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Galactagogue effects of *Musa x paradisiaca* flower extract on lactating rats

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ABSTRACT

Objective: To investigate the potential of *Musa x paradisiaca (M. x paradisiaca)* flower extracts in promoting milk production of lactating rats and its effects on growth of the suckling pups. **Methods:** Galactagogue activity was evaluated in terms of quantity of milk produced from the rats treated with petroleum ether, ethanol or water extracts of the flower. Lactating rats (n=5) of Spraque Dawley with six pups each were administered with the extracts in the amount of 500 mg/kg body weight, while the control rats were given an equivalent amount of distilled water. The rats were daily administered via oral feeding starting from Day 5 until Day 14 and the performance of milk production was measured along the experimental period by weight-suckle-weight method. Results were statistically analyzed using SPSS by means of ANOVA at 0.05 and was expressed as their mean±standard deviation. The rates of pups' growth were measured as the weight gain along the experimental period. **Results:** The rats treated with aqueous extract produced higher milk than control and ethanol groups. Aqueous extract was identified to increase milk production by 25%, while petroleum ether extract by 18%. The mean of yields produced by the rats during suckling period for aqueous, petroleum ether, ethanol and control were 4.62±2.45, 4.37±1.93, 3.65 ±1.89 and 3.69±1.79, respectively. Growth rates of pups for the rats treated with control, aqueous, ethanol extract and petroleum ether were (1.85±0.49), (1.78±0.56), (1.65±0.46) and (1.56±0.42) g/pup, respectively. **Conclusions:** The present study reveals the potential of *M. x paradisiaca* flower to enhance milk production of nursing mothers which could be exploited for commercialization of the isolated extract.

1. Introduction

Bananas and plantains are among the popular and cheapest foods throughout the tropical and sub-tropical regions of the world. Although banana is one of the most important commercial crops in the world, it is estimated that 87% of the production is purposely for local consumption[1]. All parts of banana plants are practically being used for various purposes such as food, beverages, fermented sugars, medicines, flavorings, silage, fragrance, rope, cordage, garlands, shelter, clothing, smoking material, and numerous ceremonial and religious uses[2,3]. Although banana family is preferred for its nutrients rather than medicinal properties, but it is believed that the plants propose some traditional medicinal value[4].

Banana flower has yet received very little attention from the world of science particularly on the medicinal value toward human health. Based on ethnomedicinal surveys around the world and supported by limited bioactivities and clinical research, it should have tremendous pharmacological value. The flowers have been traditionally used to alleviate heart pain, asthma, diabetes mellitus, menorrhagia, painful menses, diarrhea and stomach cramps[5]. Extracts from the flowers have been reported to have medicinal properties for illness such as diabetes mellitus, oxidative stress[6] and malaria[7].

Previous research showed that many plants have been identified to possess galactopoietic effects such as fenugreek, goat's rue, milk thistle, aniseed, *Asparagus racemoses (A. racemoses)*, Grap sap, fennel seeds, dill, borage, comfrey and Lamiaceae. They are commonly used in the world to enhance milk supply[8,9]. These herbs could be either fresh prepared or added as spices to foods. Since

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most galactagogue herbs normally applied as infusions or decoctions, thus its actions are unnoticeably. Traditionally in India and Indonesia, women have used fenugreek [*Trigonella foenum-graecum (T. foenum-graecum)*] as spice and eat torbangun [*Coleus amboinicus (C. amboinicus)* Lour] soup, respectively to increase the flow of milk[8,10]. Despite, most of the plant substances have not been scientifically evaluated but ethnomedicinally has suggested them as safe and show its efficacy.

Taking into consideration of the effectiveness of the galactagogue herbs, *Musa x paradisiaca* (*M. x paradisiaca*) flower were investigated for its functional properties of milk production on lactating rats.

2. Materials and methods

2.1. Chemicals

All the chemicals used are analytical grade obtained from SYSTERM include petroleum ether (40:60 ℃), ethanol (95%) and Tween 20.

2.2. Collection and preparation of plant materials

The study was carried out on the flower of *M. x paradisiaca* (Pisang Nipah). The sample was obtained from cultivated local farmland in Jerantut, Pahang and was identified by a Botanist from the Institute of Biosciences, University Putra Malaysia. The flowers were separated into florets and bracts, then dried in the oven for seven days at 40 ℃. After dried, the samples were ground into powder using grinder. The dried samples were then stored in air-tight container before extraction.

2.3. Sample extraction

For each 500 g samples, it was extracted with petroleum ether and ethanol (95%) respectively using soxhlet method. The samples were extracted until the solvents become colourless. The solvents were evaporated to dryness under vacuum using rotary evaporator. In water extraction, the sample was shaken in water bath at 60 ℃ for 6 h followed by filtration and centrifugation at 4 000 rpm for 10 mins. The precipitate was discarded and the supernatant was freeze-dried to get powder extract.

2.4. Animal

All the experiments were carried out with Spraque Dawley rats purchased from the University Putra Malaysia. The rats were kept in the animal room with a constant temperature at (21 ± 2) ℃. They were kept on wood shavings in plastic boxes with wire covers and the lighting was adjusted with 14 h of lightness and 10 h of darkness in a day. They were fed with commercial feeds (BARASTOC from Ridley Agriproducts Pty.Ltd., Victoria, Australia) and tap water *ad libitium* prior to and throughout the experiment. All experiments were approved by the animal ethics committee of International Islamic University Malaysia.

2.5. Intervention procedures

Twenty female rats at age three months old with the weight of 200-350 g were housed and mated with male rats. The rats were allowed to deliver their youngs, and the day of parturition was designated as Day 1 of lactation. All the lactating rats were randomly divided into four groups of five rats each (n=5). Each mother was adjusted to have only six pups per litter within 48 h. The groups treated with crude extract were administered with any one of these; petroleum ether, ethanol or aqueous extract and the controls were given distilled water. The dose applied to each mother was 500 mg/kg of the body weight. All the treatments samples including control were added with 0.05% of Tween 20. The groups were administered with either plant extracts or control via oral using animal feeding tubes.

The treatments to dam were daily administered at 1 600 h starting from Day 5 to Day 14 of lactation period. The milk productions were measured daily after 12 hours of treatment starting from Day 6 until Day 15. Prior to the first treatment, the pups were separated for 2 h on the second day and the separation period were increased gradually to 6 h on Day 5 of postpartum. Litters of the pup were isolated from their dams for 6 h before milking. The weights of the litters before and after 60 min of suckling were measured to estimate milk yield. The differences in weight of the litters were considered as the amount of yield. The measurement of milk production and weight gain of littermates along the experimental period were 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 (Mettler Toledo).

2.6. Statistical analysis

The result was expressed as their mean ± standard deviation. The differences in mean value amongst the treatment groups were analyzed by one-way ANOVA followed by the least square difference (LSD), using statistical package of SPSS (version 17 for Windows). $P<0.05$ was considered to be statistically significant.

3. Results

3.1. Milk production

Milk production of aqueous, petroleum ether, control and ethanol group were (4.62 ± 2.45), (4.37 ± 1.93), (3.69 ± 1.79) and (3.65 ± 1.89) g/pup/d, respectively. Estimated milk production for rats that were subjected to different treatments of solvent extracts and distilled water are presented in Table 1.

The aqueous extract group was found to give significant yield compared to control (P<0.05). Statistically, the amount of milk produced in the rats treated with petroleum ether extract was not significant compared to control and aqueous extract. Milk production of the ethanol group is comparable with the control group along the experimental period.

Total milk production during 10 d of the lactation for the

rats treated with the extract of aqueous, petroleum ether, and ethanol were 217.27, 214.25 and 178.93 g, respectively. The rats treated with any of the extracts showed higher total milk production than the control, which was only 177.31 g.

The aqueous extract group which was determined to produce significant milk production also indicated the highest quantity of milk during peak lactation time. The lactation peak was regarded as the time at which the yield of milk secretion was maximum. During the course of lactation in rats, milk secretions gradually increase to the peak and then decrease after the peak of lactation. Both aqueous and control showed peak lactation time at Day 13, meanwhile for petroleum ether at Day 10 and ethanol at Day 14. The amount of milk produced by day of lactation and the peak lactation time for all treatment groups are shown in Figure 1. The amount of milk produced at lactation peak for the rats treated with aqueous was the highest, followed by petroleum ether, control and ethanol.

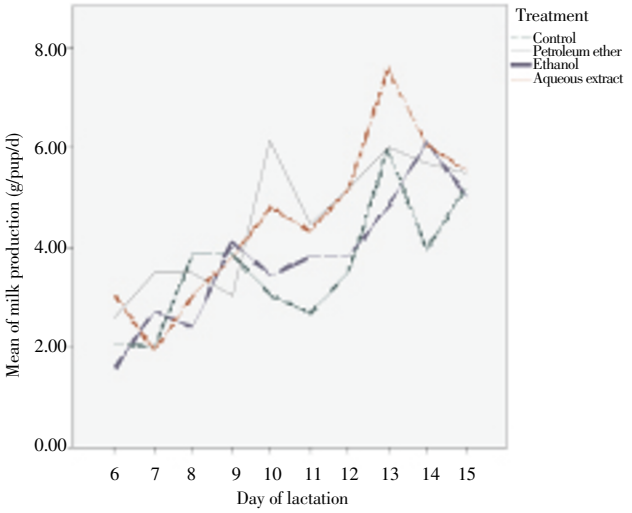

Figure 1. Effect of various solvent extracts of BF on milk production during 10 d of experimental period.

Relatively, the aqueous and petroleum ether extract groups produced 25% and 18% more milk than the control. The percentage of milk produced by the rats treated with ethanol extract was a little bit lower (−1%) than the control.

3.2. Growth rate and weight gain of pups

Daily weight of all the suckling pups linearly increases over the period of 10 d of observation as shown in Figure 2. The slope of regression was measured as the r^2 value. The estimated r^2 value for ethanol, aqueous, control and petroleum ether groups are 0.838, 0.814, 0.799 and 0.649, respectively.

The pups body weight increased by (0.82−2.88), (0.66−2.58), (0.86−2.45) and (0.82−2.40) g/pup per day for control, aqueous, ethanol extract and petroleum ether, respectively. The daily weight gains were (1.85 ± 0.49), (1.78 ± 0.56), (1.65 ± 0.46) and (1.56 ± 0.42) g/pup respectively. The changes of body weight of the suckling pups during lactation period for all treatment groups are shown in Figure 2.

Table 2 shows the measurement of initial and final weight and also the weight gain of the suckling pups. The rats treated with aqueous extract indicated no difference in weight gain of their suckling pups compared to control (P<0.05). Lower growth rate found in the pups of the rats treated with petroleum ether and ethanol extracts. The increment of pup's body weight by different treatments along the experimental period is presented in Figure 3.

The highest increment of body weight was for the pups of control, followed by aqueous, ethanol and petroleum ether extract. The mean of weight gain for control was the highest, followed by aqueous, ethanol and petroleum ether extracts.

Table 1
Milk production of various solvent extracts during 10 d of lactation.

Treatment group	Mean of milk production (g/pup/d)	Total milk production during 10 d (g)	Quantity of milk at peak lactation time (g/d)	Percent increment of milk produced (%)
Aqueous extract	4.62 ± 2.45[a]	217.27	38.56	25
Petroleum ether extract	4.37 ± 1.93[ab]	214.25	32.28	18
Ethanol extract	3.65 ± 1.89[b]	178.93	22.86	−1
Control (distilled water)	3.69 ± 1.79[b]	177.31	29.91	–

Reported values are based on n=5, with 6 pups per litter. Values are mean±standard deviation. Means followed by different superscript letters in the same column represent significant difference (P < 0.05).

Table 2
Comparison between initial and final weight, and weight gain of pups.

Treatment	Mean of initial weight (g)	Mean of final weight (g)	Weight gain (g/pup)
Control (distilled water)	10.67 ± 1.67[a]	27.35 ± 3.76[a]	1.85 ± 0.49[a]
Aqueous extract	12.07 ± 1.36[a]	28.06 ± 3.36[a]	1.78 ± 0.56[ab]
Ethanol extract	11.71 ± 1.29[a]	26.55 ± 3.62[a]	1.65 ± 0.46[bc]
Petroleum ether extract	12.17 ± 2.98[a]	26.26 ± 3.89[a]	1.56 ± 0.42[c]

Reported values are based on n = 5, with 6 pups per litter. Values are mean± standard deviation. Means followed by different superscript letters in the same column represent significant difference (P < 0.05).

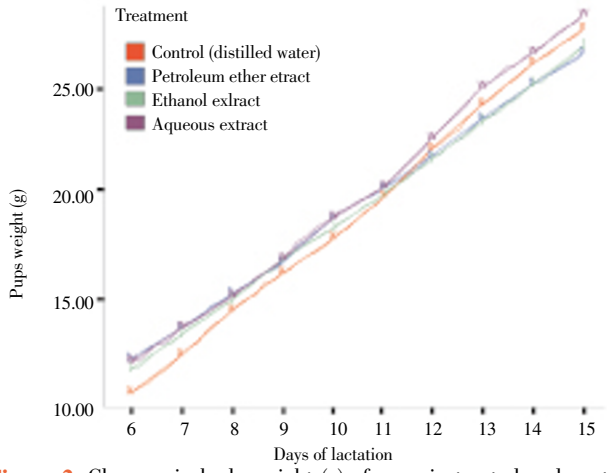

Figure 2. Changes in body weight (g) of pups in treated and control groups throughout the experimental period.

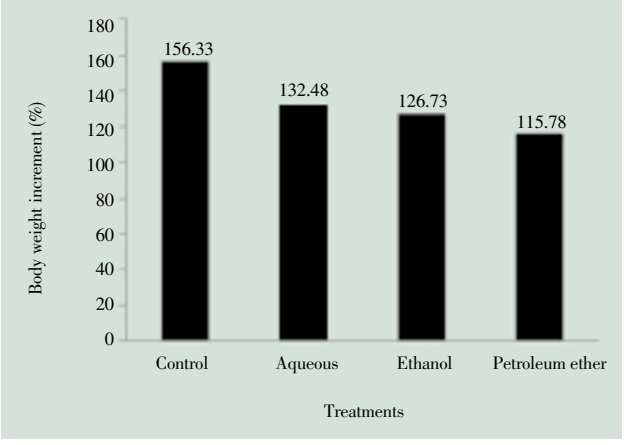

Figure 3. Percentage of body weight increment of pups for various treatment groups.

4. Discussion

Milk production of 20 rats was relatively measured within ten days of their lactation by daily weighing of their litters. At Day 16, measurement of milk production was terminated due to the pups have started to eat solid food supplied to dam.

Measurements of milk production were not corrected for any weight loss associate with metabolic process of the suckling pups. Correction to the weight could perhaps exaggerate the amount of milk measured due to the pups generally do not defecate or urinate after a few hours of separation from their mothers.

Since there was no significant difference in milk production between petroleum ether and aqueous extract, therefore it is likely that petroleum ether extract give significant yield if the higher dose applied to the rats.

The increase of milk production in lactating rats was assumed due to the increase of cells proliferation in their mammary gland after intervention of the extract. Galactagogue herbs was reported to have a profound effects on the mammary secretory cells proliferation[11] which is used as an indicator for the activity of the secretory cells in secreting milk[12,13]. Although some galactagogues were identified to act as dopamine antagonist, but the mechanism of action for most is simply unknown[8].

The rate of milk secretion during lactation in rats could be due to mammary secretory cell population and cellular activity[12,14]. Despite the decrease of milk production is due to the decrease of mammary cell numbers, it remains to be determined whether significant cell turnover occurs during lactation. Most species share almost the same pattern of milk flow[15]. Milk output at first start with a rapid increase until the point of peak lactation is reach. Since aqueous extract has significantly affected the milk production in rats, thus it is assumed that the extract contains bioactive constituents that promote galactagogue.

Previous study on phytochemicals constituents in *M. paradisiaca*flower showed that it contains of alkaloids, saponins, glycosides, tannins, flavanoids and steroids [16]. The presence of these compounds such as saponins, tannins, alkaloids and flavanoids in *Hibiscus sabdariffa* L. was assumed to the increase of serum prolactin level, the hormone that associates to milk secretion[17]. Due to the fact that most polar compounds should have dissolved in polar solvent of extraction, thus it can be concluded that the compounds contain in the aqueous extract are the polar compounds. The presence of saponins and tannins in the aqueous extract of *Musa* flower[16] indicates that at least one of the two compounds should have influenced to the effects of galactagogue in this study.

There is considerable variation of pups growth during the first 3 or 4 days of life due to dehydration of the young and stabilization of the mothers habit toward lactation, thus data collection was only started on Day 6 of post-partum. The gain of pup was identified constant from day 5 to 15 of lactation. Thus, when this condition is met, growth rates can also be estimated from the slope of pup weight regressed against day of lactation. In most of the time, litter weight-gain per day is assumed to be approximately proportional to the milk production during lactation, thus was sometimes used as indicator of milk production in rats.

Milk consumed by pups is mainly for body maintenance and growth. Therefore, it is assumed that rapidly growing pups must have relatively consumed large amount of milk than more slowly growing pups. It is very important that the mother should be well-fed during lactation period. At early lactation, enough feed is required to meet the energy demand of lactation and maintenance requirement[18]. Another thing to consider in measuring milk yield is the number of pups per litter and the separation interval between the mother and the pups.

The litter requires small number of pups in order to obtain enough milk to grow at its maximum potential. This is because the growth of the mouse was strongly influenced by the quantity of the milk available during the suckling time.

In most species, intake of food by mothers during lactation is dependent on the litter size. As the litter size increases, the milk secreted must be divided between more and more offspring which consequently reduce the pups body masses[19]. The separation had to be long enough so that the quantity of milk accumulate in the dams' mammary glands could be measured accurately.

Result of this study showed although milk production in aqueous extract had increased significantly compared to control groups but the increase was not consistence with the increase of weight gain of the pups. Thus, we could not rely on the daily weight gain of suckling pups to determine milk production of lactating rats as it gives us inaccurate and unreliable estimation. Measurement of milk production by weight-suckle-weight method was identified as an accurate method which employs natural process of milking.

The results also support the ethnomedicinal use of the flower among nursing women for galactagogue purpose. Aqueous extract of *M. x paradisiaca* flowers have shown a significant galactagogue in rats. This finding would raise confidence among consumers toward the effectiveness and safety of the extract since water was known as free risk from any chemicals. The flower of *M. x paradisiaca* may have a potential use not only for human but also for ruminants in promoting milk production.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Dzomeku BM, Ankomah AA, Quain MD, Lamptey JNL, Anno-Nyako FO, Aubyn A. Agronomic evaluation of some IITA *Musa hybrids* in Ghana. [Online]. Available from: http://www.cabdirect.org/abstracts/20103268268.html [Accessed on 2007].

[2] Nelson SC, Ploetz RC, Kepler AK. *Musa* species (banana and plantain). Musaceae (banana family). *Species Profiles Pac Island Agroforestry* 2006; **15**: 15.

[3] Ploetz RC, Kepler AK, Daniells J, Nelson SC. Banana and plantain—an overview with emphasis on Pacific island cultivars. [Online]. Available from: http://www.agroforestry.net/tti/Banana-plantain-overview.pdf. [Accessed on March 23, 2010].

[4] Alisi CS, Nwanyanwu CE, Akujobi CO, Ibegbulem CO. Inhibition of dehydrogenase activity in pathogenic bacteria isolates by aqueous extracts of *Musa paradisiaca* (Var Sapientum). *Afr J Biotech* 2008; **7**: 1821-1825.

[5] Sumathy V, Lachumy SJ, Zakaria Z, Sasidharan S. *In vitro* bioactivity and phytochemical screening of *Musa acuminata* flower. *Pharmacologyonline* 2011: 118-127.

[6] Vinaykumar T, Sumanth MH, Suman L, Vijayan V, Srinivasarao D, Sharmila A, et al. Reno protective and testicular protective effect of *Musa paradisiaca* flower extract in streptozotocin induced diabetic rats. *J Innov Trends Pharm Sci* 2010; **1**: 106

[7] Bagavan A, Rahuman AA, Kaushik NK, Sahal D. *In vitro* antimalarial activity of medicinal plant extracts against *Plasmodium falciparum*. *Parasitol Res* 2011: **108**: 15-22.

[8] Dog TL. The use of botanicals during pregnancy and lactation. *Altern Ther Health Med* 2009; **15**: 54-59.

[9] Zapantis A, Steinberg JG, Schilit L. Use of herbals as galactagogues. *J Pharm Pract* 2012.

[10] Damanik R, Wahlqvist ML, Wattanapenpaiboon N. Lactagogue effects of Torbangun, a Bataknese traditional cuisine. *Asia Pac J Clin Nutr* 2006; **15**: 267.

[11] Lompo-Ouedraogo Z, van der Heide D, van der Beek EM, Swarts HJ, Mattheij JA, Sawadogo L. Effect of aqueous extract of *Acacia nilotica ssp adansonii* on milk production and prolactin release in the rat. *J Endocrinol* 2004; **182**: 257.

[12] Sternlicht MD, Kouros-Mehr H, Lu P, Werb Z. Hormonal and local control of mammary branching morphogenesis. *Differentiation* 2006; **74**: 365-381.

[13] Capuco AV, Akers RM. Management and environmental influences on mammary gland development and milk production. In: *Managing the Prenatal Environment to Enhance Livestock Productivity*. International Atomic Energy Agency: Springer; 2010.

[14] Wall EH, McFadden TB. Triennial Lactation Symposium, A local affair: how the mammary gland adapts to changes in milking frequency. *J Anim Sci* 2011; **90**: 1695-1707.

[15] Hadsell D, George J, Torres D. The declining phase of lactation: Peripheral or central, programmed or pathological? *J Mammary Gland Biol Neoplasia* 2007; **12**: 59-70.

[16] Mahmood A, Ngah N, Omar MN. Phytochemicals constituent and antioxidant activities in *Musa x Paradisiaca* flower. *Eur J Sci Res* 2011; **66**: 311-318.

[17] Okasha MAM, Abu Bakar MS, Bako IG. Study of the effect of aqueous *Hibiscus Sabdariffa* Linn seed extract on serum prolactin level of lactating female albino rats. *Eur J Sci Res* 2008; **22**: 575-583.

[18] Baumgard LH, Odens LJ, Kay JK, Rhoads RP, Van Baale MJ, Collier RJ. Does negative energy balance (NEBAL) limit milk synthesis in early lactation. [Online]. Available from: http://animal.cals.arizona.edu/swnmc/Proceedings/2006/Baumgard_SWNMC2006.pdf [Accessed on March 23, 2006].

[19] Speakman JR. The physiological costs of reproduction in small mammals. *Philos Trans Royal Soc Lond B Biol Sci* 2008; **363**: 375-398.