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Formulation and Evaluation of a *polyherbal formulation (lacto-4)* for its Galactogogue activity in wistar rats

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Abstract: With the aim to increase the milk production in mammalian species, we performed the experiment with alcoholic extract of polyherbal formulation (lacto-4) in lactating wistar rats. According to best of our knowledge nobody has conducted this study on the alcoholic extract of herbs what we selected (Asparagus racemosus, Musa paradisiaca, Cyperus rotundus, Psidium guava). We measured gain in pup weight, increase in milk yield, and pup's serum cholesterol for 15 days treatment. Control group results were compared with individual drug groups and poly herbal group and it was found that above mentioned parameters were more in polyherbal treated group. The treatment with lacto-4 increased the mean body pup weight up to 12.899±2.590 gm/pup and increase was significant ($p<0.001$), the mean milk yield was also significant ($p<0.001$) with 2.3124±0.3432gm/pup and serum cholesterol levels up to 56.588±2.590mg/dl after 15 days treatment. This study demonstrates that alcoholic extract of lacto-4 can stimulate milk production in female rats and could consequently have the properties claimed for lactating women.

Keywords: Galactogogue, Asparagus racemosus, Musa paradisiaca, Cyperus rotundus, Psidium guava.

Introduction

[1][2] Lactation is the process of milk production from mammary glands in female mammals. Galactogogues are either synthetic (domperidone[3], metoclopromide), plant derived (asparagus, fenugreek etc.,) or endogenous (prolactin) substances increase the milk production .The chief function of lactation is to provide nutrition and immune protection to babies. Most of those discovered have been found to interact with dopamine. An inadequate or low supply of breast milk is may be due to single or combined effect of inadequate or infrequent removal of milk, hormonal imbalances, damaged mammary tissue (polycystic ovarian syndrome is cause of poor mammary tissue development), scarring, stress, malnutrition, modern life style and antigalactogogues (sage, parsely, thyme, peppermint etc.,)

(Various types of hormones that influence the milk duct system and lactation are,

- Progesterone — influences the growth in size of alveoli and lobes. Progesterone levels drop after birth. This triggers the onset of copious milk production.[2]
- oestrogen — stimulates the milk duct system to grow and become specific. Oestrogen levels also

drop at delivery and remain low for the first several months of breastfeeding.[2] It is recommended that breastfeeding mothers avoid oestrogen-based birth control methods, as a spike in estrogen levels may reduce a mother's milk supply.

- Follicle stimulating hormone (FSH)
- Luteinizing hormone (LH)
- [3] Prolactin — contributes to the increased growth of the alveoli during pregnancy.
- [4]Oxytocin — contracts the smooth muscle of the uterus during and after birth, and during orgasm. After birth, oxytocin contracts the smooth muscle layer of band-like cells surrounding the alveoli to squeeze the newly-produced milk into the duct system. Oxytocin is necessary for the *milk ejection reflex*, or *let-down* to occur.
- Human placental lactogen (HPL) — From the second month of pregnancy, the placenta releases large amounts of HPL. This hormone appears to be instrumental in breast, nipple, and areola growth before birth.

Galactopoiesis is the maintenance milk production. This stage requires prolactin and oxytocin. The release of the hormone oxytocin leads to the milk ejection or let down reflex. Oxytocin stimulates the muscles surrounding the breast to squeeze out the milk.

The first stage of milk that develops during pregnancy is called colostrums (thick & yellow).

Two to four days after the baby is born, transitional milk replaces colostrums.

Ten to fifteen days following baby's birth, the production of mature milk begins, often appears light bluish in colour (fore milk) and turns white towards end of feeding (hind milk) as fat increases.

Breast milk contains – proteins (60% whey, 40% casein), lactoferrin, secretoryIgA [5], Lysozyme, bifidus factor, fats, vitamins, carbohydrates.

The main advantage of herbal galactogogue is that they-

- Provide effective control by acting on various factors and pathways that lead to oligogalactia.
- Enhance both quality and quantity of milk.
- Completely natural, safe, and effective thus can be continued for long periods.

Materials and methods

Collection and authentication of plant materials

Cyperus rotundus was collected from the paddy field of Thimmasamudram (a village in Andhra Pradesh). Asparagus roots were collected from a nursery in Vijayawada . Guava is collected from Guava orchards in ongole . Musa paradisiaca was collected from local area at Mettupalayam. All the drugs were authenticated from Botanical survey of India , Agriculture University, Coimbatore [Tamilnadu].

Preparation of plant extracts

All the four drugs Asparagus racemosus [AR], Musa paradiasica[MP], Cyperus rotundus[CP], Psidium guava[PG] were sun dried and coarsely powdered. Individual Soxhlet ethanolic (ethanol : water-1:4) extracts were prepared for seven cycles at 35-40ºC and evaporated in petry plates on water bath maintined at 60ºC.

Preparation of galactogogue formulation (LACTO-4) [6][7] (Table-1)

All the extracts were concentrated and made into a powder form and they were formulated.

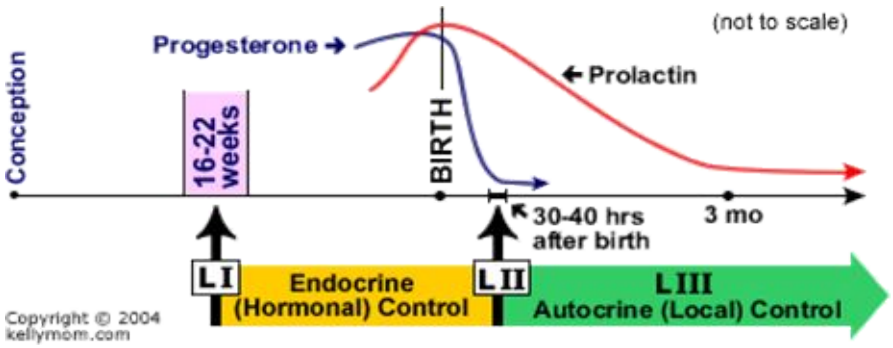

Table-1

Sr. No	Ingredients	Weight per capsule
1	Asparagus racemosus	100 mg
2	Cyperus rotundus	100 mg
3	Musa paradisiac	100 mg
4	Psidium guava	100 mg
5	Microcrystalline cellulose	60 mg
6	Polyvinyl pyrolidine	10 mg
7	Sodium benzoate	0.5 mg
8	Starch	79.5mg
9	Sodium starch glycolate	60 mg
10	Talc	12.5 mg
11	Magnesium stearate	7.5 mg
	Total weight	630 mg/capsule

Preparation of granules:

-A required amount of all the granulating agent and excipients were weighed accurately.

-The weighed quantity of polyvinyl pyrolidine was to be soaked in sufficient quantity of isopropyl alcohol (at least 1 hr before starting the granulation).

-All the excipients used in the formulation were passed through 100 no mesh and then used for granulation.

-All the weighed fine powder of granulating excipients (microcrystalline cellulose, starch) were uniformly mixed with the extract powder, which already weighed sieved and mixed well.

-The above mixed material that contains extract and granulating excipients were blended into a slight clumpy mass by using granulating agent polyvinyl pyrolidine solution.

-The obtained clumpy mass was made into granule by passing through 16 no mesh. The obtained granules were collected and dried at normal room temperature for 10 min.

-After that the granules were dried under sunlight and open air for 30 min.

-After that the granules were dried in oven at 25 ºC for 25 min with door of the oven opened and with constant gentle moving for every 5 minute.

-After that the temperature of oven was increased to 45 ºC and dried for 1 hr with constant and gentle mixing until the granules were free from moisture.

-Then these granules were filled in empty capsules and cleaned the surface. The accurately weighed quantity of the capsule were packed and sealed. The Lacto-4, 630 mg were suspended in 0.3 % CMC (carbonated) and given to rats per kilogram body weight by oral gavage.

Animals

Healthy, adult female wistar rats (180-220 gms) were obtained from the central animal house facility from J.S.S. college of pharmacy, ootacamund, Tamilnadu. The animals were kept in a well ventilated room and the animals had exposed to 12 hours day and night cycle with a temperature of between 20±3°c. The animals were housed in large spacious, hygienic polypropylene cages during the course of experimental period. The animals were fed with water and rat feed *ad libitum,* supplied by this institution. All the experiments were performed after obtaining prior approval from **IAEC.** The animals were housed in suitable environmental conditions with nutritional feed and filtered tap water *ad libitum*.

Acute toxicity study of galactogogue formulation (lacto4). (As per OECD guide lines number 423) [8]

Female wistar rats of weight (180-220g) were taken for the study and kept for overnight fasting. Next day, body weight was taken and lacto-4 was administered orally. Then the animals were observed for mortality and morbidity at 0,1/2, 1, 2, 4, 6, 8, 12, and 24 hours. Feed was given to the animals after 4 hours of dosing and body weight was checked 6 hours after dosing. No signs of Morbidity like convulsions, tremors, grip strength and pupil dilatation were observed.

Grouping of animals

Animals were divided into six groups of four female rats in each group.

Group 1 : Control (solvent).
Group II :Asparagus recimosus (extract- 100 mg/kg).
Group III : Musa paradisiaca (extract- 100/kg).
Group IV : Cyperus rotundus (extract- 100 mg/kg).
Group V : Psidium guava (extract- 100 mg/kg).
Group VI : Galactogogue formulation (lacto-4) (400 mg/kg).

Experimental protocol [9][10]

Lactating rats, with litters adjusted to 10 pups per dam on day 2 of lactation were assigned to the experimental groups. All the feeding females were divided into 6 groups of four each and received 100mg/kg (group-I to V); 400mg/kg of polyherbal formulation (group-VI).

1. Milk production estimation (Table – 2 &3)

All animals were treated daily, starting on the evening of day 2 lactation. The extracts were administered orally with a gavage syringe each day at 18:00h. Milk production was estimated 15h after gavage. Milk production was measured from day 3 to day 15 of lactation.

Every day during the study period, the pups were weighed at 09:00h (w1) and subsequently isolated from their mother for 4h (Sampson & jansen 1984). At 13:00h the pups were again weighed (w2), returned to mother and allowed to feed for 1h. At 14:00h, they were weighed (w3). Milk yield after 15h after gavage was estimated as w3-w2.

2. Pups body weight estimation (Table- 4 &5)

For this estimation, the pups were weighed at 09:00hrs (w1) daily from day 1 to 15. This weight was recorded to assign and find out the pups body weight gain (or) change during the experimental procedures with an electronic balance (Sartorius basic plus) accurate to 0.01g. None of the animals or pups showed any signs of morbidity or mortality during study periods.

Table: 2 Effect of Asparagus racemosus, Musa paradisiaca, Cyperus rotundus and Psidium guava ethanolic extracts and Lacto-4 on milk production after 18 hours of treatment for 15 days.

Sr. No	No: of Days	Milk yield (gm/pup/day) (Mean value)					
		Control	Asparagus racemosus	Musa paradisiaca	Cyperus rotundus	*Psidium guava*	Lacto-4
1	1	0.312	0.300	0.399	0.311	0.349	0.340
2	2	0.324	0.360	0.401	0.398	0.312	0.530
3	3	0.336	0.490	0.305	0.410	0.402	0.600
4	4	0.418	0.750	0.498	0.510	0.576	0.750
5	5	0.728	0.800	0.534	0.590	0.589	0.900
6	6	0.769	1.000	0.715	0.730	0.812	1.140
7	7	0.823	1.350	0.800	0.900	0.890	1.900
8	8	0.853	1.605	0.991	1.190	1.278	2.200
9	9	1.120	1.900	1.432	1.426	1.432	2.700
10	10	1.212	2.100	1.601	1.512	1.512	2.900
11	11	1.324	2.350	1.779	1.760	1.922	3.200
12	12	1.232	3.183	2.143	2.100	2.176	3.480
13	13	1.534	3.400	2.288	2.255	2.197	3.600
14	14	1.712	3.600	2.566	2.580	2.423	4.600
15	15	2.124	3.900	2.770	2.900	2.986	4.900

Table: 3 Effect of Asparagus recimosus, Musa paradisiaca, Cyperus rotundus and Psidium guavaethanolic extracts and Lacto-4 on mean milk production per day.

Sr. No	Treatment Groups	Milk yield (gm/pup)
1	Control	0.9112 ± 0.1312
2	Asparagus recemosus	1.960 ± 0.2624***
3	Musa paradisiacal	1.226 ± 0.1924 ns
4	Cyperus rotundus	1.278 ± 0.2123ns
5	Psidium guava	1.939±2887***
6	Lacto-4	2.3124 ± 0.3432 ***

Mean milk production per day. The values are mean ± S.E.M, ***P<0.001, ns P> 0.05 (not significant) when compared to control groups, (ANOVA followed by Bonferroni multiple comparison test.)

Table: 4 Effect of Asperagus recemosus, Musa paradisiaca, Cyperus rotundus and Psidium guavaethanolic extracts and Lacto-4 on pup weight gain up to treatment for 15 days.

Sr. No	No: of days	Pups weight (gm/pup)					
		Control	Asparagus recemosus	Musa paradisiaca	Cyperus rotundus	Psidium guava	Lacto-4
1	1	4.235	5.231	4.121	4.323	4.729	4.834
2	2	4.443	5.624	4.543	5.445	5.235	5.479
3	3	4.934	5.745	5.121	6.009	5.889	5.906
4	4	5.133	6.136	5.956	6.560	7.112	6.839
5	5	5.679	7.345	6.766	7.345	7.189	6.986
6	6	6.554	8.967	7.543	7.977	7.867	8.431
7	7	7.322	9.109	8.434	8.862	8.234	11.431
8	8	7.899	9.222	8.532	8.234	9.167	13.864
9	9	8.123	10.437	9.451	9.098	9.329	15.076
10	10	8.876	11.576	10.325	10.456	9.867	17.254
11	11	9.654	13.567	11.328	10.456	10.775	19.324
12	12	10.324	14.768	12.673	11.089	11.224	20.387
13	13	11.125	15.764	13.564	11.123	11.778	23.098
14	14	13.098	16.986	15.565	12.975	12.324	25.312
15	15	15.232	18.321	16.344	13.456	13.344	25.541

Table: 5 Effect of Asparagus recimosus, Musa paradisiaca, Cyperus rotundus and Psidium guavaethanolic extracts and Lacto-4 on Mean weight gain of pups for 15 day.

Sr. No	Treatment Group	Weight Gain (gm/pup)
1	Control	8.3453 ± 0.8676
2	Asparagus recemosus	11.343 ± 1.342**
3	Musa paradisiacal	9.985 ± 1.354ns
4	Cyperus rotundus	8.643 ± 1.398ns
5	Psidium guava	11.7652±0.7223**
6	Lacto-4	12.899 ± 2.590***

Mean weight gain of pups per day. The values are mean ± S.E.M, ***P<0.001, *P<0.05, ns P>0.05 (not significant), when compared to control groups, (ANOVA followed by Bonferroni multiple comparison test.)

3. Serum cholesterol estimation (Table - 6)

Pups were anaesthetised with pet ether and blood was collected from pups through ocular puncture. Plasma was separated by subjecting the sample to centrifugation at 2500rpm for 10 min. Plasma was separated and Cholesterol was estimated by micro lab 200(Merck) Auto analyser, in plasma by Ecoline diagnostic kit.10 µl of plasma is mixed with reagent solution 1000 µl and incubated for 10 minute at + 37°C.Read absorbance of sample and standard against blank. The cholesterol level in plasma was expressed in mg/ dl.

Table: 6. Effect of Asparagus recimosus, Musa paradisiaca, Cyperus rotundus and Psidium guavaethanolic extracts and Lacto-4 on pups serum cholesterol levels on 15 day.

Sr. No	Treatment Group	Serum Cholesterol (mg/dl)
1	Control	33.267±1.113
2	Asparagus racemosus	48.478 ± 1.484**
3	Musa paradisiacal	40.439± 1.776ns
4	Cyperus rotundus	35.324 ± 1.453ns
5	Psidium guava	38.389±0.5622ns
6	Lacto-4	56.588 ± 2.590***

Mean serum cholesterol levels of pups. The values are mean ± S.E.M, ***P<0.001, *P<0.05, ns P>0.05 (not significant), when compared to control groups, (ANOVA followed by Bonferroni multiple comparison test.)

Statistical analysis

Data were analysed by one-way ANOVA followed by Bonferroni selected comparison test. All the values were assigned as ±S.E.M. The statistical analysis was performed by GRAPH PAD INSTANT-3. P<0.05 was considered significant.

Results

Milk production (Table-2&3)

Milk productions of all the drug treatment groups were higher than that of the control (untreated) group. Milk yield increased from 0.312 gm/pup per day to about 2.124 per day for controls, and those received Asparagus racemosus (AR) 100 mg/kg the milk yield increased from 0.300 gm/pup per day to about 3.900 per day on 15th day. The Musa paradisiaca (MP) 100 mg/kg treatment increased the milk yield from 0.399 to 2.770 gm/pup per day. The extract of Cyperus rotundus (CR) 100 mg/kg treatment increased the milk yield from 0.311 ± to 2.990 gm/pup per day. The Psidium guava (PG) 100 mg/kg treatment increased the milk yield from 0.349 to 2.986 gm/pup per day. The Lacto-4, 400 mg/kg treatment increased the milk yield from 0.340 to 4.900 gm/pup per day on 15th day. The differences in milk production were significant for AR and Lacto-4 treatment groups (ANOVA followed by Bonferroni). The mean milk yield was significant P<0.001 with 1.960 ± 0.2624 gm/day per pups for AR extract treated groups. The mean milk yield was significant P<0.001 with 2.3124 ± 0.3432 gm/day per pups for Lacto-4 treated groups.

[11][12]Body weight (Table 4&5)

All pups gained weight during the study period. The rate of weight gain for the treatment groups was higher than that of control. Body weight gain was 8.3453 ± 0.8676 gm/pup for the control. For AR extract treated groups it was 11.343 ± 1.342 gm/pup. Those received MP extract; the pup weight was increased to 9.985 ± 1.354gm/pup up to 15 days. The treatment with CR extract group showed 8.643 ± 1.398 gm /pup to 15 days. Those received GP extract; the pup weight was 11.7652±0.7223 gm/pup up to 15 days The treatment with Lacto-4 (400mg/kg) increased the weight up to 12.899±2.590gm/pup during 15 days treatment. The pup body weight increase was highly significant (P<0.001) for lacto-4 during the treatment periods.

[13][14]Serum cholesterol estimation (Table 6)

The estimation of cholesterol was made and the increase in cholesterol was noted for both AR and lacto-4 treated groups. For control group it was 33.267±1.113 mg/dl and for AR extract treated groups it was 48.478 ± 1.484 mg/dl. Those received MP extract it was 40.439± 1.776 mg/dl and for CR it was 35.324 ± 1.453 mg/dl up to 15 days. The treatment with PG extract group showed 38.389±0.5622 mg/dl after 15 days. The treatment with Lacto-4 (400mg/kg), the serum cholesterol levels up to 56.588 ± 2.590 mg/dl during 15 days of treatment.

Discussion

Lactation is a complex and essential process which encloses a natural invaluable food for the developing child. The estimation of milk production levels in rat is a difficult and tedious process. The milk yield estimation for rats by means of pup weight and weight gain have been used in several studies. It has to be noted that, the purpose of this study was essentially to determine whether the individual plant extracts, or the polyherbal formulation (Lacto-4) is lactogenic or not. As expected, the milk production in the Lacto-4 treated group was significantly higher than in the control or MP or CR treated animals. The AR and PG extract also showed a rise in lactogenic action. In addition milk yield appears to be significantly stimulated after 6-8 days of drug administration and the pup growth rate was significantly higher in Lacto-4 treated groups. This suggests a possible effect of the

Lacto-4 on milk components. And also by evaluating the cholesterol levels of pups which were treated with the test drugs for the period of 15 days, it is clear that Lacto-4 can significantly increase the cholesterol levels. Likewise, extract of AR and PG has been observed to stimulate milk production in rats and increased pup weight. However, the suggestion can be confirmed only by studying the composition of the milk. On the other hand, treatment with other two extract like MP, CR did not improve the pup growth rate in rats. But it slightly increased the milk yield. These finding suggest the possibility of these drugs to stimulate prolactin level during treatment.

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