Effect of Aqueous Extract of *Carica papaya* Dry Root Powder on Lactation of Albino Rats

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**Abstract**

Effects of *Carica papaya* root (CP) on lactation of rats were studied using the aqueous extract from dry powder in various doses for 15, 21 and 30 days. The lactating rats were fed with distilled water and the aqueous extract of CP twice a day at doses of 400, 500 and 1,000 mg/kg/day. The level of alkaline phosphatase (ALP), prolactin and total protein in mammary gland were measured. In case of rats fed with CP for 15 days, the average weights of mammary glands of fed rats at all doses were significantly higher (p<0.05) than those of the control groups. The level of ALP in rats fed with CP at 500 mg/kg/day was highest while the level of prolactin in rats fed with CP at 400 mg/kg/day was significantly higher than other groups. Rats fed with CP at 400 and 1,000 mg/kg/day contained higher protein content than the control groups. As for rats fed with CP at 400 mg/kg/day for 21 days had higher level of ALP and the average weight of their mammary glands was significant higher than the control groups. The levels of prolactin in rats fed with CP at 400 and 500 mg/kg/day were significant higher than the control ones. The mammary glands of rat fed with CP at all doses contained higher protein content than the control groups. For rats fed with CP for 30 days, there were no significant differences in weight of mammary gland, ALP, prolactin and protein contents among the fed and control rats. It was concluded that feeding lactating rats with CP for 15 and 21 days could activate higher lactating ability by increasing the weight of mammary gland, ALP, prolactin and protein content.

**INTRODUCTION**

In Thailand, papaya (*Carica papaya*) is widely grown and has herbal properties. Many Thai people found that *Carica papaya* root has effect on lactation (Mudchachip, 1991). It is therefore very interesting to study the effect of CP on mammary gland. Primary study using the aqueous solution from dry powder fed to rats for 15 days during lactation, indicated that the mammary gland weight of CP treated group was significantly higher than those of the control (Tossawanchuntra, 2000).

This study aims to study the effects of *Carica papaya* root on lactation of rats. Rats were fed with 3 doses of aqueous solution from dry CP powder for three different trials (15, 21 and 30 days). Oxytocin-receptor concentrations in the rat mammary gland were determined by alkaline phosphatase staining using histochemistry method (Suwanjarat, 1994). Oxytocin causes milk ejection by mammary gland (Crowley and Armstrong, 1992). Enzyme-linked immuno sorbent assay (ELISA) have been used in immunology to measure the quantity of prolactin (Signorella and Hymer, 1984). Prolactin caused the development of mammary gland. Kjeldahl method was used to determine the quantity of protein in mammary gland (Samasil, 2001).
MATERIALS AND METHODS

Carica papaya Root Powder

*Carica papaya* roots were incubated at 60°C until dry and ground to powder. Dry powder was dissolved in distilled water to obtain various concentrations.

Animal

Wistar rats were used in this experiment. Vaginal tissue was taken by vaginal smear to detect estrous cycle. Female rats were mated with male rats and sperms in vagina were detected. If sperms were present, it was counted as the first day of pregnancy. After the day of parturition, there would be one lactating rat per five pups. One hundred and twenty rats were divided into 3 sets of 40 rats. Each set was divided into 4 groups of 10 rats. The four groups were fed with distilled water, 400, 500 and 1,000 mg/kg/day of aqueous solution of dry powder of CP twice a day. The first, second and third sets were fed for 15, 21 and 30 days respectively.

Alkaline Phosphatase

Mammary gland was sectioned to 0.5 x 0.5 cm. and fixed with acetone at 4°C. Parafin sections were serially cut at 7 µm thick. Alkaline phosphatase activity was detected by calcium cobalt method by the appearance of black precipitation of cobalt sulfide. Determination of ALP activity was done by random selection of 160 fields from 32 sections and observed the black precipitation of cobalt sulfide at 4 level of intensities of black colour of the precipitates.

ELISA

Blood samples were taken from the heart. They were left at room temperature for 1 hour and centrifuged at 3,000 rpm for 30 minutes. Serum samples were assayed for prolactin by competitive indirect ELISA method as described by Signorella and Hymer (1984). The assay involved 8 steps: (1) Wells were coated with prolactin at the concentration of 20 ng/100 µL in coating buffer (at 4°C, 16 hours). (2) (2.1) Tubes containing 125 µL each of primary sample and 125 µL antibody (rabbit anti rat prolactin) dilution 1:10,000. (2.2) Standard curve: prolactin concentration 0, 10, 100, 1,000 and 10,000 ng/mL 125 µL/tube and 125 µL/tube rabbit anti rat prolactin (1:10,000) (at 4°C, 16 hours). (3) Wash plate 4 times with 200 µL/well of washing buffer. Add 100 µL/well 1.5% BSA into well (37°C, 1 hour) and wash 4 times. (4) Add 200 µL of the mixture from step 2 to well (step 3) (at room temperature, 4 hours) wash 4 times. (5) Added 50 µL of conjugate (horseradish peroxidase conjugated goat anti-rabbit immunoglobulin (HRP), 1:1,000) (at room temperature, 2.5 hours) wash 5 times. (6) Add 200 µL of substrate OPD for HRP in the dark room temperature. (7) Terminate the reaction by adding 50 µL of 4 N sulfuric acid to each well. (8) Measure the absorbance at 492 nm.

Kjeldahl Method

Protein content was estimated by Kheldahl method. One gram of mammary gland tissue was digested by adding 10 ml concentrated sulfuric acid and 5 g selenium reaction mixture enzyme. The digestion was done with a digestion unit (BUCHI Model 426) at 110°C for 90 min. When the temperature of mixture reached the room temperature, 20 ml distilled water and 32% sodium hydroxide were added. The reaction mixture was distilled using a distillation unit (BUCHI Model 323) for 5 min. Distilled solution was dropped into 50 ml solution A (2% boric acid, 0.1% methyl red and 0.025% methylene blue) and titrated with standard 0.1 N hydrochloric acid until the color of reagent became purple. Protein content was calculated from the following formula:
Protein content (% in 100 g of mammary gland) = $\frac{NV \times 1.4 \times 6.25}{W}$

Where $N =$ concentration of HCl (N)
$V =$ volume of HCl used for titration (ml)
$W =$ weight of mammary gland

RESULTS AND DISCUSSION
The average weights of mammary glands in the 15-day feeding group, the average weight of mammary gland of rat fed with CP at every dose was higher than that of the control group ($p<0.05$) (Fig. 1) which agrees with our previous work (Tossawanchuntra, 2000). For the 21-day group, only rat fed with CP at dose 400 mg/kg/day had higher mammary gland weight than the control group ($p<0.05$). On the other hand, there was no significant difference in mammary gland weight among rat fed with distilled water and CP at every dose for 30 days (Fig. 1). It was concluded that CP could be used for increasing weight of mammary gland during the first 21 days of lactation.

Alkaline Phosphatase
Table 1 shows the level of alkaline phosphatase (ALP) in mammary gland of rat fed with CP at various doses and periods. It could be noticed that mammary glands of rats fed with CP for 15 and 21 days had higher ALP than those of the control groups, especially the groups that were fed with CP at 500 mg/kg/day for 15 days and at 400 mg/kg/day for 21 days. For the rats fed with CP for 30 days, their mammary glands contained slightly higher level of ALP than those of the control group. The increase of ALP indicated that feeding the rat with CP for 15 and 21 days could activate lactation. The level of ALP was directly related to the increase of oxytoxin-receptor in mammary gland which plays an important role in lactation (Soloff and Weider, 1983).

Prolactin
In the 15-day group, rats fed with CP at 400 mg/kg/day had serum with higher amount of prolactin than that of the control group ($p<0.05$). In the 21-day group, serum of rats fed with CP at doses of 400 and 500 mg/kg/day contained higher amount of prolactin than that of the control ($p<0.05$). However, in the 30-day group, serum of rats fed with CP at 400 mg/kg/day showed only slightly higher amount of prolactin (Fig. 2). This increase of prolactin, the key substance in milk biosynthesis during lactation (Flint and Gardner, 1994), indicated that feeding with CP for 15 and 21 days was able to increase the degree of lactation.

Protein
In the 15-day group, rats fed with CP at 400 and 1000 mg/kg/day had mammary glands with higher amount of protein than that of the control group ($p<0.05$) (Fig. 3). Mammary glands of rats fed with CP at every dose contained higher amount of protein than of control ($p<0.05$). For the 30-day group, there was no significant difference in protein content in mammary glands of the control and CP-fed rats. However, the increasing trend of protein content in CP-fed rats could be noticed. The increasing protein content in the mammary gland during lactation is the index for the increasing degree of lactation (Greenbaum and Slater, 1956). The increase of protein content in this experiment agreed with the increase of mammary gland weight and prolactin amount. It could be concluded that CP was able to activate the mammary gland of lactating rat to promote higher quality and amount of lactation.

CONCLUSION
The aqueous solution from dry powder of CP given to the rats for 15 and 21 days could improve milk production and secretion. The mammary gland weight, ALP, prolactin and protein in the mammary gland also increased. It tended to improve
mammary gland activity. However, in 30 day group the result was not obvious.

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Literature Cited


**Tables**

Table 1. Level of alkaline phosphatase (ALP) in mammary gland of rat fed with distilled water (control) and the aqueous extract of *Carica papaya* (CP) at various doses for 15, 21 and 30 days.

<table>
<thead>
<tr>
<th>Feeding Period (days)</th>
<th>CP Dose (average from 10 rats)</th>
<th>Level of alkaline phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>control</td>
<td>+++♠</td>
</tr>
<tr>
<td></td>
<td>400 mg/kg/day</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>500 mg/kg/day</td>
<td>+++</td>
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<td>1,000 mg/kg/day</td>
<td>++</td>
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<tr>
<td>21</td>
<td>control</td>
<td>++</td>
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<tr>
<td></td>
<td>400 mg/kg/day</td>
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<td>500 mg/kg/day</td>
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<td></td>
<td>1,000 mg/kg/day</td>
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<tr>
<td>30</td>
<td>control</td>
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<tr>
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<td></td>
<td>500 mg/kg/day</td>
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<td></td>
<td>1,000 mg/kg/day</td>
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</tr>
</tbody>
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♠ +++ mean highest level of alkaline phosphatase

**Figures**

Fig. 1. Weight of mammary gland of rat fed with distilled water (control) and the aqueous extract of *Carica papaya* (CP) at various doses for 15, 21 and 30 days.
Fig. 2. Amount of prolactin in mammary gland of rat fed with distilled water (control) and the aqueous extract of *Carica papaya* (CP) at various doses for 15, 21 and 30 days.

Fig. 3. Protein content of mammary gland of rat fed with distilled water (control) and aqueous extract of *Carica papaya* (CP) at various doses for 15, 21 and 30 days.