

PHYTOCHEMICAL CONSTITUENT OF *Picria fel-terrae* Lour. ETHANOL EXTRACT AND ITS TERATOGENIC EFFECT

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ABSTRACT

The objective of this study is to determine phytochemical constituent in the ethanol extract of *Picria fel-terrae* leaves as well as to examine the teratogenic effect toward the fetus. There are five groups consists of pregnant rats. Group 1 was the healthy control; groups 2, 3, 4 received extract at the dose of 125, 500 and 1000 mg/kg respectively, group 5 was positive control group (received gabapentin 50 mg/kg). The treatment was performed during the organogenesis period. On the 19th day, maternal laparotomy was carried out, and the fetus was removed. Observations were including reproduction and uterus performance as well as external malformation. Phytochemical screening revealed that the extract consists of flavonoids, tannins, glycosides, saponins and steroids/triterpenoids. Administration extract at the dose of 125, 500, 1000 mg/kg revealed significant effect to the weight loss, fetal body length, number of dead fetuses, resorption and haemorrhages compared to healthy control ($p < 0.05$). The uterus abnormality was found in the group of extract dose of 500 mg/kg as well as 1000 mg/kg. All groups undergo external malformations. The research concluded that all doses of the ethanol extract of *P. fel-terrae* leaves had the teratogenic effect on the fetus.

Keywords: *Picria fel-terrae*, teratogenic, reproduction, organogenesis, uterus, fetus

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INTRODUCTION

Picria fel-terrae Lour. or pugun tanoh is one of the medicinal plants of the Linderniaceae species. Some researches have been carried out to this plant and reported has many activities such as a diuretic, acetylcholinesterase inhibitor, hepatoprotective, anticancer, antidiabetic, antiasthma as well as anthelmintic.¹⁻⁹

The various benefits derived from *P. fel-terrae* as a medicinal plant are potential to be developed into a safe and easy-to-use herbal medicine preparation including for pregnant women. To obtain herbal medicine which safe for human use, a series of toxicity tests are required. Several toxicity tests has been carried out to this extract including acute toxicity test and subchronic test. However, the teratogenic test has never been conducted before.¹⁰⁻¹² The teratogenic test is a specific toxicity test designed to evaluate the specific effects of a compound on a fetus during pregnancy. Since this extract has wide activities toward many diseases or disorders, therefore it is important to test the toxicity in the pregnancy. The period of organogenesis is a critical period in pregnancy because there is an extremely intensive cell differentiation process to form tissues and organs, making it very susceptible to teratogenic substances. Regarding that, it is essential to to evaluate the teratogenic effect of *P. fel-terrae* leaves ethanol extract on the reproduction during the organogenesis period.

EXPERIMENTAL

Plant Collection and Authentication

*Picria-fel terra*e Lour. leaves were obtained from Tiga Lingga Village, Dairi, North Sumatra and have been identified by Medanense Herbarium Number 993/MEDA/2017. The dried *P. fel-terrae* were macerated with ethanol for a couple of days protected from light while occasionally stirring. After that,

the macerate was collected and then concentrated using a rotary evaporator until obtained ethanol extract of *P. fel-terrae*.

Chemicals and Reagents

Gabapentin 300 (Novell Pharmaceutical Laboratories, Indonesia), sodium carboxymethyl cellulose (Sigma-Aldrich), methylene blue solution (Sigma-Aldrich) for the dye of vaginal smear, bouin solution for external malformation observation.

Phytochemical Screening

Phytochemical screening was conducted using a standard method for detection of alkaloids, flavonoids, tannins, glycosides, saponins and steroids/triterpenoids.¹³

Animal

Animals used in this experiment were healthy white female rats weighing 150-185 grams with an age of about 2-3 months in a nullipara state (at which has never given birth). These rats were acclimatized for 14 days. Recommendations for ethical research approval was from the Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara Number 499/KEPH-FMIPA/2017.

General Procedure

Determination of Estrus Cycle and Pregnancy

The fertile female rats were mated to male rats in the evening for one night. The next day the female rats were separated, and the vaginal smears were being checked. If a yellowish disc was found in the vaginal and the presence of sperm cells in the swab, it is marked as the day 0 of pregnancy.

Inside the vaginal, 0.9% NaCl was inserted. The vaginal fluid which was successfully obtained with the pipette then transported onto the object-glass to let it dry and then 0.1% methylene blue dye was added. Afterward, it was examined under a microscope with 10x10 magnification. Female rats in the estrus cycle were characterized by more dominant cornified epithelial cells than nucleated epithelial cells.¹⁴

Treatment

The rats which were proven to mate, were kept in individual cages and divided into healthy control received 0.5% CMC-Na, treatment groups received ethanol extract of *P. fel-terrae* with the dose of 125, 500 and 1000 mg/kg respectively, and the positive control group received gabapentin 50 mg/kg. The treatments were given during the organogenesis period which was during 6-15 days of pregnancy. On the 19th day, laparotomy was conducted to remove the fetus from the mother's uterus. The observation started from the adult rats reproductive performance including the development of the parent's body weight, fetus length and weight, the number of fetuses (life, death, resorption), haemorrhage, and the number of mothers with uterus abnormalities.¹⁴

Statistical Analysis

Data were analyzed by using ANOVA followed by Tukey test.

RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemical screening result showed that the ethanol extract of *P. fel-terrae* contained flavonoids, tannins, glycosides, saponins and steroids/triterpenoids.

Determination of Estrus Cycle and Pregnancy

Rat proved to mate, i.e. with sperm in the vaginal smears as seen in Fig.-1.

Litter Weight

The results showed that the litter in groups received *P. fel-terrae* leaves dose of 125, 500, 1000 mg/kg and the positive control group which received gabapentin 50 mg/kg undergo inhibition of weight gain for 10

days pregnancy i.e. 33.1; 33.8; 18.9; 27.7 grams consecutively, and significantly different with healthy control which has weight gain about 37.8 grams in 10 days ($p < 0.05$). The higher the dose, the higher the effect of reducing litter weight. It proves that *P. fel-terrae* leaves have an impact on litter body weight during pregnancy.

The Number of Living, Dead and Resorption of Fetuses

P. fel-terrae leaves ethanol extract dose of 1000 mg/kg affects up to 26.6% of fetal deaths with delayed growth and 10% of the resorption remnants in adult rats. This value was found to be the highest compared with other dose groups. Group of the dose of 500 mg/kg and gabapentin dose of 50 mg/kg each had resorption of 4% and 8.3% consecutively without fetal death observed. Increasing the dose of extract, decrease the number of fetuses, raise the death of fetuses as well as the resorption.

A red clump characterizes resorption in the uterus that gives no response when touched. Resorption indicated that there was no development of the embryo into a normal fetus. It happens due to morphological errors through various defects that might result in death.¹⁵

Table-1: Effect of *P. fel-terrae* Ethanol Leaves Extract of on Reproductive Performance of Pregnant Female Rats

Parameter	Treatment Groups				
	Healthy control	EEPL 125 mg/kg	EEPL 500 mg/kg	EEPL 1000 mg/kg	Gabapentin 50 mg/kg
Amount of litter	5	5	5	5	5
Mean weight gain of litter (g)	37.8 ± 10.85 ^b	33.1 ± 10.06 ^a	33.8 ± 2.13 ^a	18.9 ± 4.29 ^a	27.7 ± 5.76 ^a
Amount of live fetus	31	34	24	19	29
Rasio of gender	M(16); F(15)	M(16); F(18)	M(13); F(11)	M(13); F(14)	M(18); F(16)
Amount of death fetus	0	0	0	8	0
Amount of resorption	0	0	2	3	3
Haemorrhage	0	5	2	3	8
Mean weight gain of the fetus (g)	5.17 ± 0.38 ^b	2.27 ± 0.26 ^a	5.53 ± 1.33	1.73 ± 0.8 ^{ab}	2.14 ± 0.37 ^a
Mean body length of litter (cm)	4.35 ± 0.19 ^b	3.19 ± 0.31 ^a	3.95 ± 0.59	2.70 ± 0.22 ^{ab}	3.07 ± 0.36 ^a
Mount of the litter with malformation uterus	0	0	1	1	0

Description: Data is performed in standard deviation, ^a): significantly different from healthy control ($p < 0.05$), ^b): significantly different from the positive control group ($p < 0.05$), EEPL: Ethanol extract of *P. fel-terrae* leaves (EEPL); M: male; F: female

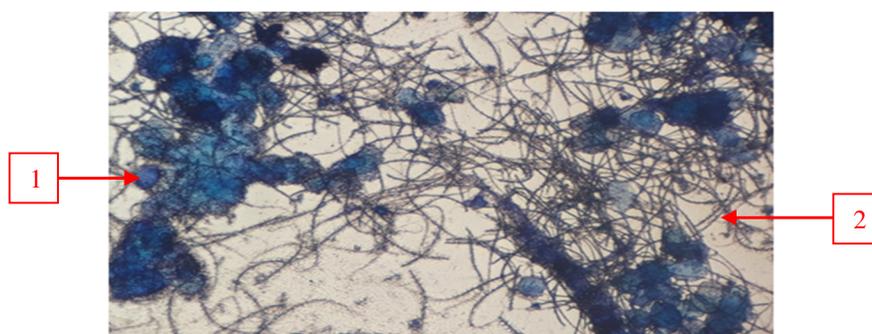


Fig.-1: Vagina smear after *coitus*
Information: 1 horned epithelial cells; 2 sperm

If a teratogen contacted an embryo in the pre-differentiation stage, this could result in significant damage to the embryonic cells which might cause the cells death.¹⁶ Active compounds such as saponin and polyphenol contained in *P. fel-terrae* leaves could trigger smooth muscle contractions in the uterus.¹⁷ These contractions might disrupt fetal growth during pregnancy. Fetus death is caused by cells death in the final proliferation stage which allowed only a portion of the cells to be repaired, as well as incomplete reabsorption process by its mother which lead to fetus defect.¹⁸

Haemorrhage

Gabapentin is found to be the most hemorrhagic agent with 27.59% of the total number of the fetus to have suffered from haemorrhage. It was followed by the dose of 1000 mg/kg(15.79%), 125 mg/kg (14.71%) and lastly, the dose of 500 mg/kg (8.33%). The haemorrhage was observed to have spread from head to the neck, lower limb and the whole body.

The presence of flavonoid and saponin through repeated *P. fel-terrae* ethanol extract administration might have caused the haemorrhage due to their ability to penetrate the placental barrier, which overall resulted in the disruption in the osmotic balance between amnion and embryonic fluid.¹⁸

Fetal Weight and Length

There was no significant difference between fetal weight and length among group dose of 125 mg/kg, 1000 mg/kg and gabapentin 50 mg/kg. The group with the treatment of 1000 mg/kg shows the lowest weight and length among the other groups with an average of 1.73 grams and 2.7 cm, respectively.

The increase in weight and length of the fetus is affected by hormones. Growth hormone is essential for embryonic growth as it affects proteins, electrolytes, carbohydrates, and fats metabolism.¹⁹The rates of fetal growth and development determine the size of the newborns. Saponin and flavonoid compounds contained in *P. fel-terrae* leaves might interfere with the hypothalamus in secreting growth hormone which in this case is Growth hormone-releasing hormone (GHRH)and growth Hormone Inhibiting Hormone (GHIH). The decrease in weight of the fetus is the smallest form of teratogenic.²⁰

Litter with a Uterus Abnormality

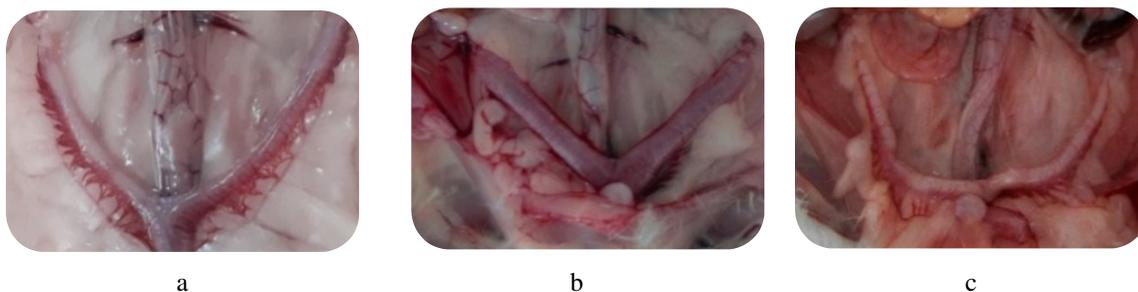


Fig.-2: Uterus Abnormalities, (a) Normal Uterus, (b) Distended Uterus, (c) Constricted Uterus

Uterus abnormalities in the litter are one indication of the occurrence of teratogenic effects. The distended uterus was experienced by two of five mothers exposed to *P. fel-terrae* ethanol extract dose of 500 mg/kg. On the other hand, the constricted uterus was observed in parent exposed to the dose of 1000 mg/kg. The exact mechanism in which extract causes uterus abnormalities is still unclear. However, it is suggested that some active compounds in *P. fel-terrae* plants which are cytotoxic are responsible for the observed defects.

The active flavonoids contained in *P. fel-terrae* ethanol extract suggested having antiestrogenic property. This substance is structurally analogous to a hormone; however, it would not stimulate the receptor. If it occupied the estrogen hormone receptors, it would reduce the action of the hormones on the target cells.^{21,22} Estrogen caused the endometrium to proliferate which resulted in swelling and thickening of the uterine wall. The lack of estrogen due to the presence of flavonoid as antiestrogen would limit the

development of the uterus. Besides, flavonoid and saponin cause continuous uterine contractions where the uterus appeared to shrink.²³ On the other hand, it was suggested that the failure of cells in the uterine wall to restore the normal uterus condition before implantation was responsible for the swollen uterus.

Table-2: Effect of Ethanol Extract of *P. fel-terrae* Lour. Leaves on the Appearance of External Malformations

Parameter	Treatment Groups				
	Healthy Control	EEPL 125 mg/kg	EEPL500 mg/kg	EEPL 1000 mg/kg	Gabapentin 50 mg/kg
Number of fetuses	31	34	24	19	29
Cranial	-	-	-	-	-
Anencephaly	-	-	-	-	-
Brachygnathia	-	-	-	-	-
Spina bifida	-	-	-	-	-
Micromelia	-	14	6	6	8
Humpback body	-	-	-	2	-
Limbs defect	-	-	-	8	-
Liver	-	-	-	-	-
Heart	-	-	-	-	-
Lungs	-	-	-	-	-
Kidney	-	-	-	-	-
Kinkeytail	-	-	-	-	-

Description: EEPL: Ethanol extract of *P. fel-terrae* leaves

The fetus experienced external malformations in the form of micromelia in all treatment groups. The highest number was experienced by the group that was administered with the dose of 125 mg/kg with 14 fetuses. It is then followed by gabapentin group of dose 50 mg/kg with eight fetuses, and lastly, the dose of 500 mg and 1000 mg/kg, both with six fetuses. Micromelia is a congenital abnormality in which the body parts are abnormally shortened. A proliferative disruption causes micromeliaduring organogenesis. Repeated *P. fel-terrae* administration containing several active compounds such as flavonoid during organogenesis is thought to be the cause of micromelia in the fetus. This result in line with the previous study conducted by Satria et al. (2017) stated that extract of *P. fel-terrae* contain high phenolic compound (92.88 ± 0.50 GAE/g) and it has high antiproliferative activity.⁵

Humpback body is an abnormality in the spinal structure that causes the fetus to appear hunched. Humpback body is only observed in the group administered with the dose of 1000 mg/kg. It might be due to the death of cells that contribute to the spine formation which causes the rate of growth between bones to be unequal, hence, the bone bent. The anti-proliferative compound contained in *P. fel-terrae* is thought to be the agent responsible for the death of the bone forming cells.⁵ Furthermore, these compounds also seemed to increase the uterus contraction which affects the fetus growth.

Limb defect is found at the dose of 1000 mg/kg where growth retardation was also experienced. Fingers and toes, along with the other parts of the body were not growing properly. The effect of cytotoxic compounds during proliferative phase disrupted and stopped the development of the fetus.

The external malformations can be seen in Fig.-2.

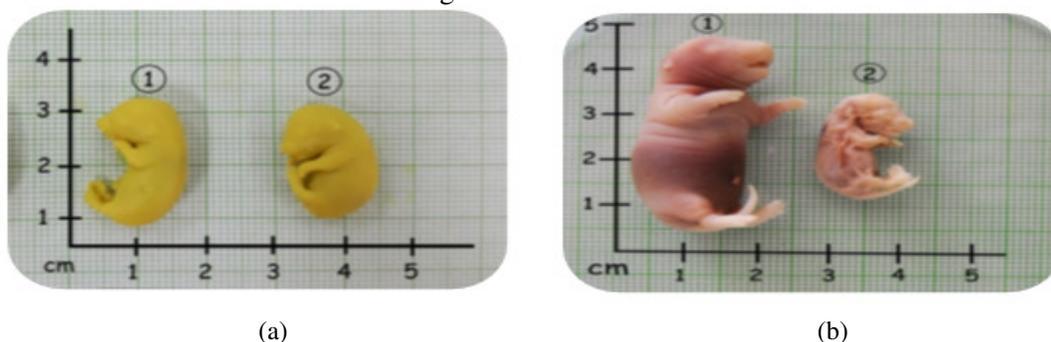


Fig.-2: External Malformations, (a) Humpback Body; (b) Micromelia; (1) Normal fetus; (2) Malformation fetus

CONCLUSION

Administration ethanol extract of *P.fel-terrae* leaves during the organogenesis period exert the teratogenic effect on the fetus. The teratogenic effects involved weight loss and fetal length, increasing number of dead fetuses, resorption, haemorrhage, as well as external malformations such as micromelia, humpback body and limb defect. This ethanol extract of *P. fel-terrae* should not be used during pregnancy.

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