The Expression of Prolactin and Oxytocin Genes in Lactating BALB/C Mice Supplemented with Mature Sauropus androgynus Leaf Extracts

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Abstract. Breastfeeding is a natural way of providing young infants with the nutrients that they need for healthy growth and development. Breast milk is the best form of nutrition for infants before they are able to eat and digest other foods. Sauropus androgynus is believed to increase breast milk production during lactation and is traditionally consumed by Indonesians. A total of 24 lactating Mus musculus strain BALB/c were divided into three groups of eight. During the lactation period, they were given different dosage treatments for 12 days. The first and second groups received a dosage of mature S. androgynus leaf extracts 173.6 mg/kg and 868 mg/kg mice body weight, respectively. The third group, as the control group, did not receive any mature S. androgynus leaf extracts. The expression of oxytocin gene in the lactating mice supplemented with 173.6 mg/kg and 868 mg/kg mice body weight mature S. androgynus leaf extracts increased significantly by 22.02-fold and 46.39-fold, respectively compared to the control group (p < 0.05, ANOVA). The prolactin gene expression level in the mice group which were supplemented with 173.6 mg/kg mice body weight mature S. androgynus leaf extracts increased significantly 14.65-fold compared to the controlled ones, whereas the second group which were supplemented 868 mg/kg increased significantly 2.42-fold compared with the controlled ones (p < 0.05, ANOVA). The increased gene expression in both treatments could be related to papaverine content in mature S. androgynus leaves that relaxed the smooth muscles and dilated the blood vessels. This also caused a smoother circulation of oxytocin hormone through the bloodstream.

Keywords: Sauropus androgynus, prolactin, oxytocin, qRT-PCR, papaverine.

1. Introduction

Breastfeeding is a natural way of providing young infants with the nutrients that they need for healthy growth and development. Breast milk serves as the best form of nutrition for infants before they are able to eat and digest other foods. It contains all of the nutrients, antibodies, hormones, immune factors, and antioxidants that an infant needs to grow during the first six months of life. Breast milk continues to provide the main nutrients source for normal growth, development and immunological protection and is recommended to remain the source of nutrition for the babies for at least two years.

However, a survey in Indonesia reported that 38% mothers stop giving breast milk because of the lack of breast milk itself [1]. Difficulty of milk production was caused by so many reasons, such as mother’s psychology factor and nutrition. Therefore, many traditional plants are consumed by lactating mother to increase the breast milk production. One of them is Sauropus androgynus, which is also known as katuk in Indonesia. S. androgynus is a shrub that belongs to Euphorbiaceae family. Results on an earlier research showed that oral infusion of S. androgynus leaves can increase the quantity of milk production in mice [2]. It was also reported that the S. androgynus leaf extract can increase the mother’s breast milk production up to 50.7% without decreasing the quality of the breast milk [1].

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The cyclical process of milk synthesis and secretion, which is called as lactation, occurs with the help of two hormones, prolactin and oxytocin. Prolactin hormone is released from the pituitary in response to nipple stimulation during suckling. Prolactin is involved in milk production and is thought to act on the central nervous system to promote maternal behaviour. Oxytocin is a nonapeptide hormone which is synthesized primarily in the supraoptic nucleus (SON) and paraventricular nucleus (PVN) of the hypothalamus. It is transported to the posterior lobe of the pituitary gland and is released into the systemic circulation. Peripherally, oxytocin acts to control parturition and milk let down. It also has important central actions on behavior [3]. Soka et al. [4] reported that the lactating mouse group that was supplemented with mature S. androgyrns leaf extracts had significant increment in the expression of prolactin and oxytocin genes.

The objective of this study is to compare the two doses of mature S. androgyrns leaf extracts on the expression of prolactin and oxytocin genes in lactating BALB/c mice using Quantitative Reverse Transcription PCR (qRT-PCR) analysis.

2. Materials and Methods

2.1. Raw Material

Mature S. androgyrns leaves were obtained from Research Institute for Medicinal and Aromatic Plants (IMACRI) at Bogor, Indonesia. The mature leaves were picked from three main leaves, the eighth-, the ninth-, and the tenth-branch from top. They have dark green color. The size of the leaves was more than 2.0 cm in width, more than 5.0 cm in length. The leaves were prepared for freeze drying. They were placed on small trays and washed by water. Then, the trays were covered with aluminum foil and placed into the freeze dry machine. The leaves were freeze dried for about 72 hours. The dried leaves were pulverized using mortar and pestle until they became powder and were stored at 4 °C.

2.2. Quantification of Papaverine in S. androgyrns Leaf Extracts

The Papaverine content in S.androgyrns leaf extracts was quantified using High-Performance Liquid Chromatography [4].

2.3. Animal Preparations

A total of 24 lactating Mus musculus strain BALB/c were purchased from Rodentia Facility of PT. Bimana Indomedical, Bogor, Indonesia. These 24 mice were divided into three groups of eight. All of them were maintained in the single cage. During the lactation period, they were given different dosage treatments for 12 days. The first and second groups received a dosage of mature S. androgyrns leaf extracts 173.6 mg/kg and 868 mg/kg mice body weight, respectively. The third group, as the control group, did not receive any mature S. androgyrns leaf extracts. The extracts were given to the lactating BALB/c mice by oral gavage method every morning at the same time. On the 13th day, all of mice were euthanized. Then, the brains were collected and stored at -70 °C. All procedures were approved by Animal Care and Use Committee at PT. Bimana Indomedical, Bogor, Indonesia.

2.4. Total mRNA Extraction

Total mice cellular RNAs were extracted from brains using QIAzol Lysis Reagent (Qiagen, The Netherland) for cell lysis, QIAshredder (Qiagen, The Netherland) for homogenization, and RNeasy Lipid Tissue Mini Kit (Qiagen, The Netherland) for separation mRNA from lipid tissue. All procedures were done according to the manufacturer’s protocols. The purity and the concentration of RNA were determined using NanoDrop (Thermo Fisher Scientific, USA) by measuring the A260/280 and A260/230 values. Total RNA templates for qRT-PCR were diluted until 50 ng/µl. After the measurement was done, the RNA was stored at -20 °C.

2.5. Quantitative Reverse Transcription PCR (qRT-PCR)

qRT-PCR was performed in iQ5 Real-Time Detection System (BioRad, USA), using iScript One-Step RT-PCR Kit with SYBR Green (BioRad, USA). The primer sequences and cycle conditions used for the amplification of oxytocin-, prolactin-, and β-actin genes were performed as described by Soka et al. [4]. The master mix for qRT-PCR consisted of 12.5 µl 2x SYBR Green RT-PCR reaction mix, 0.75 µl forward primer 10 pmol, 0.75 µl reverse primer 10 pmol, 8 µl nuclease-free water, 2.5 µl RNA template 50 ng/µl, and 0.5 µl iScript reverse transcriptase for one-step RT-PCR.

2.6. Data Analysis

Gene expression level was calculated based on the value of a cycle threshold (Ct) using the formula [5]:

\[2^{-\Delta\Delta Ct}}\]
\[ \Delta Ct \text{ (treatment)} = Ct \text{ (treatment)} - Ct (\beta \text{-actin}) \]
\[ \Delta Ct \text{ (control)} = Ct \text{ (control)} - Ct (\beta \text{-actin}) \]
\[ \Delta \Delta Ct = \Delta Ct \text{ (treatment)} - \Delta Ct \text{ (control)} \]
Respective gene expression level = \(2^{-\Delta \Delta Ct}\)

3. Results and Discussion

In this study, the expression of prolactin and oxytocin genes in lactating BALB/c mice was measured using qRT-PCR method by its mRNA. Both of gene expressions were compared between mice groups that were supplemented with two different doses of mature \(S. \text{ androgynus}\) leaf extracts and water as control during the lactation period.

The result, as shown in Figure 1, indicated that the expression of oxytocin gene in the lactating mice supplemented with 173.6 mg/kg and 868 mg/kg mice body weight mature \(S. \text{ androgynus}\) leaf extracts increased significantly by 22.02-fold and 46.39-fold, respectively compared to the control group (\(p < 0.05, \text{ANOVA}\)). Based on the result, the mouse group that was supplemented with 868 mg/kg mature \(S. \text{ androgynus}\) leaf extracts had a higher increment of the oxytocin gene expression compared to the other groups. On the other hand, the prolactin gene expression level in the mouse group which were supplemented with 173.6 mg/kg mice body weight mature \(S. \text{ androgynus}\) leaf extracts increased significantly 14.65-fold compared with the controlled ones, whereas the second group which were supplemented with 868 mg/kg increased significantly 2.42-fold compared with the controlled ones (\(p < 0.05, \text{ANOVA}\)). This might indicate that a higher concentration of mature \(S. \text{ androgynus}\) leaf extracts lowered the level of prolactin’s gene expression.

The increase of both gene expressions might be due to a correlation between the increased hormone concentration during lactation period and papaverine content in \(S. \text{ androgynus}\) leaf. According to Neumann et al. [6], the intranuclear oxytocin is elevated during parturition and suckling, for about 10 days of lactation in rats. During puerperal lactation, prolactin levels fluctuate widely owing to a release in association with suckling. Papaverine is one of the metabolic secondary compounds in \(S. \text{ androgynus}\) leaf. In the previous research, Padmavathi & Rao [7] has reported that the papaverine content in 100 gram of \(S. \text{ androgynus}\) fresh leaves is about 580 mg. Soka et al. [4] also reported that papaverine in mature \(S. \text{ androgynus}\) leaf might be contained in a higher amount than the younger ones. This could be the reason for the higher expression level of prolactin and oxytocin genes in the group of mice that was supplemented with mature \(S. \text{ androgynus}\) leaf extracts. The papaverine concentration in this mature \(S. \text{ androgynus}\) leaf extracts was 6.3 \(\mu\text{g/mL}\).

According to Chadwick et al. [8], papaverine has been used to treat complications related to vasospasm during various neurosurgical operations. Therefore, as a vasodilator, papaverine can dilate the blood vessels so that the blood flow will increase. Thus, a higher concentration of papaverine can help the circulation of oxytocin hormone through the bloodstream become more smoothly.

The other content of \(S. \text{ androgynus}\) leaf that might give effect in the increasing of milk production is sterol [9]. This compound has a specific function in intracellular signal transduction [10]. As well as cAMP, sterol can act as secondary messenger in cell signaling process, which can relay signals from receptors on the cell surface to target molecules inside the cell. It relays the signals of hormones and growth factors, and causes some changes in the activity of the cell. Therefore, the sterol content in \(S. \text{ androgynus}\) leaf also help to increase the signal transduction of oxytocin hormone. According to Suprayogi et al. [11], the \(S. \text{ androgynus}\) leaf can increase the expression level of oxytocin gene.

![Fig. 1: The expression level of prolactin and oxytocin genes in lactating BALB/c mice that were given two doses of mature S. androgynus leaf extracts more than the control group (p < 0.05).](image-url)
**androgynus** leaf nutrients can also increase the milk production by increasing the glucose metabolism for lactose synthesis.

The lower expression of prolactin gene at higher dosage of mature *S. androgynus* leaf extracts might be related to dopamine. Dopamine plays a predominant role in the regulation of prolactin secretion. Through a direct effect on anterior pituitary lactotrophs, dopamine inhibits the basally high-secretory tone of the cell. It accomplishes this by binding to D2 receptors expressed on the lactotroph cell membrane, which results a reduction of prolactin exocytosis and gene expression by inhibiting cAMP/PKA signaling via Gi-mediated inactivation of adenylyl cyclase [12].

Dopamine- and cAMP-regulated phosphoprotein, Mr 32 kDa (DARPP-32), was initially identified as a major target for dopamine and protein kinase A (PKA). In other words, it is a major target for the cAMP/PKA signaling cascade [13, 14]. Activation of PKA stimulates DARPP-32 phosphorylation at Thr[34] and thereby converts DARPP-32 into a potent inhibitor of protein phosphatase-1 (PP-1) [14]. The inhibition of PP-1 thereby controls the phosphorylation state and activity of many downstream physiological effectors, including various neurotransmitter receptors and voltage-gated ion channels. Mice lacking DARPP-32 are deficient in their molecular, electrophysiological, and behavioral responses to dopamine, drugs of abuse, and antipsychotic medication, indicating an essential role for DARPP-32 in dopaminergic signaling [15].

Phosphodiesterases (PDEs), an enzyme which degrade cAMP and downregulate cAMP/PKA signaling, control the dopaminergic signaling. PDE10A predominantly regulates DARPP-32 phosphorylation, thus inhibits PP-1 and affects dopaminergic signaling [16]. Papaverine has function in inhibiting PDE10A, thus increases phosphorylation of cAMP-dependent substrates by activating cAMP/PKA signaling and leading to the inhibition of dopamine D2 receptor signaling [17]. Papaverine blocks the dopamine receptors, and then can stimulate prolactin release.

It is well established that prolactin affects its secretion by regulating its own hypothalamic control through a short-loop feedback mechanism [18]. Elevation of serum levels of prolactin increases hypothalamic dopamine synthesis and the concentration of dopamine in hypothalamo-hypophysial portal blood [19]. These statements provide an explanation on the higher gene expression level at the lower dosage compared to the higher dosage. The higher dose of mature *S. androgynus* leaf extracts is given, the higher papaverine is consumed. It enhances prolactin secretion, thus enhances dopamine secretion. A higher dopamine secretion causes an inhibition of prolactin secretion.

4. **Conclusion**

This study compared the gene expression levels between mice which were supplemented with mature *S. androgynus* leaf extracts either 173.6 mg/kg or 868 mg/kg mice body weight and non-treatment mice as control. The expression of oxytocin gene in lactating mice supplemented with 173.6 mg/kg and 868 mg/kg mice body weight mature *S. androgynus* leaf extracts increased significantly by 22.02-fold and 46.39-fold, respectively compared to the control group. On the other hand, expression of prolactin gene in lactating mice supplemented with mature *S. androgynus* leaf extracts 173.6 mg/kg and 868 mg/kg mice body weight increased significantly by 14.65-fold and 2.42-fold. The increasing of gene expression in both treatments could be related to papaverine content in mature *S. androgynus* leaves that will relax the smooth muscles and dilate the blood vessels, the circulation of oxytocin hormone through the bloodstream will be more smoothly.

Moreover, the prolactin gene expression was correlated inversely with the dosages of *S.androgynus* which were given to the mice. The higher concentration of papaverine consumed, the more prolactin was released. The release of prolactin enhances dopamine secretion, which inhibits prolactin secretion. It can be described as feedback inhibition mechanism of prolactin release dy was supported by Atma Jaya Catholic University of Indonesia Research Centre.

5. **Acknowledgements**

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6. **References**

