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Original Article

Evaluation of lactogenic activity of *Triumfetta rhomboidea* L. root: Validating its traditional usage

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ABSTRACT

Objectives: The aim of the study was to investigate the lactogenic activity of aqueous extract of *Triumfetta rhomboidea* L. on nursing rats. **Materials and methods:** Rats (200-250 g) with suckling pups were selected and were divided into five groups (n=6). Group I treated as control (distil water); Group II treated as standard (Domperidone) and Group III, IV and V were orally administered with aqueous extract of *T. rhomboidea* roots (ATRR) at 125, 250 and 500 mg/kg body wt. respectively and continued for 14th day of parturition. Milk yield, the weight of pups and mother were measured daily. On 15th day, the total protein/carbohydrate contents from mammary tissue and serum prolactin/cortisol level from blood sample were measured and compared with control. **Results:** Oral administration of ATRR increases the milk yield, body weight of pups as well as mother rat, glycogen and protein content as well as serum prolactin and cortisol level as compared to the control animals. In addition, the lactogenic effect of ATRR was followed dose dependent manner as compared to control. **Conclusion:** The present study revealed that the ATRR possesses significant lactogenic activity by enhancing milk production and prolactin concentration in nursing rats.

KEY WORDS: *T. rhomboidea,* lactogenic, milk yield, serum prolactin/cortisol, glycogen.

INTRODUCTION

Milk is accredited by the quantity and quality of nutrients and it contains proteins and significant quantities of inorganic salts like phosphorus, calcium which are structural materials for the skeleton [1]. In case of neonates, mother's milk (colostrums) is an important diet for survival, proper development and growth of body. Specially, colostrums contain non-nutrient substances (such as antibodies and bioactive factors) which are great biological significance on neonate [2]. Prolactin is the key hormone for milk biosynthesis, which exerts direct effect on the mammary gland. Low prolactin level in nursing mother affects the milk production. The secretion of prolactin is affected by environmental stimuli, dopamine and internal milieu like the effect of the chemistry of lactation [3, 4]. Today, low supply of milk is one of the most common reasons for discontinuing breast feeding. Galactagogue are the medications or substances to assist initiation, maintenance and augmentation of maternal milk production. On the basis of toxicity, the plant source is one of the preferred directions of research for galactagogue.

Triumfetta rhomboidea L. (Tiliaceae) is an annual herb; distributed in India, Ceylon, Malaya, Africa and America [5]. Various parts of this plant are therapeutically active. In Ayurvedic system of medicine, the roots are regarded as bitter, tonic, acrid, styptic, galactagogue, aphrodisiac, immunomodulator, cooling, diuretic. Both the fresh leaves and barks are used to treat dysentery, tumors, diarrhoea, wounds and intestinal ulcer. The leaves, fruits and flowers of this plant are astringent, mucilaginous and demulcent and are used in treating gonorrhoea, leprosy and to promote parturition [6-8]. In Kenya and Tanzania, the root infusion of plant was applied the affected site of snake bite [9]. Earlier scientific investigation suggested that the plant possesses antibacterial [10], antiulcer [11], ecbolic [12, 13], cytotoxic [14], antimicrobial [15], antitumor and antioxidant [16], antidiabetic [17], diuretic [18] etc. Phytochemically, the plant contains carbohydrates, glycosides, phytosterols, steroids, flavonoids, tannin, phenolic compounds and triterpenoid [19-21]. The root of this plant has been used as galactagogue by various traditional practitioners of India [21-23]. There is no scientific study from the root of *Triumfetta rhomboidea* on lactogenic effect till date. Therefore, the present study was aimed to evaluate the lactogenic effect on nursing wistar rats.

MATERIALS AND METHODS

Collection of plant materials

The fresh roots of *T. rhomboidea* were collected from local region of Bhopal, Madhyapradesh, India. Further taxonomic identification and authentification was conducted at the Department of Botany, Jiwaji University, Gowalior, MP, India where a plant specimen was deposited in the herbarium for further reference.

Preparation of extract

The collected roots were allowed to dry and powdered to coarse consistently in a grinder mill. Then the coarse powder was passed through 40 mesh size and stored in an airtight container at room temperature. Accurately 250 gm of coarse

powder was weighed and extracted in water by adopting a simple maceration procedure at room temperature, for seven days, in conical flasks with occasional shaking and stirring. Then the extract was filtered and concentrated to dryness at room temperature to avoid decomposition. The yield of the aqueous *T. rhomboidea* root (ATRR) extract was found to be 19.41% w/w and preserved in refrigerator for further use.

Experimental animals

Adult *wistar* albino rats (200 – 250 gm body wt) of either sex were housed in standard conditions of temperature (22 ± 2°C) and relative humidity (55 ± 5%). The rats were kept on wood shavings in plastic boxes with wire covers and the lighting was adjusted with 14 hrs of lightness (06.00 a.m.-08.00 p.m.) and 10 hrs of darkness (08.00 p.m. - 06.00 a.m.) in a day with standard pellet diet (Lipton India Ltd., Mumbai, India) and water *ad libitum*. The experimental protocol was approved by Institutional Animal Ethical Committee of Vedica College of Pharmacy, Bhopal, Madhya Pradesh, India as per the guidance of Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India.

Effect of oral treatment of ATRR on milk production

Thirty female rats were housed and mated with male rats. The rats were allowed to deliver their young and the day of parturition was designated as Day 1 of lactation. All the lactating rats were randomly divided into five groups of six rats each (n=6). Each mother was adjusted to have only six pups per litter within 48 hrs. Group I treated as control and received distil water; Group II treated as standard and received Domperidone (2.5 mg/kg body wt.) and Group III, IV and V treated as test extract and were orally administered with ATRR at 125, 250 and 500 mg/kg body wt. respectively. The ATRR and domperidone were suspended in vehicle and administered daily using animal feeding tube to lactating mother at 07.00 a.m. starting from 5th to 14th day of lactation period. The milk productions were measured daily after 12 hrs of treatment. The weights of the litters before and after 60 minutes of suckling were measured to estimate milk yield. The differences in weight of the litters were considered as the amount of yield. The milk yields and weight gain of littermates as well as mother rats along the experimental period were measured and compared between the treatment groups and their respective control group. All the measurements of weight were read with accuracy of 0.01 g using electronic balance [24].

Estimation of serum prolactin and cortisol level

On 15th day of parturition, the blood samples collected from lactating rats through retro-orbital plexus. The blood samples were centrifuged and allowed to separate the serum. From the serum sample, the prolactin and cortisol level were estimated by using enzyme immunoassay [25].

Estimation of glycogen and 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 [25]. 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 phenol–sulphuric acid method. Total protein content was estimated using total protein kit.

Statistical analysis

The result was expressed as Mean ± Standard Deviation. The differences in mean value amongst the treatment groups were analyzed by one-way ANOVA followed by Tukey-Kramer post hoc test (intra coefficient of variation), using statistical package of SPSS (version 17 for Windows). $P \leq 0.05$ was considered to be statistically significant.

RESULTS

Effect of ATRR on milk production of lactating rats

The milk production of control, domperidone and the groups of ATRR was measured daily (Table 1). The result showed that there was significant increase ($P < 0.001$) of milk yeild in ATRR treated groups as compared to control. Again, ATRR treated groups were showed dose dependent increase of total milk yeild during 10 days of the lactation period. During this lactation period, total milk production was the highest in ATRR (500 mg/kg) as compared to standard group. The ATRR at 125, 250, 500 mg/kg and standard group produced 23%, 39%, 55% and 54% increase of milk yield respectively as compared to control. The percentage of milk production during 10 days was found significant at $P < 0.01$ and < 0.05 in ATRR groups and also indicated the highest quantity of milk during peak lactation time.

Effect of ATRR on body weight of pups and lactating mother

The changes of body weight of the suckling pups during lactation period for all treatment groups were observed (Table 2). Daily body weight of all the suckling pups was linearly increased over the period of 10 days of observation. In ATRR treated groups, the body weight of pups were nearly doubled at the end of the study period and the rate of weight gain in ATRR groups were significantly higher than the control and domperidone treated groups. Similarly, ATRR significantly increased the body weight of lactating mother as compared to the control animals. By visual observation, there was prominently enlargement of mammary gland's size in ATRR treated groups as compared to control.

Table 1. Effect of ATRR on milk production during 10 days of lactation in rat

Treatment groups	Milk yeild on last day (g/pup/day)	Total milk yeild (g) during 10 days	% of increase in milk production
Untreated control	4.11 ± 1.23	241.32	-
Domperidone 2.5 mg/kg	6.69 ± 1.52 ***	372.19	54.23**
ATRR 125 mg/kg	4.93± 1.18 **	298.41	23.65*
ATRR 250 mg/kg	6.12 ± 1.36***	337.15	39.71**
ATRR 500 mg/kg	7.27 ± 1.13 ***	375.97	55.79 **

Values are expressed as Mean ± Standard Deviation (n = 6). ANOVA (Tukey-Kramer post hoc test); untreated control versus ATRR/Domperidone (***P<0.001; **P<0.01, *P<0.05).

Table 2. Effect of ATRR on body weight of pups and mother rats

Treatment groups	Body wt of pups		% of wt gain in pups	Body wt of Mother		% of wt gain in mother
	On 5th day	On 15th day		On 5th day	On 15th day	
Untreated control	12.41±1.63	26.78±2.12	115.79	247.45±10.05	253.11±10.07	2.36
Domperidone 2.5 mg/kg	11.64±1.86	33. 49±2.41	187.71**	231.54±13.39	244.13±9.89	5.43**
ATRR 125 mg/kg	10.13±2.48	27.87±2. 74	175.12*	236.18±12.67	244.21±7.32	3.39*
ATRR 250 mg/kg	12.16±2.19	32.11±1.67	164.06**	205.49±13.16	213.34±11.21	3.82*
ATRR 500 mg/kg	10.67±1.72	31.90±2.43	198.96**	222.33±14. 83	233.09±9. 42	4.83**

Values are expressed as Mean ± Standard Deviation (n = 6). ANOVA (Tukey-Kramer post hoc test); untreated control versus ATRR/Domperidone (**P<0.01, *P<0.05).

Effect of ATRR on the serum prolactin and cortisol level in lactating rats

The results obtained in this experiment showed that the ATRR had increased serum prolactin and cortisol level significantly (P < 0. 001) in lactating rats (Figure 1). The serum prolactin and cortisol level in ATRR at 250 and 500 mg/kg was found higher as compared to domperidone treated group. Again, the serum prolactin and cortisol level was increased more than two fold in ATRR at 500 mg/kg group as compared with control.

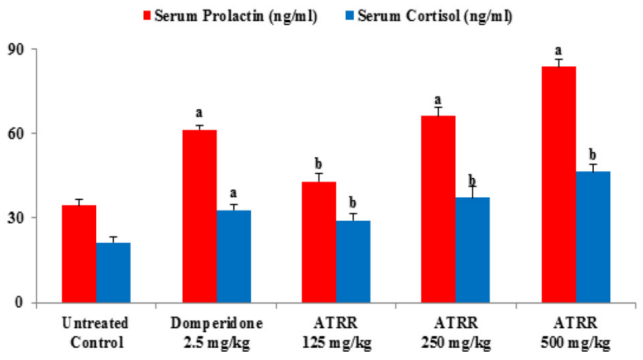

Figure 1. Effect of ATRR on serum prolactin and cortisol level in lactating dams at Day 15.
Values are expressed as Mean ± Standard Deviation (n = 6). ANOVA (Tukey-Kramer post hoc test); untreated control versus ATRR/Domperidone (a- P < 0.001; b - P < 0.01).

Effect of ATRR on total protein and glycogen content of mammary gland tissue

The status of milk protein and glycogen were significantly increased in mammary tissue of ATRR treated mother rats as compared with control (Figure 2). Total protein and glycogen content were increased in dose dependent manner and found significant at P < 0.01 and P < 0.05 as compared to control. The total protein and glycogen content was increased by two fold in ATRR at 500 mg/kg group as compared with control.

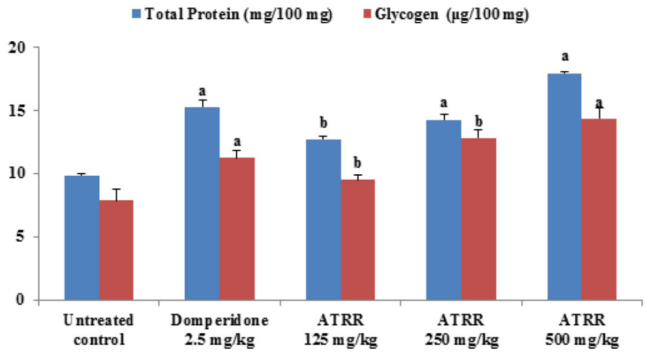

Figure 2. Effect of ATRR on total protein and glycogen contents from mammary homogenate of lactating dams at Day 15.
Values are expressed as Mean ± Standard Deviation (n = 6). ANOVA (Tukey-Kramer post hoc test); untreated control versus ATRR/Domperidone (a- P < 0.001; b - P < 0.01).

DISCUSSION

At present, discontinuation of breast feeding is mainly due to low supply of milk in lactating mothers. The breastfeeding is influenced by certain nutritional and nonnutritional factors like endocrinologic imbalance, health and climate which cumulatively affect milk synthesis and secretion. These factors also modulate various physiological disease conditions in breastfeeding women like agalactias and hypogalactias [26]. Domperidone is a synthetic galactagogues, acting by blocking the dopamine receptor and increase the production of prolactin. Therefore,

domperidone has been used as reference galactagogue in various preclinical studies [2, 25]. In this study, the milk production was significantly as well as linearly increased in ATRR treated animals than the untreated control. This increase of milk production in lactating rats was assumed due to the increase of cells proliferation in their mammary gland after intervention of ATRR. Galactagogue herbs have a profound effect on the mammary secretory cells proliferation which is used as an indicator for the activity of the secretory cells in secreting milk [27]. So, the increase of rate of milk secretion in ATRR 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 ATRR 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]. Result of this study showed the weight gain of lactating rats up to the termination period. It is clearly indicated that ATRR acts as a health promoter to mother rats during lactation period.

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 ATRR 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 [2, 33] Again, ATRR increased the protein and glycogen content of mammary gland as compared to untreated control which results improvement of body wt in pups as well as in mother rat. The cortisol level was also increased in ATRR 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 [25]. The lactogenic activity is 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 [34]. The presence of flavonoids, steroids and tannins in the ATRR indicates that cumulatively these above compounds should have influenced to lactogenic effect in this study.

Thus, ATRR 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.

CONFLICT OF INTEREST STATEMENT

There is no conflict of interest of authors.

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