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Effect of bitter melon (*Momordica charantia* Linn) fruit juice on blood prolactin level and histological change of mammary gland in lactating mice

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Abstract. Breastfeeding has nowadays been promoted. Meanwhile, lactation insufficiency is a significant problem. Thus, herbs for promoting milk are interesting. In Thai local wisdom, *Momordica charantia* Linn is claimed as a galactagogue. Experiments were performed in lactating mice with 6 litters each and metoclopramide (2 mg/100 g BW), a hyperprolactinemia inducing drug, was used as a reference drug. The drug and *M. charantia* fruit juice (400 and 600 mg/100 g BW) were orally administered during day 1-day 21 of lactation. Results were found that both the drug and the fruit juice significantly increased blood prolactin levels in lactating mice. A significant increase in the number, size and function of alveoli (milk sacs) in mammary tissue also were found. It was concluded that *M. charantia* fruit juice has a lactogenic activity by increasing blood prolactin level and inducing increased growth and function of mammary gland.

Key Words: *Momordica charantia*, mammary gland, prolactin, lactogenic activity.

Introduction. Eventhough, breast milk is being promoted for baby feeding, a number of women have lactation insufficiency. Thus, herbs used to augment milk are commonly found in rural peoples in eastern culture (Brown 1977). Many herbal galactagogues are reported such as flowers of *Borago offcinalis* (Al-Saidi et al 2006), seeds of *Hibiscus sabdarifa* (Okasha et al 2008), rhizomes of *Asparagus racemosus* and leaves of *Cissampelos pareira* (Preciado et al 2011; Tanwar et al 2008). Milk production is a complex physiological process involving the interaction of multiple hormones where prolactin is the principle lactogenic hormone among progesterone and oxytocin (Behera et al 2013). Some women have lactation insufficiency due to prolactin deficiency (Freeman et al 2000).

Momordica charantia Linn. (bitter melon) is a flowering vine in the family Cucurbitaceae (Smitinand & Santisuk 2008). In Thailand, its leaves and fruits are commonly consumed as a vegetable. Meanwhile, it also has been used in alternative medicine for type II diabetic treatment because of its high potential hypoglycemic properties (Grover & Yadav 2004; Krawinkel & Keding 2006; Kumar et al 2010). In addition, it also has been claimed that the fruits and leaves have galactogenic properties (Preciado et al 2011; Behera et al 2013; Kumar et al 2010). However, scientific studies to support this are still lacking. Interestingly, important chemicals were found in *M. charantia* fruit such as saponins and alkaloids, which were also found in *H. sabdariffa* seeds, and these increased serum prolactin (PRL) levels in lactating rats (Okasha et al 2008; Grover & Yadav 2004). The purpose of this study is to evaluate the galactogenic properties of *M. charantia* juice in lactating mice by determining blood PRL level, histological changes and morphometric studies of the mammary gland after oral administration for 21 days.

Material and Method

Chemicals and drugs. Prolactin radioimmunoassay kit (125/IRMA kit, ICN Biomedicals, Inc. Costa Mesa, CA926261) and metoclopramide (Pharma Supply Co. Ltd., Thailand) were used.

M. charantia fruit juice preparation. Fresh *M. charantia* fruits were purchased from a local market in Khon Kaen province, Thailand. They were cleaned, seeds removed and air dried, then mixed with distilled water (1:1) and minced by electronic blender. The fruit juice was obtained, then filtered through cotton mesh and evaporated in a hot air oven at $45^{o}C$ until dry. The mass of fruit juice was minced to a powder and reconstituted with distilled water at concentrations of 800 and 1200 mg mL^{-1} before oral administration (0.5 ml/100 g BW).

Animals. Adult female mice, ICR strain, 8 weeks old and 35-40 g were obtained from the National Laboratory Animal Center, Nakornpathom province, Thailand. They were housed under a 12:12 light-dark cycle and at $25\pm1^{o}C$. A pellet diet (Chareanpogapan Ltd., Thailand) and water *ad libitum* were freely provided. The experiments were performed after the experimental protocol had been approved by the Institutional Animal Ethics Committee, Khon Kaen University, Thailand (Reference No. 0514.1.12.2196).

Histological and morphomertic study. Mammary glands were collected at the areas under skin around nipples. They were immediately fixed in Bouin's solution for 48 hours, and then were processed by the paraffin method. They were sectioned at 5 µm thickness and stained with periodic acid shiff (PAS). Sections were examined under light microscope for detecting galactogenesis in alveoli (milk sacs) by positive result from PAS staining. The diameter of alveoli and content ratio of alveoli and adipose tissue were also evaluated.

Hormonal determination. Blood samples were collected by cardiac puncture and centrifuged at 1,700 rpm for 5 min at room temperature. Plasma samples were collected for PRL assay.

Experiments. Thirty pregnant mice were obtained by mating with male mice. On the first day of delivery, they were separately caged individuals with 6 litters each. The experiments were undertaken on 4 groups of 6 lactating animals each. Group I served as a negative control group receiving distilled water at 0.5 ml/100 g BW and group II served as a positive control group receiving metoclopramide (a hyperprolactinemia-inducing drug) at 2.0 mg/100 g BW and groups III and IV received *M. charantia* fruit juice at 400 and 600 mg/100 g BW, respectively, for 21 days of May 2012, starting from day 1 to day 21 of lactation. At the end of the treatment, body weight and blood prolactin levels of all groups were evaluated, and the mammary glands were collected for histological and morphometric studies.

Statistical analysis. All data are expressed as mean and standard deviation ($\bar{X} \pm$SD). Each data was separately analysed by one-way ANOVA and Duncan's multiple range test used to compare the different results among groups. Values of $p < 0.05$ were considered significantly different (Zar 1999).

Results and Discussion. Effect of *M. charantia* fruit juice on blood PRL levels of lactating mice was shown in Figure 1. The fruit juice of *M. charantia* at 400 and 600 mg/100 g BW significantly increased blood PRL level in lactating mice as compared to the control group ($p < 0.05$). It is noticed that the increase is dose dependent. In regard to metoclopramide treated group, it increased blood PRL level similarly to the group receiving a high dose of the fruit juice (600 mg/100 g BW). Since, metoclopramide increases PRL secretion by inhibiting dopamine-mediated hypothalamic secretion of prolactin inhibiting factor (PRL-IF) (McCallum et al 1976). This implies that the potency of the fruit juice at high dose is almost the same as such a drug. This finding coincides to the study of Okasha et al (2008), who used *H. sabdariffa* seeds extract. They claimed that the presence of saponin, tannins, alkaloids and flavonoidi in *H. sabdariffa* seeds may be responsible for the increase in serum prolactin level in lactating rats. Meanwhile, these chemicals were also found in *M. charantia* fruit (Raman & Lau 1996).

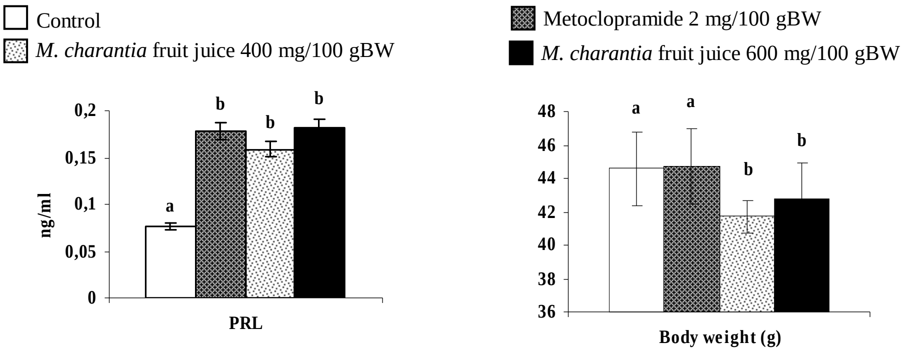

Figure 1. Effect of *M. charantia* fruit juice on prolactin levels and body weight (g) in lactating mice (same letter within figure means non-significant difference ($p > 0.05$) and different letter within figure means significant difference ($p < 0.05$).

Effect of *M. charantia* fruit juice on histological change of mammary tissue was presented in Figures 2 and 3.

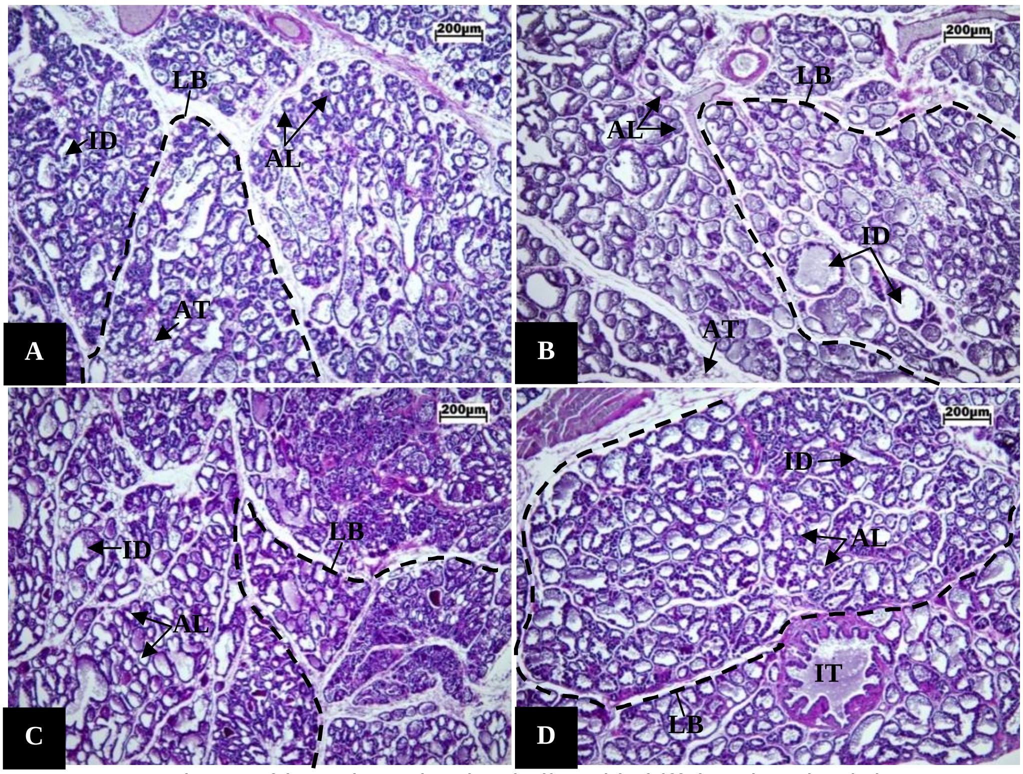

Figure 2. Mammary tissue of lactating mice (periodic acid shiff (PAS) stained, bar = 200 µm): A, negative control group received distilled water; B, positive control group received metoclopramide at 2 mg/100 g BW; C & D, treated groups received *M. charantia* fruit juice at 400 and 600 mg/100 g BW, respectively, for 21 days. (ID = intralobular duct; IT = interlobular duct, AT = adipose tissue; AL = alveoli, LB = lobule of gland).

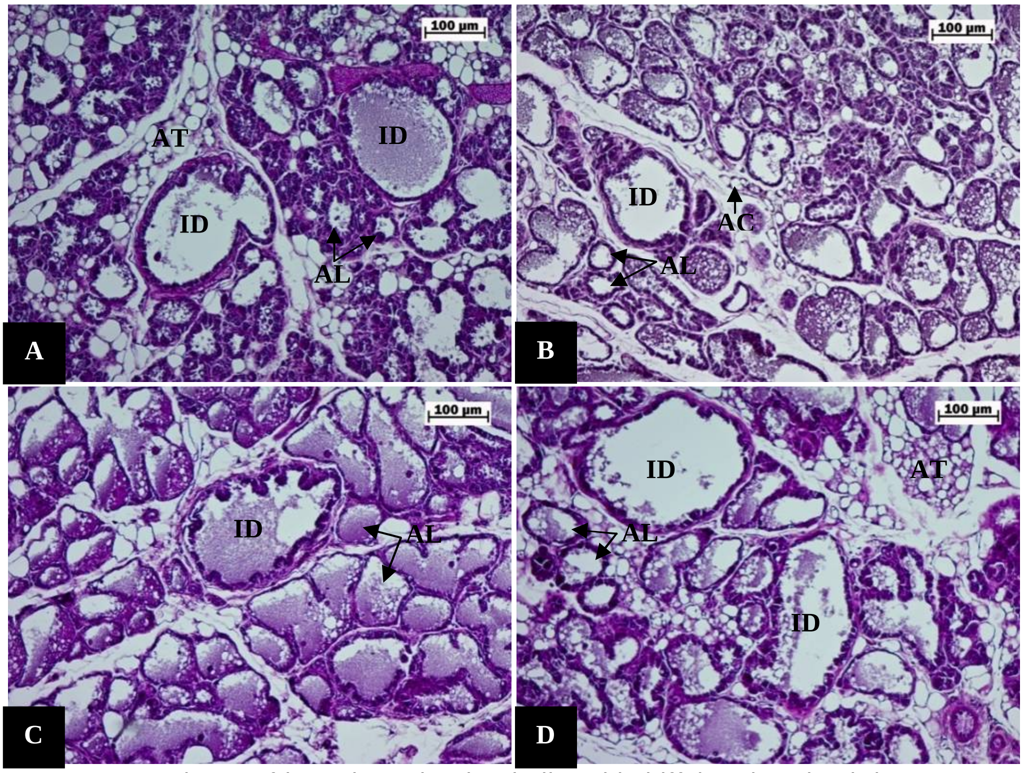

Figure 3. Mammary tissue of lactating mice (periodic acid shiff (PAS) stained, bar = 100 μm); A, negative control group received distilled water; B, positive control group received metoclopramide 2 mg/100 g BW; C & D, treated groups received *M. charantia* fruit juice 400 and 600 mg/100 g BW, respectively. (ID = intralobular duct; AT = adipose tissue; AD = adipocyte, AL = alveoli).

The mammary tissue of the control group exhibited small lobules filled with large amounts of adipose tissue and alveoli (Figure 2A). An increase in lobular size was found in groups receiving metoclopramide (2 mg/100 gBW) and the fruit juice (400 and 600 mg/100 g BW) (Figures 2B-2D). The lobules were packed with alveoli with a corresponding decrease in the adipose tissue as compared to the control group (Figure 3A). These alveoli are dilated and their lumens are filled with milk secretion, which was confirmed by positive results for PAS stained in the alveoli (Figures 3B-3D). The morphometrical study of mammary tissue is presented in Table 1.

Table 1

Effect of *M. charantia* fruit juice (MCJ) on morphometry of mammary gland in lactating mice

Treatment (mg/100 g BW) N = 6	*Mammary tissue ($\bar{X}$ ± SD)*			
	Adipose tissue (%)	*Alveoli (%)*	*Alveoli/ adipose tissue*	*Diameter of alveoli (μm)*
0	11.95 ± 2.56^{a}	88.05 ± 2.56^{a}	7.37	95.24 ± 3.32^{a}
Metoclopramide 2	$7.77 \pm 3.44^{a,b}$	$92.23 \pm 3.44^{a,b}$	11.07	105.33 ± 1.85^{b}
MCJ 400	$8.08 \pm 3.21^{a,b}$	$91.92 \pm 3.21^{a,b}$	11.37	103.64 ± 7.84^{b}
MCJ 600	5.05 ± 3.26^{b}	94.95 ± 3.26^{b}	18.80	106.35 ± 6.52^{b}

N = number of animals per group; same letter within column means non–significant difference (p > 0.05), different letter within column means significant difference (p < 0.05).

The diameters of alveoli were significantly increased in the group receiving metoclopramide and the groups receiving the fruit juice as compared to the control group ($p < 0.05$). The mammary tissue of the control group had content ratio of alveoli and adipose tissue of approximately 7.37. Meanwhile, there were 11.07, 11.37 and 18.80 in groups receiving metoclopramide and in both groups receiving fruit juice, respectively. This result supported the histological study that the fruit juice of *M. charantia* increased the growth of alveoli and decreased content of adipose tissue in mammary tissue. This implies that the fruit juice of *M. charantia* Linn. had a prolactin like action and lactogenic properties by inducing the growth and function of mammary tissue. This study conincides with previous works such as Sabins et al (1968), who reported that systemic administration of the extract of *Asparagus racemosus* rhizomes in weaning rats increase the volume of the milk secretion due to the action of released prolactin. Ushiroyama et al (2007) also claimed that Xiong-gui-tiao-xue-yin (a traditional herbal medicine) promote physiological lactation of humans by regulating secretion of prolactin. However, this study also found that the doses of *M. charantia* fruit used decreased mother weight when compared to the control group. The weight loss of mothers were shown in Figure 1. It may be caused by the increase in breast milk production. Lactogenic effect of herbs have been reported in many plants such as leaves of fenugreek (*Trigonella foenum graecum*), flowers of aniseed (*Pimpinella anisum*), flowers of borage (*B. officinalis*), seeds of rosselle (*H. sabdariffa*), rhizomes of shatavari (*A. racemosus*) and leaves of Torbangun (*Coleus amboinicus*) (Al-Saidi et al 2006; Okasha et al 2008; Behera et al 2013; Al-Khateeb 1996; Al-Saady 1997; Damanik et al 2006). However, galactogenic properties of *M. charantia* have not been reported before. This finding supports the belief of local Thai people in using the fruit of *M. charantia* for galactogenic augmentation during lactation.

Conclusions. It can be concluded that the fruit juice of *M. charantia* Linn. has a lactogenic activity by increasing blood prolactin level which is the principle lactogenic hormone. Meanwhile, it also induces growth and function of mammary tissue. Therefore, *M. charantia* fruit juice may be introduced as a galactagogue in lactating women.

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