

## Prenatal Development of Fetus in Mice Treated with Leaves Extract of Jeruju (*Acanthus ilicifolius* L.)

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### ABSTRACT

Jeruju (*Acanthus ilicifolius* L.) is perennial shrub of wet land areas that have long been used as traditional medicine in Indonesia. The aim of the study was to determine the effect of jeruju leaf extract given to pregnant mice (*Mus musculus* L.) on the prenatal development of the fetus. By using a completely randomized design four groups of female mice (5 mice each) were treated orally with jeruju leaves extract of different dosage namely: 3.75 mg/30g BW; 7.5 mg/30g BW; 15 mg/30 g BW and distilled water as control. The results showed that administration of jeruju leaves extract had a significant effect on decreasing the average length of the cranium, sternum, tails, anterior and posterior extremities in fetal mice. Jeruju leaf extract given to pregnant mice at a toxic dose, 15 mg/30grBB, was potentially to negatively affect prenatal development in fetal mice.

**Keywords:** Jeruju, *Acanthus ilicifolius*, fetal development, teratogenic effect, prenatal development

### I. INTRODUCTION

Currently, medicinal plants are still used as an alternative treatment for Indonesian people. The use of traditional medicinal plants from nature has been known for a long time from our ancestors from generation to generation. This traditional medicine is in great demand and is well developed as a safer alternative, provides lower side effects and provides optimal results when consumed according to the purpose of treatment [1].

One of the plant that has the potential as a medicine is jeruju (*Acanthus ilicifolius* L.). It was known that jeruju leaves are efficacious as aphrodisiacs (libido stimulants), asthma, diabetes, diuretics, hepatitis, leprosy, neuralgia, roundworms, rheumatism, skin diseases, stomach aches, antifertility, tumors and ulcers (resin) [2]. Jeruju plants contain glucosides, alkaloids, flavonoids,

fatty acids, steroids, lignans, phenolic and terpenoid components [3].

In an effort to utilize the jeruju plant as a medicinal plant, it is necessary to carry out a safety test of the jeruju leaf extract, especially when consumed by pregnant women. Safety test is a test by giving certain factors or substances to see the effect on the fetus of the test animals due to the administration of these substances. Thus, this study was conducted to prove the effect of jeruju leaf extract (*Acanthus ilicifolius* L.) on cranium length, sternum length, tail length, anterior and posterior extremity length of fetal mice (*Mus musculus* L.).

### II. MATERIALS AND METHODS

#### 2.1 Preparation of jeruju leaf extract

Jeruju leaf extract obtained by evaporation method. Jeruju leaves are cleaned, washed, dried in the sun (without sunlight) to dry (oven). After drying, the jeruju is then ground to a powder. Then maceration is carried out by soaking 500 grams of jeruju powder in 5 liters of ethanol solution for 24 hours and then filtered [4]. The filtered liquid is then concentrated by evaporation using a rotary evaporator for 4 hours with a temperature of 50°C and a pressure of 120 atm. Then the extract (200 ml) obtained was considered as stock. All the process above conducted in November-December 2021 at the Zoology Laboratory, Faculty of Mathematics and Natural Sciences, University of Lampung.

#### 2.2 Preparation and treatment of test animals

Twenty male mice and 20 female mice weighing 30 grams each were mated after being acclimatized for a week. After pregnancy occurred, the mice were divided into 4 groups with each group consisting of 5 mice as replicates. On the 6th to 17th day of pregnancy, the mice were given jeruju leaf extract with the following treatment doses: The control was given 0.3 ml distilled water (K), a dose of 3.75 mg/30grBB in 0.3 ml distilled

water (P1), a dose of 7.5 mg/ 30grBB in 0.3 ml of distilled water (P2) and a dose of 15mg/30grBB in 0.3 ml of distilled water (P3).

### 2.3 Observation

Using a set of surgical tools on the 17<sup>th</sup> day of pregnancy, all mice from both the control and treatment groups were anesthetized using chloroform. Mice were dissected and the fetus was removed from the uterus, then cleaned with running water. Furthermore, observations were made of the parameters measured including cranium length, sternum length, tail length, fore extremity length and rear extremity length using vernier calipers.

### 2.4 Data Analysis

Research data was obtained to determine the differences of the effect between treatments, processed statistically using analysis of variance (ANOVA). If there is a significant difference, then a further test will be carried out with a significant difference test (LSD) at the 5% level.

## III. RESULTS AND DISCUSSION

### 3.1 Cranial development

Impact of the plant extract of jeruju on the prenatal cranial development parameters of mice fetus are presented in Table 1.

Table 1 Fetus cranial length of mice treated with jeruju leaf extract (*Acanthus ilicifolius* L.)

Dose (mg/30grBW)	Fetus cranial length (mm) X±SD*
Control (K)	13.420 ± 0.130 <sup>a</sup>
3.75 (P1)	12.340 ± 0.240 <sup>b</sup>
7.5 (P2)	11.620 ± 0.083 <sup>c</sup>
15 (P3)	10.540 ± 0.054 <sup>d</sup>

\*Values followed by the same superscript are not statistically different at 5% LSD test.

From Table 1, it is known that the administration of jeruju leaf extract affect cranial development of fetus by decreasing the length of the cranium with an increase in the dosage of the extract. This is thought to be due to flavonoid compounds.

### 3.2 Sternum development

Table 2 depicts the effect of the plant extract of jeruju on the prenatal sternum development parameters in fetal mice.

Table 2 Fetus sternum length of mice treated with jeruju leaf extract (*Acanthus ilicifolius* L.)

Dose (mg/30grBW)	Fetus sternum length (mm) X±SD
Control (K)	22.400 ± 0.158 <sup>a</sup>
3.75 (P1)	21.280 ± 0.178 <sup>b</sup>
7.5 (P2)	20.600 ± 0.100 <sup>c</sup>
15 (P3)	20.360 ± 0.089 <sup>d</sup>

\*Values followed by the same superscript are not statistically different at 5% LSD test.

It is clear from Table 2 that the plant extracts of jeruju exhibit negative effects on the prenatal development of the fetus sternum in a dosage base-manner. Jeruju leaf extract supposed to inhibit ossification of sternum. Ossification process accompanied with the accumulation of bone matrix as a critical stage that is susceptible to teratogens influenced by several tannins, saponins and alkaloids [5].

Based on previous study it was found that administration of bitter melon extract as much as 30 mg/30 gBW can cause a decrease in the length of the sternum in the fetus [6]. This is because

bitter melon contains alkaloids, flavonoids and saponins [7]. Alkaloid compounds and saponins cause a lack of osteoblasts in bone formation [8]. So that the decrease in the length of the sternum in the fetus is thought to be caused by the toxic compounds of alkaloids, flavonoids and saponins contained in jeruju leaves.

### 3.3 Tail development

Prenatal tail development parameters of mice following treatment of plant extracts of jeruju are presented in Table 3.

Table 3 Fetus tail length of mice treated with jeruju leaf extract (*Acanthus ilicifolius* L.)

Dose (mg/30grBW)	Fetus tail length (mm)	
	X±SD	
Control (K)	12.440 ± 0.114 <sup>a</sup>	
3.75 (P1)	12.320 ± 0.083 <sup>b</sup>	
7.5 (P2)	11.620 ± 0.083 <sup>c</sup>	
15 (P3)	10.400 ± 0.070 <sup>d</sup>	

\*Values followed by the same superscript are not statistically different at 5% LSD test.

From Table 3, it's found that the administration of jeruju leaf extract significantly affect the prenatal development of the mice tail where as the concentration of the extract increases the development of the tail is increasingly hampered.

This may occur due to the toxic factors contained in the extract of jeruju leaves. The toxicity of a chemical or compound can increase or decrease in an organism depending on the dose and duration of administration [9]. And the teratogenic properties of a substance depend on the dose of drug or chemical substance when the fetus is in the organogenesis phase [10].

Saponins are commonly known to have cytotoxic properties against cells that are undergoing development. Jeruju leaves contain flavonoids, alkaloids and saponins [11]. Thus, these cytotoxic compounds make the tail length of the fetus shorter than that of normal mice (control).

#### 3.4 Development of anterior fetal extremities

The teratogenic effects of plant extract of jeruju on the prenatal development of fetus anterior extremities were assessed by measuring the length of the forelimbs of the mice fetuses which results are presented in Table 4.

Table 4. Fore limbs length of mice treated with jeruju leaf extract (*Acanthus ilicifolius* L.)

Dose (mg/30grBW)	Fore limbs length of fetus (mm)	
	X±SD (Right)	X±SD (Left)
Control (K)	6.400 ± 0.158 <sup>a</sup>	6.400 ± 0.158 <sup>a</sup>
3.75 (P1)	6.300 ± 0.223 <sup>a</sup>	6.300 ± 0.223 <sup>a</sup>
7.5 (P2)	5.560 ± 0.089 <sup>b</sup>	5.560 ± 0.089 <sup>b</sup>
15 (P3)	5.080 ± 0.044 <sup>c</sup>	5.080 ± 0.044 <sup>c</sup>

\*Values followed by the same superscript are not statistically different at 5% LSD test.

Data in the Table 4 showed that the administration of jeruju leaf extract resulted in a decrease in the length of the anterior extremity of the fetus which was significantly different between the control and the treatment dose of 3.75 mg/30grBW (P1), a dose of 7.5mg/30grBW (P2) and a dose of 15mg /30grBW (P3), but there was no significant difference between the control (K) and the treatment dose of 3.75 mg/30grBW (P1).

However the length of the right and left anterior extremities were the same in length and not significantly different, so that the effect given by the toxic compounds from jeruju leaf extract worked equally on both the right and left anterior extremities.

The alkaloids, flavonoids and saponins contained in the nutmeg rhizome extract make the nutmeg rhizome extract have a cytotoxic effect

(Lawal and Adebola, 2009). Jeruju leaves also contain alkaloid compounds, flavonoids and saponins [12]

The decrease in the length of the anterior extremities of the fetus in this study was thought to be caused by these compounds. Additionally, the decrease in the length of the anterior extremities can be caused by an inhibited ossification process at the organogenesis stage [13].

#### 3.5 Development of posterior fetal extremities

Measurement results on the length of the hind limbs of mice fetuses following treatment of plant extract of jeruju in order to evaluate its teratogenic effects on the prenatal development of posterior extremities in mice are presented in Table 5.

Table 5 Hind limbs length of mice treated with jeruju leaf extract (*Acanthus ilicifolius* L.)

Dose (mg/30grBW)	Hind limbs length fetus (mm)	
	X±SD (Right)	X±SD (Left)
Control (K)	7.400 ± 0.158 <sup>a</sup>	7.400 ± 0.158 <sup>a</sup>
3.75 (P1)	6.320 ± 0.192 <sup>b</sup>	6.320 ± 0.192 <sup>b</sup>
7.5 (P2)	6.440 ± 0.089 <sup>b</sup>	6.440 ± 0.089 <sup>b</sup>
15 (P3)	5.440 ± 0.450 <sup>c</sup>	5.440 ± 0.450 <sup>c</sup>

\*Values followed by the same superscript are not statistically different at 5% LSD test.

From Table 5, it is known that the administration of jeruju leaf extract showed a significant effect on the length of the posterior extremities of the mice fetuses in comparison with the control. In addition, there were no different results between the right and left posterior extremities.

Based on the previous study reported by Rosa et al. (2016), it was found that feeding nutmeg rhizome extract to pregnant mice caused a decrease in the length of the posterior extremities in the fetus. Nutmeg rhizome extract contains alkaloid compounds [14], flavonoids and saponins. Alkaloids compounds were suggested to be able to stop zygote division at the metaphase stage so that the mitotic process stops [15].

Jeruju leaves contain alkaloids, flavonoids and saponins. Thus, this is the cause of a decrease in the posterior extremities in the fetus. In addition, a decrease in the length of the embryo's body segments, especially the length of the posterior extremities, can occur due to stress and other disorders which affect the lack of food, oxygen and blood intake in the embryo resulting in the fetus being born imperfect and small [9, 16].

#### IV. CONCLUSIONS AND RECOMMENDATIONS

These research findings suggest that administration of jeruju leaf extract to pregnant mice affect prenatal development of mice fetuses by decreasing in the length of the cranium, sternum, tails, anterior and posterior extremities. It is recommended therefore to carry out further investigation on the types and compounds in the pant extract of jeruju (*Acanthus ilicifolius* L.) which are likely to have a major effect on the growth and development of the fetus in mice (*Mus musculus* L.) and to review the dosage of the extract. jeruju leaves so that it can determine the dose which is very influential on the growth and development of the mouse fetus.

#### Compliance with ethical standards

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##### Disclosure of conflict of interest

The authors declare no conflict of interest.

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