

Ethanollic Extract of *Pluchea indica* Less Leaf Increases Serum Growth Hormone in Lactating Rats

Rul Afyah Syarif^{1,2*}, Nungki Anggorowati³, Mia Munawaroh Yuniyanti¹,
Mae Sri Hartati Wahyuningsih^{1,2}

¹ Department of Pharmacology and Therapy, Faculty of Medicine Public Health and Nursing, Universitas Gadjah Mada, Indonesia

² Herbal Medical Center, Faculty of Medicine Public Health and Nursing, Universitas Gadjah Mada, Indonesia

³ Department of Anatomical Pathology, Faculty of Medicine Public Health and Nursing, Universitas Gadjah Mada, Indonesia

ABSTRACT

The objectives of the study were to evaluate the effect of ethanolic extract of *P. indica* Less leaf (EPI) on serum growth hormone (GH), milk yield, body weight gain of dams, and dam's organ weight in lactating rats. A total of 25 lactating rats with six pups were randomized and distributed to one of five treatments (control (RO water), standard (domperidone 2,5 mg/kg BW/day), EPI 250 (250 mg/kg BW of EPI), EPI 500 (500 mg/kg BW of EPI), and EPI 750 (750 mg/kg BW of EPI)). The treatment was administered daily starting from the 2nd until the 15th day of lactation. On the 16th day serum growth hormone level, body and organ weight of rats were measured. Serum GH levels in the EPI 750 group (1963.25 ± 360.91 pg/ μ L) increased significantly compared to the domperidone (409.46 ± 28.80 pg/ μ L) and the control group (723.40 ± 95.78 pg/ μ L, $p < 0.05$). The total milk yield in the EPI 750 group was not significantly higher when compared to the control group ($p > 0.05$). There was significant body weight gain in the EPI 750 group compared with the domperidone group. There was no significant difference in organ weight gain in each group. The study revealed that ethanolic extract of *P. indica* Less leaf can increase serum growth hormone in lactating rats.

Keywords: *Pluchea indica*; growth hormone; milk production; body weight

INTRODUCTION

Pluchea indica (L.) Less is a wild perennial plant that grows in South-East Asia, India, Philippines, and Australia. The common names are *luntas* (Javanese), *beluntas* (Indonesia, Malaysia), and *kalapini* (Philippines). In Indonesia and Malaysia, the leaves of *P. indica* are empirically used as a medicine for abdominal pain, a cough medicine (Valkenberg *et al.*, 2001), and an appetizer (Susila, *et al.*, 2012). In India and Indo-China, fresh leaves and roots are used especially as diaphoretic and antipyretics (Valkenberg *et al.*, 2001). The fresh leaves possess an aromatic and astringent taste and are used as a vegetable in South-East Asia (Suriyaphan, 2014).

Pharmacological studies found that *P. indica* induces nasopharynx cancer cells apoptosis (Kao *et al.*, 2015), has anti-inflammatory (Buapool *et al.*, 2013) and antibacterial activities (Pargaputri *et al.*, 2016), inhibits spermatogenesis (Amalina *et al.*, 2010), and has antidiabetic activity (Widyawati *et al.*, 2015). In Yogyakarta, Indonesia, the leaves of *P. indica* are one of the medicinal plants used as a

galactagogue, an agent to induce, maintain, and augment maternal milk production (Sumanth & Narasimharaju, 2011) beside the famous one, *katuk* (*Sauropus androgynous*) leaves (Nahdi *et al.*, 2016; Sa'roni, 2004).

The main hormone which regulates milk secretion is prolactin. Other hormones that play important roles in milk production are somatotropin or growth hormone (GH), estrogen, progesterone, insulin, leptin, oxytocin, and thyroid releasing hormone (Tabares, 2014). A study performed by Alamer and Basiouni (2005) found an increase in milk production in goats feeding with fenugreek (*Trigonella foenum graecum*). This paralleled an increase in serum GH, which is synthesized by somatotrophs cells in the anterior pituitary. The release and production of GH are controlled by growth-releasing hormones and somatostatin produced in the hypothalamus. It is known that GH is essential for the growth process and increases protein synthesis in peripheral tissue especially bone, muscle, and connective tissue. This study aimed to evaluate the effect of ethanolic extract of *P. indica* Less leaf (EPI) on serum GH, milk production, body weight gain of dams, and dam's organ weight in lactating rats.

*Corresponding author : Rul Afyah Syarif
Email : rulafyah@ugm.ac.id

METHODOLOGY

This study was experimental laboratory research conducted at the Pharmacology and Therapy Laboratory, Faculty of Medicine Public Health and Nursing, Universitas Gadjah Mada. The study was approved by the Medical and Health Research Ethics Committee Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada (No KE/FK/0478/EC/2020).

Preparation of ethanolic extract of *P. indica* leaf

P. indica Less leaves were collected from the Special Region of Yogyakarta, in March 2020 and identified in the Pharmaceutical Biology laboratory, Faculty of Pharmacy, Universitas Gadjah Mada (No 12.18.08/UN1/FFA/BF/PT/2020). The leaves were dried in an oven at 50° C then powdered by mechanical grinding. The powder (200 g) was macerated by soaking in 70% ethanol for 24 hours. The filtrate was filtered using vacuum filtration and the residue was re-macerated twice by renewing the solvent. The filtrates were combined and evaporated to dryness at room temperature, and 40 g of *P. indica* extract (or 20% of extract rendement) was obtained from the maceration. Rendement of the extract is determined by dividing the final weight (weight of extract produced after evaporation) with the initial weight (weight of biomass used) multiplied by 100%.

Animals

The Wistar pregnant rats, 6-8 weeks old, were obtained from the Faculty of Pharmacy, Universitas Gadjah Mada. The animals were housed in an animal room with a temperature of 25-26°C, 12/12 of the light-dark cycle, humidity 69-70%, and given free access to food and water. The day when female rats delivered their pups was designated as Day 1 of lactation.

Dams body weight measurement

Twenty-five lactating rats weighing 150-250 g were divided into five experimental groups of five rats each (n=5) and administered 2 mL of reverse osmosis waters as controls, domperidone 2.5 mg/kg BW as standard, and ethanolic extract of *P. indica* Less (EPI) at 250, 500, and 750 mg/kg BW orally, respectively. Each dam was adjusted to suckle only six pups per litter. The animals were treated daily at 6:00 pm starting from the 2nd day to the 15th day of the lactation period. The dams were fed AD-II pellets at 10 g/100gBW daily, morning and evening. Every morning the bodyweight of the dam and pups was measured

at 7:00 am with an electronic balance (Mettler, Toledo) with 0.01 g accuracy. Weight gain of dams was calculated by the difference between the 16th day bodyweight and the 2nd day body weight.

Relative organ weight

On day 16th rats were euthanized and organ weights were measured using an electronic balance. The relative organ weight (brain, heart, lung, pancreas, kidney, spleen, ovary, and uterus) of each dam were calculated as:

$$\frac{\text{absolute organ weight (g)}}{\text{bodyweight of rat on sacrifice day (g)}} \times 100$$

Total milk yield

The calculation of milk production followed the method of Lompo-Ouedraogo *et al.* (2004). Each dam was limited to breastfed only 6 pups. On the second day of labor, the weight of the dams and pups were weighed and continued with treatment. Milk production was measured at 18 and 23 hours after treatment. At 07:00 the pups were weighed (W1) then separated from the dam for 4 hours. At 11:00 the pups were weighed (W2) then reunited to the dam to be breastfed for 1 hour. After being breastfed, at 12:00 the pups were weighed again (W3). Pups were separated again for 4 hours. At 16:00 the pups were weighed (W4) and reunited with the dam for 1 hour to be breastfed. At 17:00 the pups were weighed again (B5) then reunited with the dam until the morning.

The milk produced after 18 hours of treatment was calculated from W3-W2. Milk produced in a day was corrected for weight loss due to the metabolic process during breastfeeding using the formula $\frac{W2-W1}{4}$. This value was multiplied by the number of hours fed per day and then added to the weight gain (amount of milk obtained) due to breastfeeding.

The milk produced after 23 hours of treatment was calculated from W5-W4. The amount of milk a day was corrected using the formula $\frac{(W2-W1) + (W4-W3)}{8}$. This value was multiplied by the number of hours fed per day and then added to the weight gain (amount of milk obtained) due to breastfeeding. The daily weight gain was calculated from body weight measurements at 07.00. The scales used are capable of measuring up to a precision of 0.01 g.

Growth Hormone measurement

Growth hormone level in serum was measured using rat Growth Hormone Elisa kit (AB clonal, catalog no. RK03678) under the manufacturer's instructions.

Table I. Milk yield 18 and 23 hours after treatment

Group	Milk yield (g/litter/day)		P-value T-test
	18 h	23 h	
Control	4.80 ± 0.56	6.35 ± 0.54	p>0.05
Domperidone	5.50 ± 0.70	7.17 ± 0.91	p>0.05
EPI 250	5.09 ± 0.50	6.20 ± 0.62	p>0.05
EPI 500	5.22 ± 0.52	6.53 ± 0.70	p>0.05
EPI 750	4.72 ± 0.61	6.82 ± 0.93	p>0.05

Values are presented as mean ± SEM. No statistical difference among groups (Anova followed by LSD post hoc test, p<0.05) and between 18 and 23 hours after treatment (independent t-test)

Table II. Serum growth hormone level of lactating rats treated ethanollic extract of *P. indica* Less.

Group	Before	After	P- value
Control	715.78 ± 84.86	723.40 ± 95.78	NS
Domperidone	606.82 ± 151.28	409.46 ± 28.80	NS
EPI 500	811.88 ± 254.90	702.68 ± 38.87	NS
EPI 750	612.85 ± 135.29	1963.25 ± 360.91	P<0.1

NS: not significant

Statistical analysis

IBM SPSS statistic version 25 was used for statistical analysis. The results were expressed as the mean and standard error of the mean (SEM). The differences of the mean for weight gain, organ weight, and growth hormone level among groups were analyzed using a one-way analysis of variance (ANOVA). The mean difference of growth hormone level before and after treatment was analyzed using paired sample t-tests. Differences were considered significant at p < 0.05.

RESULTS AND DISCUSSION

This study aimed to determine the effect of ethanollic extract of *P. indica* Less on total milk yield and serum GH levels. Milk yield per day was calculated after 18 and 23 hours of treatment based on pups' body weight gain. Domperidone was chosen as a positive control because, as a dopamine receptor antagonist, it has the effect of increasing the amount of breast milk production (Grzeskowiak *et al.*, 2018). The milk yield was increased in domperidone and EPI-treated groups at both 18 and 23 hours after treatment. The highest milk yield was found in the group given domperidone (5.50 ± 0.70 and 7.17 ± 0.91 g/litter/day at 18 and 23 hours after treatment, respectively). EPI 750-treated group produced a milk yield, at 23 hours after treatment of 6.82 ± 0.93, higher than the control group (6.35 ± 0.54 g/litter/day) although there was no significant difference. There was a tendency for higher milk yield in the higher dose of EPI (Table I).

The effect of EPI administration on GH levels is presented in Table II and Figure 1. After 14 days of treatment, the EPI 750 serum GH level was significantly higher (p < 0.05) compared to the control group even though the SEM of EPI 750 GH level was high. This may be due to the sample size of this study which was not large enough or human error while doing the Elisa assay procedure. There was no significant difference in GH levels between before and after treatment in the control, domperidone, and EPI 500 groups although hormone levels after treatment were found to be lower in the domperidone and EPI 500 groups.

The increase of serum GH level in EPI 750 may be related to phytoestrogen compounds contained in *P. indica* as demonstrated in the results of research conducted by Jeng *et al.*, (2010) who found that genistein and resveratrol phytoestrogens administrated for 10 weeks caused a 6-fold increase in rat GH levels. The phytoestrogens contained in *P. indica* include quercetin (Valkenburg & Bunyapraphatsara, 2001). The effect of phytoestrogens on the increase of GH level may depend on the concentration of serum phytoestrogens because genistein which had higher serum concentration increased serum GH levels and this did not occur at low concentrations of daidzein (Jeng *et al.*, 2010). Thus, the increase of milk yield in the group receiving EPI may be correlated with the increase of serum GH levels.

Growth hormone is needed for the growth and development of the mammary glands during

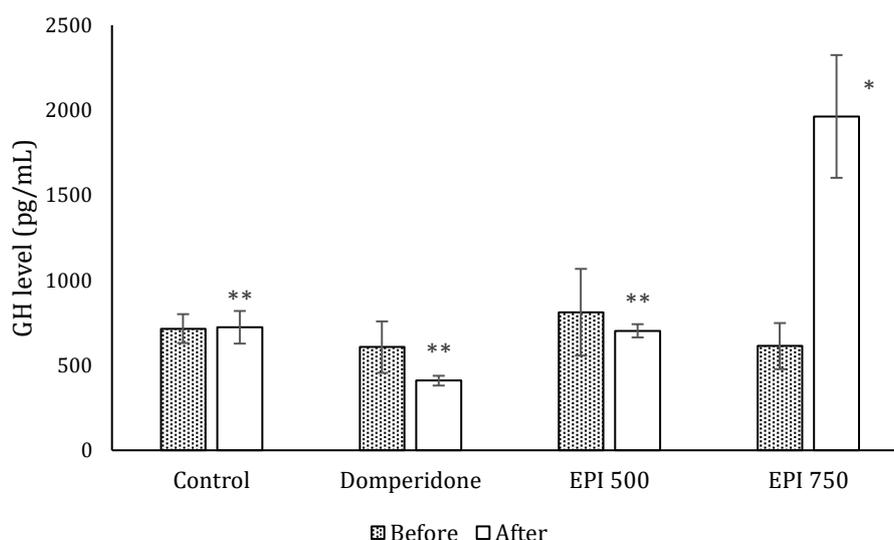


Figure 1. Serum growth hormone level of lactating rats treated ethanol extract of *P. indica* Less. Values were presented as mean \pm SEM.

* statistically significant difference with control; ** statistically significant difference with EPI 750 (Anova followed by LSD post hoc test, $p < 0.05$).

Table III. Body weight gain of Wistar rat dams that received ethanol extract of *P. indica* Less

Group	Dams body weight gain(%)
Control	3.43 \pm 1.83
Domperidone	0.66 \pm 2.93
EPI 250	1.18 \pm 3.67
EPI 500	-1.71 \pm 1.59
EPI 750	8.44 \pm 3.04*

Values are presented as mean \pm SEM. * statistically significant difference with domperidone group (Anova followed by LSD post hoc test, $p < 0.1$)

pregnancy and breastfeeding. Research conducted by Madon *et al.* (1986) showed that in the absence of prolactin, GH can maintain milk production by as much as 50%. The absence of stimulation of GH and prolactin causes breast milk to not be produced. Increasing levels of GH will increase the proliferation and differentiation of mammary myoepithelial cells, thereby increasing milk production (Ni, 2020). The effect of GH in increasing milk production acts through the somatotrophic axis by increasing insulin-like growth factor-1 (IGF-1) expression (Bao *et al.*, 2016). Our study found that there was no significant difference in GH levels between before and after treatment in the domperidone group and the GH level in the domperidone group was lower than EPI 750. These findings are likely because domperidone increased milk production, not through the somatotrophic axis, but the lactotropic axis by increasing prolactin secretion.

Bodyweight gain of dams in the domperidone, EPI 250, and EPI 500 groups were insignificantly lower than in the control group. A study reported an increased weight gain in 12% of mothers taking domperidone to enhance milk production. The weight gain is related to the domperidone's ability to increase appetite (Hale *et al.*, 2018). Our study showed that domperidone did not increase weight gain because the study was conducted on animals that have different biological characteristics than humans. The EPI 750 group body weight gain was greater than the control or domperidone groups. There was no significant difference in body weight gain between groups ($p > 0.05$) except for the EPI 750 group with domperidone ($p < 0.1$). Thus, the highest dose of the extract (EPI 750) caused the greatest increase in body weight compared with the lower dose extracts (EPI 250 and EPI 500) (Table III). Studies conducted both in animals and humans indicated

Table IV. Relative organs weight of Wistar rats after received ethanollic extract of *P. indica* Less

Organ	Control	Domperidone	EPI 250	EPI 500	EPI 750
Brain	0.81 ± 0.03	0.75 ± 0.03	0.79 ± 0.04	0.80 ± 0.03	0.84 ± 0.04
Heart	0.36 ± 0.02	0.36 ± 0.01	0.36 ± 0.02	0.37 ± 0.01	0.36 ± 0.01
Lungs	0.66 ± 0.03	0.62 ± 0.04	0.64 ± 0.03	0.58 ± 0.04	0.64 ± 0.03
Liver	4.34 ± 0.16	4.19 ± 0.15	4.65 ± 0.29	4.86 ± 0.28	4.60 ± 0.10
Spleen	0.31 ± 0.03	0.32 ± 0.05	0.29 ± 0.04	0.33 ± 0.02	0.26 ± 0.02
Kidneys	0.78 ± 0.02	0.79 ± 0.01	0.77 ± 0.02	0.80 ± 0.05	0.84 ± 0.04
Pancreas	0.42 ± 0.07	0.41 ± 0.04	0.34 ± 0.07	0.45 ± 0.07	0.44 ± 0.07
Ovaries	0.07 ± 0.00	0.06 ± 0.00	0.16 ± 0.09	0.16 ± 0.09	0.07 ± 0.00
Utery	0.18 ± 0.07	0.12 ± 0.02	0.23 ± 0.08	0.13 ± 0.01	0.13 ± 0.00

that phytoestrogens cause weight loss because phytoestrogens inhibit life cycle adipocytes and reduce fat accumulation by inhibiting the life cycle of adipocytes (Desmawati and Sulastri, 2019). However, it did not apply to the highest dose of *P. indica* in this study.

Table IV shows that there was no significant difference in relative organ weight between the groups given domperidone or the extract compared to the control group for organs of the brain, heart, spleen, kidney, pancreas, ovaries, and uterus. This result was not different from another study that found organ weights of rats were not significantly affected by phytoestrogens (Jeng *et al.*, 2010).

The results showed that GH levels, weight gain, and milk production were higher in the EPI 750-treated rats (rats receiving *P. indica* extract at dose 750 mg/kgBW). The maximal dose of extract given to the rats in this study is safe since the LD50 of methanollic extract of *P. indica* in rats was 3.2 g/kgBW (Pramanik *et al.*, 2006). Growth hormone may act as a mediator of EPI on milk production and weight gain. GH will bind to the receptors and will increase the production of IGF-1 in the liver, thereby increasing growth in the organs. Apart from going through the IGF-1 pathway, GH will also increase cell growth by binding to the GHR receptors in the endoplasmic reticulum which activates the JAK-STAT signal. JAK-STAT signals will increase the expression of genes related to growth (Hull, 2014).

Tabares *et al.* (2014) stated that the direct effect of GH on milk secretion in mammary glands is proposed to be mediated indirectly via IGF-1. GH acts similar to Recombinant Bovine Somatotropin (rBST), a synthetic hormone analog, which binds to somatotropin receptors in mammary epithelial cells and then stimulates JAK2/STAT5 pathway. The binding of IGF-1 to GF-1R forms IGF-1R/IGF-1 complex that upregulates Ras/Raf/(MAPK/ERK) and IRS/PI3K/(AKT/PKB)/mTOR pathways.

They will induce mammary epithelial cells proliferation and increase milk protein synthesis (Tabares *et al.*, 2014). To know the action mechanism of *P. indica* in JAK2/STAT5 pathways, further research is needed.

Milk secretion is not only affected by GH but also affected by the development of the mammary gland during pregnancy which is regulated by systemic hormones such as prolactin, estrogen, progesterone, insulin, glucocorticoids, and triiodothyronine (Javid & Lteif, 2013) and local factors produced by stromal cells, including fibroblast and epidermal growth factors and IGF-1 (Sternlicht *et al.*, 2006). A further study on the factors that influenced milk production needs to be conducted to get confirming evidence to develop *P. indica* as a candidate of galactagogue herbal medicine.

CONCLUSIONS

Ethanollic extract of *P. indica* Less leaf increased serum growth hormone and tended to increase milk production in lactating rats. Future research on the action mechanism and factors influencing the production and secretion of milk should be conducted to obtain the scientific evidence required to develop the potential of the herbal medicine of *P. indica*.

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