < 0.05$ is considered as significant; * indicate $P < 0.01$, ** means $P < 0.002$ and *** indicate $P < 0.001$

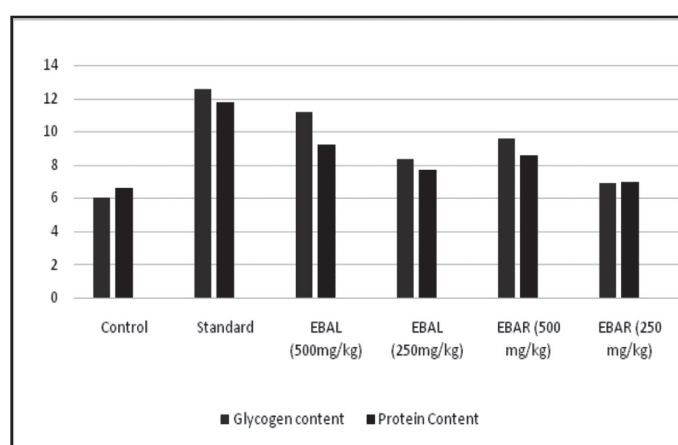


Fig. 1. Effect of EBAR and EBAL extract on total protein and glycogen contents from mammary homogenate of lactating dams at Day 15. Values are expressed as Mean \pm Standard Deviation ($n = 6$). ANOVA (Tukey-Kramer *post hoc* test)

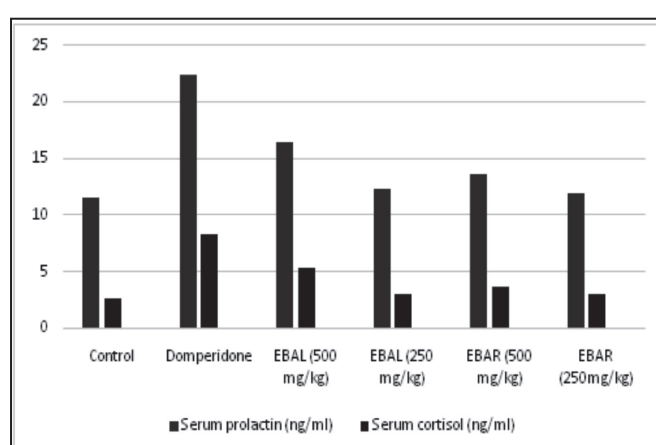


Fig. 2. Effect of EBAR and EBAL extract on serum prolactin and cortisol level in lactating dams at Day 15. Values are expressed as Mean \pm Standard Deviation ($n = 6$). ANOVA (Tukey-Kramer *post hoc* test).

4. DISCUSSION

In the present era, discontinuation of breastfeeding is a major problem in lactating mothers due to low secretion of milk. The breastfeeding is influenced by certain nutritional and non-nutritional factors such as endocrinologic, imbalance, health and climate which cumulatively affect milk synthesis and its production [6]. Secretion of prolactin plays a major role during lactation. The marketed galactagogues are acting by blocking the dopamine receptor and increase the production of prolactin

[26]. In this study, the milk production was significantly increased in EBAL and EBAR extract-treated animals than the untreated control. This increase of milk production in lactating mother was assumed due to the increase of cells proliferation in their mammary gland after interference *Basella alba* extract. Galactagogues have a profound effect on the mammary secretory cells proliferation which is used as an indicator of lactogenic activity [27]. Hence, the increase of rate of milk secretion in EBAR and EBAL extract treated

rat could be due to mammary secretory cell population and cellular activity [28, 29]. Milk consumption is responsible for body maintenance and growth of neonates. The growth of the pups was strongly influenced by the quantity of the milk available during the suckling time. The pup growth rate was significantly improved in EBAL and EBAR as well as domperidone-treated groups as compared to control group. At early stage of nursing mother, enough feed is required to meet the energy demand of lactation and maintenance requirement [30]. The results of this study showed the weight gain in lactating rats at termination period is statistically significant at $P < 0.01$ and 0.05 . It is clearly indicated that *Basella alba* root and leaf extract acts as a health promoter to mother rats during lactation period. The EBAL extract show more efficacy in milk production than the EBAR extract in the lactating mother and also increase the pups body weight.

Generally galactagogues stimulate the synthesis of lactogenic hormones (prolactin, growth hormone and cortisol), β -endorphin and β -casein in the mammary gland [31]. After parturition, prolactin stimulates the synthesis of milk proteins in the epithelial cells and proliferation of secretory cells [27, 32]. Our studies indicate that EBAL and EBAR increased the serum prolactin which stimulates the mammary gland development and the differentiation of the lobulo-alveolar system from the lobular buds. These results agreed with the earlier observations of mammary gland development in lactating rats [33]. Again, EBAL and EBAR increased the protein and glycogen content of mammary gland as compared to untreated control which results improvement of body wt of pups as well as in mother rat. The cortisol level was also increased in EBAL and EBAR treated groups which contribute to the feeling of calm, wellbeing and maintaining the mood of mother rats during suckling period. The increase in milk yield in this study is due to the increased serum prolactin level and maintaining the balance of serum cortisol, which encourage the biosynthesis of milk [34]. The lactogenic activity depends upon various phytochemicals present in herbs. Previous study reported that the presence of saponins, tannins, cardiac glycosides, alkaloids, flavanoids and steroidal ring was assumed to the increase of serum prolactin level, the hormone that associates to milk secretion. The presence of flavonoids, steroids and tannins in the EBAL and EBAR indicates that cumulatively these above compounds should have influenced to lactogenic effect in this study.

Thus, *Basella alba* extract acts as a promising source of galactagogue by stimulating milk production and prolactin synthesis as well as release in the rat. These finding would raise confidence among consumers towards its valid use and effectiveness of the extract. However, further studies are needed to investigate the bioactive agents and their molecular mechanisms for lactogenic activity.

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