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Research Article

THE EFFECT OF LIMAN LEAVES EXTRACT ON FETAL RAT DEVELOPMENT

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ABSTRACT

Objective: The objective of the study was to observe the effect of liman leaves extract on fetal rat development and provide safety information for its use during pregnancy.

Methods: Estrus cycles of female Wistar rats were observed and mated to male rats, 0 day of pregnancy determined after finding a vaginal plug or in a vaginal smear, there was sperm. Sample was administered orally using sonde with a single administration on the 11th day of pregnancy, at doses of 3750 mg/kg bw, 1185 mg/kg bw, and 375 mg/kg bw. On the 19th day of pregnancy, the rats were sacrificed and then observed the number of implantation, corpus luteum, intrauterine death, and fetal abnormalities.

Results: The dose of 375 mg/kg bw exposed the highest average implantation rate (13.20%) and the largest number of corpus luteum (13.90%). The highest total intrauterine death was presented by dose of 3750 mg/kg bw and significantly different ($p<0.05$) compared to the control group. The dose of 375 mg/kg bw expressed the highest percentage of embryos with resorption (19.03%), while the lowest average fetal body was shown by 3750 mg/kg bw compared to the other groups. The highest percentage of external abnormalities was given by the dose of 375 mg/kg bw (12.91%), which abnormalities found were dwarf, cleft palate, hydrocephalus, short sleeve, and hematoma.

Conclusion: Liman leaves extract was mild teratogenic on Wistar rat fetus.

Keywords: Fetal rat development, Teratogen, Liman leaves.

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INTRODUCTION

Liman (*Elephantopus scaber* L.) is belonged to Asteraceae that is often used as a traditional medicine for women health, such as pregnancy complaints, menstrual disorders, aphrodisiac, contraception, body care, and puerperium [1]. Liman has pharmacological effects that have been studied such as hepatoprotective activity on alcohol induced liver damage in mice [2], significantly hepatoprotective by reducing hepatocellular injury in mice induced by D-galactosamine lipopolysaccharide [3], anti-inflammatory and hepatoprotective effects by inhibiting signal p38 mitogen-activated protein and cyclooxygenase in Sprague-Dawley mice [4], significantly reduced bronchospasm induced by histamine and acetylcholine and prevented mast cell degeneration in Guinea pigs [5], effectively as wound healer by reducing chronic inflammation of the cells, reducing edema, and increasing collagenation [6], Liman leaves extract has an antimicrobial effect [7], acetone extract of liman leaves reduced blood glucose levels in streptozotocin-induced diabetic rats [8].

Several studies demonstrated that liman also had inhibitory effect of proliferation due to deoxyelephantopin from liman suppress the growth of mammary adenocarcinoma in mice [9,10]. Deoxyelephantopin from liman significantly reduced the growth rate of primary tumors *in vivo* of stably transfected human cervical carcinoma [11]. Deoxyelephantopin and isodeoxyelephantopin from liman decreased availability of tumor cell L-929 (IC_{50} 2.7 µg/ml and 3.3 µg/ml) and *in vivo* showed significant effects as antitumor for tumor cells Dalton's lymphoma ascites [12]. Therefore, the use of liman by women during the conception and pregnancy period is feared can influence growth and development of conception result, so it is necessary to test teratogenicity of this plant and provide its safety information of used during pregnancy.

MATERIALS AND METHODS

Materials

Liman was collected from Lembang-Bandung, Indonesia, and determined at Herbarium Bandungense, School of Life Sciences and Technology, Bandung Institute of Technology, extracted by reflux method using 96% ethanol solvent. Wistar rat came from the animal laboratory of the School of Life Sciences and Technology, Bandung Institute of Technology.

Methods

Wistar virgin female rat weighing 200–220 g was observed estrous cycles by microscopic observation of the vaginal smears, during the proestrus phase of female rats mated to male rats, in the ratio of one male to three females. The day of 0 pregnancy is determined after finding a vaginal plug or there is sperm in the vaginal smear. The pregnant rat is then weighed and inserted into a separate cage. The rat mother condition was observed every day during the test period, for death and other clinical conditions [13-15].

The sample was administered orally using sonde, the volume was 1 ml/100 g bw, the rats were given a single administration on the 11th day of pregnancy, the doses of 3750 mg/kg bw, 1185 mg/kg bw, and 375 mg/kg bw weighed during the test sample and before surgery.

On the 19th day of pregnancy, the mothers were sacrificed with 100% CO_2 and then surgery performed. The mother's uterus was examined macroscopically for counting number of implantation (number of live fetuses, dead fetuses, and resorption), ovaries, and corpus luteum which was observed by separating ovaries from the uterus, opened the membrane and corpus luteum of pregnancy was calculated. Further, live fetuses were examined such as body weight, sex, and the possibility of

abnormalities which can be seen macroscopically. Fetal abnormalities were observed by looking at all external parts of the fetus from the head to the extremities using a surgical microscope [13-16].

RESULTS

The observations were performed on reproductive performance of mother included number of implantation, percentage of pre-implantation loss, intrauterine death which was consisted of dead fetus and resorption embryo, number of live fetuses, weight of live fetuses, and number of live fetuses with external abnormalities. The result of observations is shown in Table 1.

The amount of implantation in all groups was relatively similar to the control group (aquabidest). The dose of 375 mg/kg bw gave the highest implantation rate (13.20%) and the highest number of corpus luteum (13.90%). The number of corpus luteum in all liman leaf extracts was not significantly different compared to the control group. Meanwhile, methotrexate showed number of implantation and number of corpus luteum which was significantly different compared to control. Based on the number of corpus luteum and the number of implantation, it can be calculated the percentage of implantation loss for each group. The percentage of pre-implantation loss in liman leaves extract 1185 mg/kg bw significantly increased (12.80%) compared to control (1.59%).

Total intrauterine deaths in liman leaves extract 3750 mg/kg bw showed the greatest percentage (19.41%) and significantly different ($p<0.05$) compared to control (2.72%). Intrauterine deaths included resorption embryo and dead fetuses are shown in Fig. 1. The liman leaves extract 3750 mg/kg bw gave a percentage of the resorption embryo was 11.20%. The highest percentage of resorption embryo was revealed by liman leaves extract 375 mg/kg bw (19.03%). Meanwhile, liman leaves extract 3750 mg/kg bw showed the highest percentage of dead fetuses (8.21%), which was significantly different from the control group, where no dead fetus found.

The percentage of live fetuses in liman leaves extract 3750 mg/kg bw (80.59%) was the most decrease and significantly different ($p<0.05$) compared to the control group (97.28%). The average fetal body weight of liman leaves extract 3750 mg/kg bw tended to be the most decrease compared to the other groups (2.04%). Observations on live fetuses with external abnormalities showed that liman leaves extract 375 mg/kg bw exposed the highest percentage of fetus with external abnormalities (12.91%), then followed by liman leaves extract 1185 mg/kg bw (8.60%) and 3750 mg/kg bw (5.91%).

It is shown in Table 2 that the types of abnormalities found were dwarf fetus (Fig. 2), cleft palate, hydrocephalus, fetus with short arm, and hematoma (Fig. 3). The most abnormalities were given by liman leaves extract 375 mg/kg bw, which found cleft palate (0.91%), hydrocephalus (2.50%), fetal hydrops with short arm (2.50%), and hematoma (8.67%). In liman leaves extract 1185 mg/kg bw, the abnormalities were dwarf (0.91%), cleft palate (2.83%), and hematoma (3.97%). Meanwhile, in liman leaves extract 3750 mg/kg bw, only cleft palate was found (0.77%).

DISCUSSION

The appearance of reproduction is reflected by the amount of implantation, loss of pre-implantation, intrauterine death which consisted of dead fetuses and resorption embryo, number of live fetuses, fetal body weight, and malformation. The reproductive appearance of mothers which were given by liman leaves extract at the

Table 1: Reproductive performance in rat mother after giving by liman leaves extract

Reproductive performance	Aquabides	Liman leaves extract (mg/kg bw)			Methotrexate 0.3 mg/kg bw
		375	1185	3750	
Number of pregnant rat	21	10	10	10	11
Number of corpus luteum	11.95±1.32	11.30±1.90	11.70±1.49	12.20±1.33	10.18±3.66
Number of implantation	11.76±1.22	13.20±1.89	11.10±2.77	12.40±1.80	8.82±3.89
Pre-implantation lost (%)	1.59	12.39	1.71	5.74	13.39
Intrauterine death					
Resorption embryo					
Mean	0.33±0.66	2.60±1.80	1.10±1.14	1.40±0.92	1.64±1.50
%	2.83	19.70	9.91	11.29	18.56
Dead fetuses					
Mean	0.00±0.00	0.00±0.00	0.10±0.30	0.80±1.54	0.45±0.82
%	0.00	0.00	0.90	6.45	5.15
Total					
Mean	0.33±0.66	2.60±1.80	1.20±1.33	2.20±1.40	2.09±2.02
%	2.83	19.70	10.81	17.74	23.71
Live fetuses					
Total					
Mean	11.43±1.25	10.60±1.80	9.90±2.62	10.20±2.89	6.73±3.77
%	97.17	80.30	89.19	82.26	76.29
Body weight (g)	2.18±0.14	2.10±0.24	2.19±0.19	2.04±0.14	2.07±0.23
With external malformation (%)	0.00	19.70	12.61	11.29	11.34

Table 2: External abnormalities in fetus after rat mother giving by liman leaves extract

Treatment group	Number of pregnant rat	Live fetuses	Type of malformation (%)				
			Dwarf	Cleft palate	Hydrocephalus	Fetal hydrops	Hematoma
Aquabidest	21	11.43±1.25	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Liman leaves extract							
375 mg/kg bw	10	8.50±3.17	0.00±0.00	0.91±0.03	2.50±0.08	2.50±0.08	8.67±0.14
1185 mg/kg bw	10	10.90±1.97	0.91±0.03	2.83±0.06	0.00±0.00	0.00±0.00	3.94±0.07
3750 mg/kg bw	10	9.60±3.35	0.00±0.00	0.77±0.02	0.00±0.00	0.00±0.00	0.00±0.00
Methotrexate 0.3 mg/kg bw	11	6.73±3.77	1.01±0.03	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

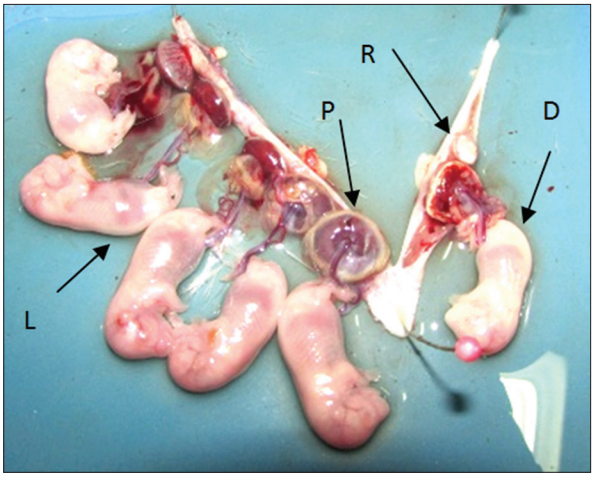

Fig. 1: Live fetuses, resorption embryo, and dead fetuses, L: Live, R: Resorption, D: Dead, P: Placenta

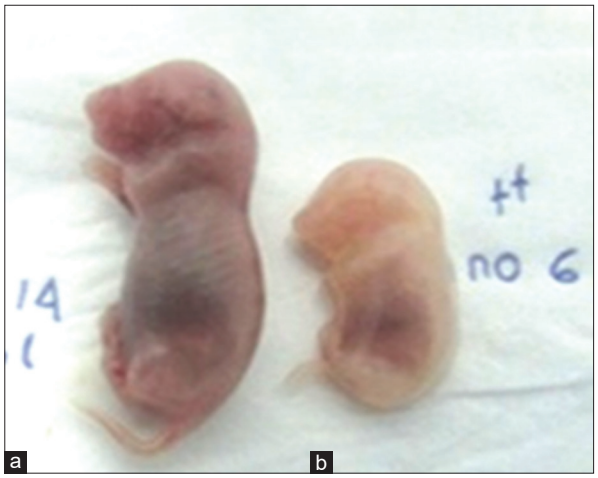

Fig. 2: Normal fetus (a) and dwarf fetus (b)

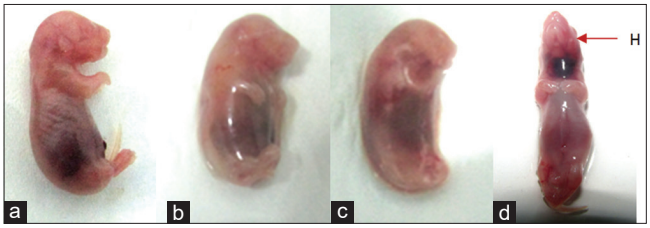

Fig. 3: Fetus with external abnormalities: Normal fetus (a), fetus with short arm (b), fetus hydrocephalus (c), fetus with hematoma (d), hematoma (h)

11th day pregnancy showed that liman leaves extract 3750 mg/kg bw was embryo lethal. It can be seen by high number of dead fetuses and high resorption embryo. Besides that, decreasing in number of live fetuses in liman leaves extract 3750 mg/kg bw was greater than the other groups. Teratogenicity was indicated by a dose of 375 mg/kg bw, which shown by the number of abnormalities present at a dose of 375 mg/kg bw which was greater than the other doses, even though mild teratogenic only. Wilson and Fraser [17] stated that a dose was expressed as teratogenic dose if the dose causes more abnormalities fetus than the dead fetus, and a dose as embryotoxic if the dose causes intrauterine death, fetal abnormalities, and developmental delay.

The reproduction performance of rat's mother which was given by liman leaves extract at the 11th day was similar to teratogenicity screening result using brine shrimp test (BST). The result of BST screening showed that high concentration of liman leaves extract (6 mg/ml) caused higher percentage of dead nauplii than the other concentrations, while developmental disorder was mostly found at low concentration (1.5 mg/ml) [18].

The external abnormality which was found in this study included very small size fetuses compared to the control group (Fig. 2). It may be due to proliferative disorders at the growth time. The phytochemical screening of liman leaves extract has been conducted in this study and the results showed that liman leaves extract contained alkaloids, flavonoids, saponins, quinones, tannins, steroids/triterpenoids, and with total alkaloid content 4.09±0.01%. The results in line with some studies which presented that the liman leaves extract contained deoxyelephantopin compound which is belonged to alkaloids group. These compounds had ability to inhibit proliferation of the growth of cancer cells [9-12]. The other studies revealed that medicine which has inhibitory effects in proliferation such as methotrexate also gave similar abnormalities of intrauterine growth and significant weight loss in both human and animal experiments [19,20]. Besides external abnormalities, fetal bleeding was also found, which was hematoma (Fig. 3). Liman leaves extract had vasodilator effect [5]. This hematoma may be influenced by increasing in vascular permeability which correlated with its vasodilator effect. Therefore, giving liman leaves extract on rat during pregnancy can cause morphological abnormalities and physiological abnormalities. However, it needs to study more deeply regarding mechanism of liman leaves extract and active compound that plays a role in the mechanism.

CONCLUSION

Based on the results described above, it can be concluded that the liman leaves extract was mild teratogenic in Wistar rat.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

REFERENCES

1. Lestari MW, Soemardji AA, Fidrianny I. Review of traditional use, pharmacological effects and toxicity of medicinal plants for women's health in Indonesia. Asian J Pharm Clin Res 2016;9:32-7.
2. Ho WY, Yeap SK, Ho CL, Abdul Rahim R, Alitheen NB. Hepatoprotective activity of *Elephantopus scaber* on alcohol-induced liver damage in mice. Evid Based Complement Alternat Med 2012;2012:417953.
3. Nataraj KS, Reddy KS, Prashanth K, Swamy BM. PP-041 Hepatoprotective potential of root extracts of *Elephantopus scaber* L. on D-galactosamine/lipopolysaccharide induced hepatitis in rats. Int J Infect Dis 2009;13:S61.
4. Hung HF, Hou CW, Chen YL, Lin CC, Fu HW, Wang JS, *et al.* *Elephantopus scaber* inhibits lipopolysaccharide-induced liver injury by suppression of signaling pathways in rats. Am J Chin Med 2011;39:705-17.
5. Sagar R, Sahoo HB. Evaluation of antiasthmatic activity of ethanolic extract of *Elephantopus scaber* L. Leaves. Indian J Pharmacol 2012;44:398-401.
6. Singh S, Krishna V, Mankani K, Manjunatha B, Vidya S, Manohara Y. Wound healing activity of the leaf extracts and deoxyelephantopin isolated from *Elephantopus scaber* Linn. Indian J Pharmacol 2005;37:238-42.
7. Avani K, Neeta S. A study of the antimicrobial activity of *Elephantopus scaber*. Indian J Pharmacol 2005;37:126-7.
8. Daisy P, Jasmine R, Ignacimuthu S, Murugan E. A novel steroid from Elephantopus *scaber* L. An ethnomedicinal plant with antidiabetic activity. Phytomedicine 2009;16:252-7.
9. Huang CC, Lo CP, Chiu CY, Shyur LF. Deoxyelephantopin, a novel multifunctional agent, suppresses mammary tumour growth and lung metastasis and doubles survival time in mice. Br J Pharmacol

2010;159:856-71.
10. Lee WL, Shyur LF. Deoxyelephantopin impedes mammary adenocarcinoma cell motility by inhibiting calpain-mediated adhesion dynamics and inducing reactive oxygen species and aggresome formation. Free Radic Biol Med 2012;52:1423-36.
11. Xu G, Liang Q, Gong Z, Yu W, He S, Xi L, *et al.* Antitumor activities of the four sesquiterpene lactones from *Elephantopus scaber* L. Exp Oncol 2006;28:106-9.
12. Geetha BS, Nair MS, Latha PG, Remani P. Sesquiterpene lactones isolated from *Elephantopus scaber* L. Inhibits human lymphocyte proliferation and the growth of tumour cell lines and induces apoptosis *in vitro*. J Biomed Biotechnol 2012;2012:721285.
13. OECD. Guideline for Testing of Chemicals: Prenatal Developmental Toxicity Study No. 414. Washington DC: OECD Publication and Information Center; 2001. p. 1-11.
14. Taylor P. Practical Teratology. London: Academic Press; 1986.
15. Sukandar EY, Kurniati NF, Fitria V. Evaluation of teratogenicity effects of ethanolic extracts of binahong leaves (*Anredera cordifolia* (Ten) steenis) in Wistar rat. Int J Pharm Pharm Sci 2014;6:422-6.
16. Ahmed M, Azmat A. Effects of unani anxiolytics (somina) on general reproductive performance and teratology in rats. Int J Pharm Pharm Sci 2017;9: 30-4.
17. Wilson JG, Fraser FC. Handbook of Teratology. General Principles and Etiology. Vol. 1. New York: Plenum Press; 1977. p. 49-72.
18. Lestari MW, Fidrianny I, Yusuf AT, Soemardji AA. Brine shrimp teratogenic screening test on some selected plants. Asian J Pharm Clin Res 2017;10:96-9.
19. Dawson AL, Riehle-Colarusso T, Reefhuis J, Arena JF, National Birth Defects Prevention Study. Maternal exposure to methotrexate and birth defects: A population-based study. Am J Med Genet A 2014;164A:2212-6.
20. Jamkhdane PG, Chintawar KD, Chdanak PG. Teratogenicity: A mechanism based short review on common teratogenic agents. Asian Pac J Trop Dis 2014;4:421-32.