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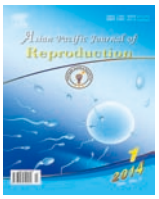

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Evaluation of safety and efficacy of Maa-Lact in lactating Holtzman rats

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ABSTRACT

Objective: To evaluate the safety & efficacy of Maa-Lact granules for its galactogougue activity in Holtzman rats and its effect on suckling pups. **Methods:** Group I rats were treated as control, group II and III rats were treated with 500 mg/kg, 1 000 mg/kg of Maa-Lact granules for 21 days. Weekly body weights of dams and pups were collected, litter survivability for 22 days and ocular blood samples were collected on 1st day of parturition and 21st day of post parturition for the estimation of prolactin levels. On 21st day blood samples were collected from retro-orbital sinus for haemotological and biochemical estimations. On the same day of weaning rats were sacrificed and subjected to necropsy and individual organ weights were recorded. **Results:** No significant difference in weekly food weight consumption, body weights between control & treated groups with normal clinical signs. There is no mortaly in dams throught the study period with no significant difference in pups weights. The percentage mortality in pups was 14.43 %, 14.07 %, and 13.42% in group I, group II and group III, respectively. The histopathological finding has shown that treated groups have less convulution and adipose tissue deposition along with increase in length and branching of lactiferous duct and alveolar size. **Conclusion:** Based on above results, it can be concluded that Maa-Lact posseses significant galctogogue activity.

1. Introduction

Milk is the primary source nutrient for development and growth of the neonates in the weaning period. The composition of milk contains water, minerals, and organic nutrients to which baby have access. Colostrum is the first milk coming out from the mammary gland after parturition contains nutrient substances and further comes a mature milk which provides non-nutrient substances like antibodies and proteins. Low or lack of production of milk was one of the reason for discontinuation of breast feeding.

Galactogogues are the substances which enhances the milk production by assisting in initiation, augmentation, and maintainence of it [1]. Breast feeding offers a benefit to the child from sudden infant death symdrome and childhood leukaemia [2].

In Ayurveda, most of the formulations are herbomineral preparations, based on the ancient scripts of Charak samhitha, Sushruta samhitha, etc. Some of the herbal drugs having the galactogogue activity were *Asparagus racemosus(A. racemosus)*[3], *Ipomea digitat(I. digitat)*[4], *Glycerrhiza glabra(G. glabra)* [5], *Leptadenia reticulate(L. reticulate), Bacopa monieri(B. monieri), Anethum sowa(A. sowa)* [6], *Centella asiatica(C. asiatica)*, fennel seeds, dill, borage, comfrey and Lamiaceae, *etc* [7,8]. Om Pharmaceuticals Limited, Bangalore, has developed a Maa-Lact preparation, a galactogogue, which contains Shatavari, Yashthimadhu, Shveta Sariva, Shunthi, Maricha , Pippali, Musta and Sharkara to stimulate the milk production, improve quality and quantity of it.

Compostion of Maa-Lact (10 g.) as follows: a. *A. racemosus*: 4 000 g; b. *G. glabra*:150 mg; c. *Hemidesmus indicus(H.*

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indicus): 150 mg; d. *Zingiber officinalis (Z. officinalis)*: 50 mg; e. Piper nigrum: 50 mg; f. Piper longum: 50 mg; g. *Cyperus rotundus (C. rotundus)*:150 mg; h. Sugar: q.s.

Various therapeutic uses have been established for *A. racemosus* such as galctogogue, aphrodisiac and demulscent activity[9]. *G. glabra* has hypolipidemic, anti-oxidant, anti-inflammatory activity [10]. *H. indicus* for antinociceptive, hepatoprotective, antiallergic action [11]. *Z. officinalis* having antiemetic, cardioprotective, and immunomodulatory activity [12]. The present study was taken up to generate the preclinical data along with it's safety to coordinate the clinical use and standardisation of the same.

2. Materials and methods

2.1. Chemicals

The chemicals were procured from the Sigma-Aldrich, Germany and serum prolactin ELISA kit was procured from IDS Ltd. USA.

2.2. Animals

A total of 18 female Holtzmann rats were procured from animal house facility NIRRH, Mumbai; and transfered to the experimentation room, under controlled environmental conditions i.e (23±1) ℃ temperature and humidity (55±5) %, and in a 14 hr light/ 10 hr dark cycle with free access to feed containing crude protein, fiber and nitrogen free extract along with fresh purified water *ad-libitum*.

Rats were divided into 3 groups, group I as control receiving a 2 % CMC solution, group II & III were orally adiministered with 500 mg/kg and 1000 mg/kg of Maa-Lact for 21 days in a 2 % CMC solution. All the experimental procedure were performed in accordance with guidelines of CPCSEA and get the approval form IAEC before starting the experiments.

2.3. Observations

2.3.1. Feed consumption of female rats

The feed consumption was recorded every day starting from day 1 to day 22 and group average was calculated.

2.3.2. Body weight of females

Weight of the female rats was recorded weekly and the group average was calculated at the end of experiment.

2.3.3. Mean Body weight of pups

Average weight of surviving pups in each litter was recoreded every day from day 1 to day 22.

2.3.4. Litter survivability

Every day the litters were observed for any mortality. The percentage mortality at the end of the study (22 day) was also calculated.

2.3.5. Blood prolactin levels

Blood was collected on 1st day of delivery and on 22nd day of weaning; serum prolactin levels were estimated using the commercial ELISA kit.

2.4. Haemotology and serum biochemistry

After collecting the blood from the retro-orbital sinus, serum was separated and haemotological and biochemical estimations were performed.

They are haemoglobin (Hb), packed cell volume (PCV), total red cell count (RBC), total white cell count (WBC), absolute erythrocyte indices like mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), differential leuckocyte count and platelet counts using automatic haemotolgy analyzer (Abacus). Biochemical parameters like total protein, albumin, alanine amino transferase (ALT), aspartate amino transferase (AST), uric acid, creatinine, cholesterol, total bilirubin, direct bilirubin, and globulin levels by automatic biochemical analyzer using commercial kits.

2.5. Necropsy and histopathology

At the end of the study i.e on 22nd day rats were sacrificed using CO_2 asphyxiation and all the organs were properly weighed and collected in 10% neutral formalin sloution and subjected for histopathology. Mammary glands were subjected to histology and observations such as length and branching of lactiferous ducts, proliferation of alveoli, alveolar size, and adipose tissue in gland was performed..

2.6. Statistical analysis

Student "*t*" test was used to comapre treatment group with that of control and *P* value less than 0.05 was considered to be significant.

3. Results

3.1. Feed consumption & weekly body weights

There was no significant ($P<0.05$) difference in the weekly feed consumption between treated groups and that of control and there is no significant ($P<0.05$) difference in weekly ponderal changes between two treatment groups (Table 1).

3.2. Clinical signs & moratlity in dams

All the animals showed normal behaviour throughout the study. No mortality was observed in control as well as treated groups during the period of lactation till weaning.

3.3. Weekly male & female pups weight

No significant (P<0.05) weight difference was observed in the weekly body weights of male & female pups, and weights between two treatment groups and that of control group (Table 2).

Table 1
Mean of weekly feed intake (g) & mean weekly body weights (g).

Group	Mean of weekly feed intake			Mean weekly body weights			
	1st week	2nd week	3rd week	1st week	2nd week	3rd week	4th week
Group I	33. 603±4.556	49.946±5.406	55.969±11.173	275.883±26.482	285.550±22.885	282.967±17.967	285.00±14.913
Group II	38.469±2.473	53.081±5.725	62.671±3.783	283.150±27.535	301.700±28.847	303.883±30.361	301.783±30.779
Group III	33.278±6.407	46.850±11.763	58.346±16.011	298.800±19.233	307.017±15.212	308.667±15.769	307.550±21.586

Table 2
Average weekly male pup weights (g) & average weekly female pup weights (g).

Group	Average weekly male pups weights				Average weekly female pups weights			
	1st day	1st week	2nd week	3rd week	1st day	1st week	2nd week	3rd week
Group I	7.216±1.020	14.653±2.207	27.532±3.996	40.625±5.654	7.038±1.000	14.423±2.311	26.795±3.372	42.392±8.490
Group II	80.152±1.461	15.968±3.969	28.825±6.488	43.971±9.410	7.411±1.708	15.816±3.952	27.906±6.537	40.700±8.235
Group III	7.344± 0.517	15.270±2.032	27.557±4.601	44.631±7.879	7.128±2.866	15.556±2.018	25.812±4.776	43.868±7.236

3.4. Percentage mortality in pups

Mortality in pups in nornal control (Group I), Maa-Lact 500 (Group II), Maa-Lact 1 000 (Group III) was found to be 14.43 %, 14.07 % and 13.42 % respectively. There was no significant difference among control and treatmed groups (Table 3).

Table 3
Average prolactin levels in blood (ng/mL) & percent mortality in pups (%)

Group	Average prolactin levels in blood (ng/mL)		Percent mortality in pups (%)
	0th day	21st day	
Group I	23.800±6.544	35.830±9.075	14.439±14.544
Group II	24.030±23.909	23.910±7.027*	14.078±10.637
Group III	31.190±13.402	14.780±5.526*	13.420±12.339

*P< 0.05 and considered as significant when compared with control group.

3.5. Serum haemotological, clinical and prolactin levels

There was no significant (P<0.05) difference in various haemotological & clinical chemistry between control and treatment groups except WBC and creatinine values. In case of treatment groups WBC count was significantly (P<0.05) decreased, creatinine values were significantly (P< 0.05) increased as compared to control, but values were within normal range. On the day of delivery the serum prolactin values were comparable in control and treated groups. However on day of weaning i.e. on 21st day a significant decrease in prolactin levels were observed in treatment group as compared to control (Table 3 & 4).

3.6. Terminal body weights & absolute organ weights

No significant difference in terminal body weight was observed in female rats of treated groups and there is no significant difference in absolute organ weights of treated and control groups except weight of ovary in group III was significantly (P<0.05) increased (Table 5).

3.7. Histopathology of mammary gland

Terminal sacrificed animals didn't show any gross pathological changes except lesions in liver, periportal mononuclear cell infiltration, cytoplasmic vaculization and hyperplasia of bronchus associated lymphoid tissue (BALT) in lungs. Histopathology of mammary gland didn't shown any signs of toxicity related pathological changes. There is a increase in increase in involution of mammary gland & adipose tissue deposition in normal control group (Figure 1). Incase of treated groups there is less involution of mammary gland & adipose tissue deposition along with increase in length and branching of lactiferous duct and alveolar size (Figure 2, 3). These results indicate of prolong lactation/milk production in treatment group as compared with control group .

Table 4
Average haemotological & biochemical values (g).

Parameter	Group I	Group II	Group III
Haemoglobin (g/dL)	15.583±0.840	15.100±0.587	15.050±0.898
RBC (10^{12}/L)	9.395±0.508	9.180±0.583	9.073±0.509
PCV (%)	48.767±2.056	50.383±2.344	48.600±2.160
MCV (pg)	51.933±1.421	54.950±1.550	53.633±2.039
MCH (fL)	16.567±0.242	16.467±0.497	16.600±0.089
MCHC (g/dL)	31.967±1.113	30.033±0.0674	31.000±1.185
WBC (10^9/L)	8.267±1.391	7.133±3.836	5.467±2.199*
Lymphocytes (%)	66.033±3.369	71.117±2.084	66.767±3.717
Neutrophils (%)	31.483±3.864	25.383±3.485	31.300±4.334
Monocytes (%)	1.900±0.885	3.167±1.389	1.300±1.026
Platelets (×10^9/L)	461.833±23.216	515.500±99.198	347.167±192.805
Total Protein (g/dL)	8.684±0.491	9.072±0.423	9.250±0.590
Albumin (g/dL)	3.128±0.198	3.282±0.070	3.176±0.251
Globulin (g/dL)	5.556±0.304	5.790±0.379	6.074±0.405
SGPT (IU/L)	155.920±46.188	183.760±53.018	154.076±67.293
SGOT (IU/L)	152.660±18.234	175.240±24.615	188.680±37.277
Bilirubin (mg/dL)	0.082±0.031	0.086±0.029	0.086±0.017
Direct Bilirubin (mg/dL)	0.046±0.017	0.054±0.0.41	0.046±0.015
Indirect Bilirubin (mg/dL)	0.036±0.015	0.032±0.015	0.040±0.007
Cholesterol (mg/dL)	119.350±19.840	131.260±14.832	126.060±10.417
Uric Acid (mg/dL)	2.730±0.433	2.976±1.299	3.056±1.121
Creatinine (mg/dL)	0.616±0.019	0.696±0.047*	0.724±0.095*

*P< 0.05 considered as significant when compared with control group.

Table 5
Terminal body weight and absolute organ weight (g).

Organ	Group I	Group II	Group III
Body weights	285.000±14.913	301.833±30.675	307.500 ±21.603
Heart	1.217±0.170	1.179±0.090	1.368±0.177
Liver	12.861±2.044	15.239±1.690	14.134±2.097
Kidney	2.101±0.266	2.211±0.320	2.269±0.217
Adrenal	0.112±0.024	0.100±0.022	0.124±0.034
Spleen	0.701±0.090	0.718±0.102	0.716±0.068
Brain	1.887±0.074	1.917±0.111	1.960±0.118
Ovary	0.145±0.030	0.176±0.037	0.205±0.054*
Uterus	0.508±0.208	0.554±0.147	0.594±0.285

*P< 0.05 considered as significant when compared with control group

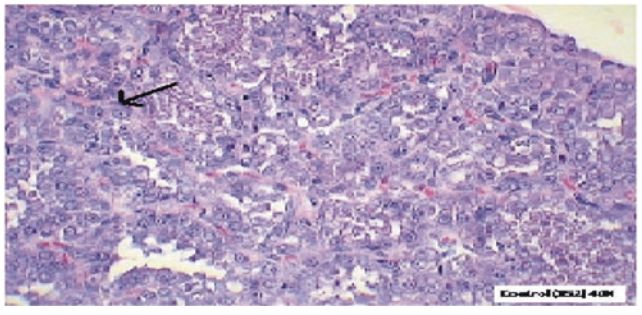

Figure 1. Group I (Vehicle control) showing involution of mammary gland and with decreased active alveoli.

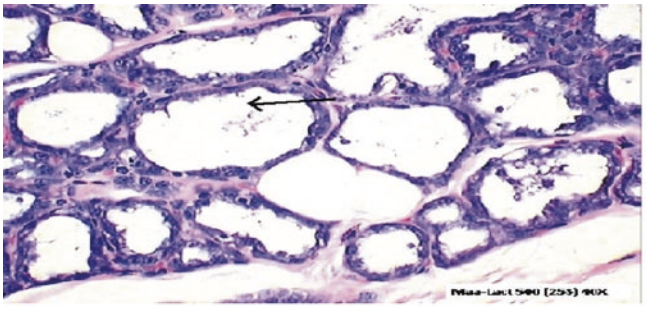

Figure 2. Group II (Maa Lact −500) showing active mammary gland and with increased active alveoli and alveolar space.

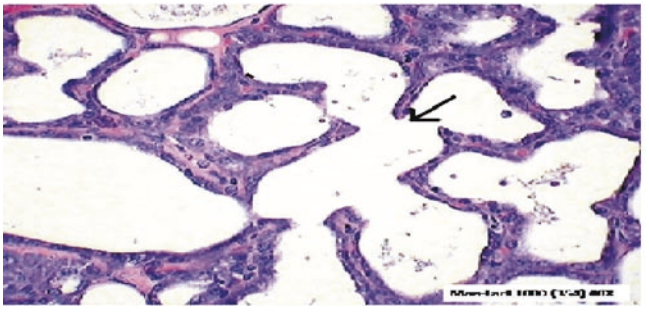

Figure 3. Group III (Maa Lact −1000) showing active mammary gland and with increased active alveoli and alveolar space & increased length and branching of alveolar length.

4. Discussion

Lactation is a natural and multiple complex process which serves as an invaluable food to baby. Several herbal medications have been used as a galactogogue along with modern medicine. These include domperidone, metoclopamide, antipsychotics sulpiride and chlorpromazine which acts through blocking dopamine receptors and subsequently increasing prolactin levels [13]. Meanwhile, some herbs like Fenugreek (*Trigonalla foenum graecum*), Galega (Goat's rue, *Galega officinalis*), Silymarin (Milk thistle, *Silybum marianum*) and Shathavari (Stanya, *Asperagus racemosus*) has been used as a galactogogues in traditional medicine [14].

Galactogogues were mainly reported that they increase the proliferation of lactiferous tubules, there by increase the milk production [15]. Moreover some herbs increase the incraese the synthesis of lactogenic hormones such as growth hormone, prolactin, cortisol and B-casein in mammary glands [16]. Another proposed mechanism of action to increase the milk production was by inhibiting the dopamine pathway [17]. Almost all species share the common pattern of milk production *i.e.*, after parturition prolactin acts on mammary glands to increase the epithelial and secretory cells of it [18].

The main aim of the present study was to determine the galactogoue activity of Maa-Lact granules in rats, by means of pup weight gain and involution in mamary gland. There is no significant differences in feed consumption and ponderal changes were observed, along with no differences in male & female pups body weights and pups mortality. Meanwhile, there is no significant differences in the serum biochemical & clinical parameters excluding WBC levels i.e., levels were significantly decreased in treated group as (5.467 ±2.199) in group III when comapred with group I as (8.267±1.391). Where as, creatinine levels were significantly ($P< 0.05$) increased in treated groups i.e., group II and group III as (0.696±0.047) and (0.724±0.095) respectively. As expected, milk production in treated groups was increased due to less involution of mammary gland & adipose tissue deposition along with increase in length and branching of lactiferous duct and alveolar size supported by histopathological examination.

Results of the present study explained taht the milk production was significantly increased in treated groups which can be ascribed to the presence of *A. racemosus* [9] and supports the Maa-Lact as a galactogogue.

Mother's milk is very important to the child as it contains plenty of nutrients required for nurturing the child. Mother's feel lack of insufficient milk production, and face numerable number of hurdles in breastfeeding mainly due to physical, mental and emotional stresses.To conclude that, our research findings corroborate and validate the galactogoue activity of Maa-Lact, a polyherbal formulation using scientifically proven methods.

Conflict of interest statement

We don't have potential conflicts of interest.

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