

## Anti-fertility effect of ethanol extract of lerak (*Sapindus rarak* DC) fruits in female Sprague Dawley Rats

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Manuscript received: 10 September 2016. Revision accepted: 24 February 2017.

**Abstract.** *Fajriaty I, Hariyanto I.H, Haryanto Y. 2017. Anti-fertility effect of ethanol extract of lerak (Sapindus rarak DC) fruits in female Sprague Dawley Rats. Nusantara Bioscience 9: 102-106.* This research aims to observe the antifertility effect of ethanol extract of Lerak fruits (SFEE) in female sprague dawley rats. Group of rats are divided into four groups, the first group is given carboxymethyl cellulose (CMC) as negative control (KN), the second group is given ethinylestradiol as positive control (KP), the third group is given SFEE with dose 50 mg/kg BW, and the fourth group is given SFEE with dose 100 mg/kg BW. The female rats in estrus phase is mated with male rats. In plug vaginal checking and smear vaginal there is sperm that signify if female rats have experienced copulation and currently on the day of the zero pregnancy. All treatment are given orally from 0 day of pregnancy to 10th day of pregnancy. Antifertility effect on treatment in rats is shown from laparotomy in the 20th day of pregnancy. Determining of the antifertility effect that to do include pre-implantation loss effect and post implementation loss effect. The result of this research shows that SFEE with dose 50 mg/kg BW has antifertility effect that is pre-implantation loss as big as 0% and post-implantation loss as big as 10.53% and SFEE with dose 100 mg/kg BW shows antifertility effect is pre-implantation loss and post implantation loss in each of 47.50% and 28.57%. This observation shows that SFEE has antifertility effect.

**Keywords:** Ethanol extract of Lerak fruits, Sprague Dawley, Antifertility effect, pre-implantation loss, post-implantation loss.

**Abbreviations:** SFEE = Ethanol extract of Lerak fruits, CMC = carboxymethyl cellulose, KN = Negative control, KP = Positive control, BW = Body Weight, TLC = Thin Layer Chromatography,

### INTRODUCTION

The use of various contraceptive method are still complained by society because can give side effect. The use of hormonal contraceptive can cause pain, psychological disorders, weight change, menstrual disorders, the risk of developing breast cancer and endometriosis. The ideal contraceptive must fulfill several requirements such as trustworthy, not cause effect which affect health, the power of work can be arranged based on needs, not cause disruption when doing coitus, not need motivation continuously, easy implementation, cheap and affordable for all segments of society and acceptable usage by concerned pair (Wiknjastro et al. 2009). Nowadays, the modern medicines have our daily life. However, lately this treatment return to medicinal plants that is used traditionally. The reason is because the medicinal plants have less side effect than modern medicine that are processed in a synthetic (Nursiyah 2013). Therefore, the use of medicine from plant must be sought and maximized its potential for the Indonesian public interest broadly.

The exploration of contraceptive source from natural, especially from plant can be used as solution as regulator of fertility. Lerak (*Sapindus rarak* DC) fruits have effect to reduce fat, eczema, psoriasis, to remove spots and that extracts have lethal dose 50 greater than 5000 mg/kg (Fajriaty et al. 2014). The subchronic toxicity treatment from extract of lerak in doses of 50, 100 and 500 mg/kg

body weight during 28 consecutive days didn't indicated any deaths or clinical signs of toxicity (Fajriaty et al. 2014).

The fruit contains saponin active compound in a lot of amount. Saponin triterpenoid is one type of saponin that are contained in its fruit. Triterpenoid is lipid derivative that are considered role as compound between steroid biosynthetic, in a lot of amount of active triterpenoid substance is assumption can cause release retardation of Luteinizing Hormon (LH) and Folicle Stimulating Hormon (FSH) that cause disruption on ovulation process (Puspitasari and Suhita 2014). Negative effect from saponin in animal reproduction is reported as abortivum, disturb the zygote formation and anti implantation (Francis et al. 2002). Based on the explanation above, it is necessary observation about antifertility effect examination about ethanol extract of Lerak fruits (SFEE) in female sprague dawley rats.

### MATERIALS AND METHODS

#### Plant material and extraction

Lerak was collected by Situbondo, East Java. This plant was authenticated in Department of Biology, Faculty of Mathematic and Science, Tanjungpura University, Pontianak. Lerak fruits had dried in oven at  $\pm 40$  °C and then extraction with insert into soxhlet that contain ethanol (95% v/v) which function as solvent.

### Experimental animal

Female Sprague Dawley strain rats by weight 100-200 g is obtained from husbandary test, UD WISTAR Yogyakarta.

### Phytochemical screening and determine pattern chromatogram of extract

To determine the chemical constituents, qualitative phytochemical screening of SFEE was carried out following standard procedures. Alkaloids, tannins, saponins (foam test, foam index test and fish index test), flavonoids, phenolic, steroid and terpenoids.

Chromatogram pattern is determined by using thin layer chromatography (TLC) using solvent extract 1%, 3%, 5% in ethanol. Move phase is used consist of ethyl acetate: methanol: water = 77: 13: 10. The observation to do in UV 254 nm and UV 366 nm.

### Antifertility study

#### Mating of rats

Female rats in estrus phase is combined with male as comparison 4: 1. The next day to do checking of vagina plug, if there is no vaginal plug so it will continue making of vaginal smear and it is checked by using microscope. The rats is stated mate if there is sperm in the liquid of vaginal smear (Sunardi et al 2010). Vaginal plug or vaginal smear there are sperm that is signed if the rats has experienced copulation and it is in the 0 day of pregnancy (Kumolosasi et al 2004).

#### The granting of material test

The treatment group consist of four groups; The first group (KN) is given carboxymethyl cellulose (CMC), the second group (KP) is given ethinyestradiol, the third group is given SFEE 50 mg/kg BW, the fourth group is given SFEE 100 mg/kg BW. The treatment is given orally for 11 days from 0 to 10th day of the pregnancy.

#### The observation of rats reproduction appereance

On the 20th day of the pregnancy all of the group of rats is laparotomy to get the data consists of number of corpora lutea, number of implantation place, number of live fetuses and the uterine weight.

#### The determination value of antifertility effect

Antifertility effect that is observed include *pre-implantation loss* and *post-implantation loss*. The pattern that is used to determine the value of antifertility effect as following (Yadav and Jain 2009):

*Pre-implantation loss* (%) =

$$\frac{\text{Total number of corpora lutea} - \text{Total number of implantation place}}{\text{Total number of corpora lutea}} \times 100$$

*Post-implantation loss* (%) =

$$\frac{\text{Total number of implantation place} - \text{Total number live fetuses}}{\text{Total number of implantation place}} \times 100$$

### Data analysis

The results of the data obtained in the form of the number of corpora lutea, implantation place, live fetuses, and weight of uterine were analyzed using ANOVA test. The analysis continued with LSD analysis if there is a significant difference.

## RESULTS AND DISCUSSION

### Phytochemical screening and pattern chromatogram of extract

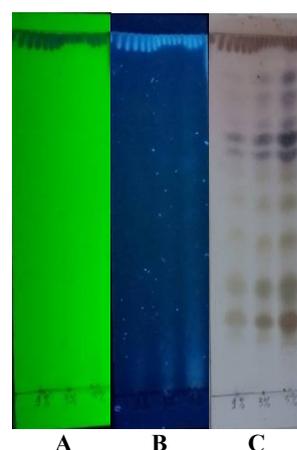
Phytochemical screening showed that ethanol extraction had more secondary metabolite compounds. SFEE revealed the presence of the following classes of chemical compounds: Alkaloids, saponins, tannins, triterpenoid and phenolic compound. Foam index of the ethanol extract of the lerak fruits has 20,000 and fish index has 8,000. The Results pattern chromatogram of extract presented in Figure 1.

### Effect of SFEE about Reproduction performance from female rats

Results effect of extracts on reproduction appearances were also investigated. Obtained results are presented in Table 1.

Based on the observation about average number of corpora lutea, there is no decrease of number with the increase of extract dose that is given to rats, it can be seen in tabel 1. Anova testing with confidence level 95% does not show if there is significant differences between group ( $p > 0.05$ ).

The observation about average number of implantation place show the decrease of number with the increase of extract dose that is given to rats it can be seen in tabel 1. However, the differences number of implantation does not show the significant differences ( $p > 0.05$ ).

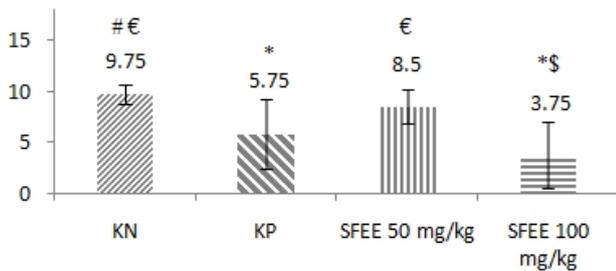


**Figure 1.** Pattern of chromatogram from SFEE fruit. (A) Plat of TLC (Thin layer chromatography) after it is eluted then it can be seen UV 254 nm; (B) Plat of TLC after it is eluted then it can be seen UV 366 nm; (C) Plat of TLC after it is sprayed with H<sub>2</sub>SO<sub>4</sub> 10% in methanol.

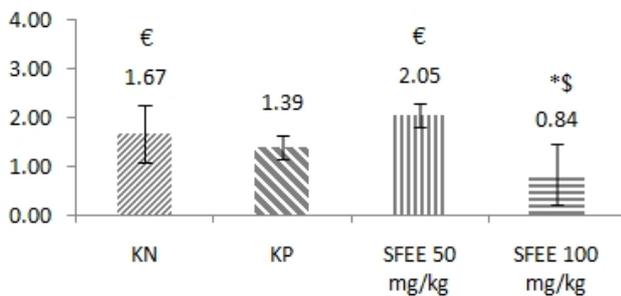
**Table 1.** Results effect of extracts on reproduction appearances

Group	Treatments	Corpora lutea (Mean ± SD)	Implantation place (Mean ± SD)	Live fetuses (Mean ± SD)	Weight of uterine g/100g (Mean ± SD)
I	KN	9.75±0.95	9.75±0.95	9.75±0.95	1.67±0.60
II	KP	8.75±0.95	6.50±4.04	5.75±3.40	1.39±0.23
III	SFEE 50 mg/kg	9.50±2.38	9.50±2.38	8.50±1.73	2.05±0.25
IV	SFEE 100 mg/kg	10.00±1.63	5.25±4.57	3.75±3.30*	0.84±0.60*

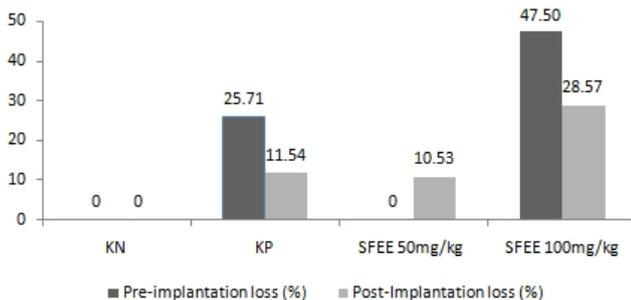
Note: \*= p<0,05 when compared between group



**Figure 2.** Effect of SFEE in live fetuses in female rats. Notes: \*= shows significant differences when compared with the KN ( $p<0,05$ ), #= shows significant differences when compared with the KP ( $p<0,05$ ), \$ = shows significant differences when compared with the SFEE 50 mg/kg ( $p<0,05$ ), € = shows significant differences when compared with the SFEE 100 mg/kg ( $p<0,05$ )



**Figure 3.** Effect of SFEE in weight of uterine in female rats. Notes: \*= shows significant differences when compared with the KN ( $p<0,05$ ), #= shows significant differences when compared with the KP ( $p<0,05$ ), \$ = shows significant differences when compared with the SFEE 50 mg/kg ( $p<0,05$ ), €=shows significant differences when compared with the SFEE 100 mg/kg ( $p<0,05$ )



**Figure 4.** Anti-fertility effect of SFEE

The decrease number of live fetusses following the level of dose from SFEE which is given, as seen as in Figure 2. In group of SFEE 50 mg/kg BW and 100 mg/kg BW found average number of live fetuses in each of them is 8.50 and 3.75. The group of KN have average number of live fetuses as big as 9.75 and group of KP have average number of live fetuses as big as 5.75. Anova testing with level of trustworthy 95% has shown significant difference between group of live fetuses ( $p<0.05$ ). The result of LSD testing ( Figure 2) in group of SFEE 100 mg/kg BW shows number of significant difference live fetuses ( $p<0.05$ ) when compared with group of KN and significant difference ( $p<0.05$ ) when compared with group of SFEE 50 mg/kg BW. The number of live fetuses in group of ethynilestradiol shows the significant difference ( $p<0.05$ ) when compared with group of KN but it does not show the significant difference ( $p>0.05$ ) with SFEE 100 mg/kg BW.

The result of decrease weight of uterine also follow the increase level of dose from SFEE. Group of SFEE 50 mg/kg BW show average weight of uterine as big as 2.05 g/100g and group of SFEE 100 mg/kg BW show average weight of uterine as big as 0.84 g/100g. Group of KN and group of KP show average weight of uterine as big as 1.67 g/100g and 1.39 g/100g. The result of Anova testing with level of trustworthy 95% shows significant difference weight of uterine between group of treatment ( $p<0.05$ ). The result of LSD testing (Figure 3) in group of SFEE 100 mg/kg BW show weight of uterine in significant difference ( $p<0.05$ ) when compared with group of KN and group of SFEE 50 mg/kg BW, but it does not show the significant difference ( $p>0.05$ ) when compared with group of KP.

**Antifertility effect of SFEE**

The determine value of antifertility effect that is determined include *pre-implantation loss* and *post-implantation loss*. The result is shown in Figure 4.

Group of KN is not found effect of pre-implantation loss or post implantation loss, so that does not show the effect of antifertility. Group KP show antifertility effect that is shown with the value of pre implantation effect as big as 25.71% and the value of post implantation effect as big as 11.54%. In group of treatment SFEE 50 mg/kg BW show the value of pre implantation effect as big as 0 % and the value of post implantation effect as big as 10.53 %. In group of treatment SFEE 100 mg/kg BW found the value of pre implantation loss effect and the value of post implantation effect as big as 47.50% and 28.57%. Based on the data, SFEE 100 mg/ kg BW show the ability to cause

the pre implantation loss and post implantation loss effect.

### Discussion

Preliminary phytochemical studies of the extract indicated the presence of alkaloids, tannin, flavanoids saponins, triterpenoid and phenolics. The determine of saponins index of SFEE to do with 2 methods, namely foam index and fish index. It is obtained the value of foam and fish index in a lot of amount. Mostly saponin has ability hemolysis and toxic to cold-blooded animals. Therefore, the concentration and potential from saponin is obtained based on toxic in fish. (Rachmawati et al. 2013). The typical properties of saponin, namely bitter, foam in water, has good detergent typical, poisonous for cold-blooded animal, has hemolysis activity (disruption hemoglobin), not poisonous for warm-blooded animal, has anti exudative and has anti inflammation (Kurniawati 2009). Saponin also has effect to obstacle estrus cycle and decrease fertility (Essien et al. 2014). The saponin triterpenoid is to be expected type of saponin that are contained in extract because show pink until violet colour on TLC after being sprayed with H<sub>2</sub>SO<sub>4</sub> 10% then it is being heated (Sharifa et al. 2012).

The influence of SFEE to reproduction performance morfologically such as number of corpora lutea, number of implantation place, number of live fetuses, and weight of uterine. Number of corpora lutea can describe number of ovum that is ovulated. The number of implantation place and number of live fetuses data is used to count antifertility effect. Weight of uterine data in rats give first describe the information of level hormone in rats.

Giving KP, SFEE 50 mg/kg BW and SFEE 100 mg/kg BW does not influence number of corpora lutea when in given after mating in female rats. Corpora lutea will form when it has ovulated. The process of ovulation had handled by several reproduction hormone namely FSH, LH and estrogen. FSH stimulate on formation follicel cell in ovarium and LH stimulate on ovulation so that it forms corpora lutea (Novriyanti et al. 2014).

The number of implantation place in female rats show decrease in giving KP and SFEE 100 mg/kg BW. That thing is allegedly because there is saponin can disturb the progesterone secretion. Decrease of progesterone hormone secretion by corpora lutea can cause uterine is not ready to receive embryo so that the embryo does not plant on uterine. Progesterone on the pregnancy has function to prepare in around of uterine and maintain the pregnancy with increase gendometrium gland secretion and disturb the miometrium movement (Yadav and Jain 2009). Less of progesterone plasma level and LH or there is unbalance ratio between progesterone and estrogen that cause the failure of implantation (Mandal and Dhaliwal 2007; Sharma et al. 2015). This thing proven there is found uterine horns in fetuses inside. So that SFEE that contain can cause pre implantation loss. Pre implantation loss also arise because the disruption of fertility or decrease cytokines production, growth factors, and several types adhesion molecule from development blastocyst or from epithelium uterin in around of implantation (Vasudeva and Sharma 2008).

Post implantation loss is signed by disruption of fetuses development on pasca phase that happen the implantation in uterine because there is resorption that can cause a compound to show the effect of blastocyst or effect of abortifacient (Dabhadkar and Zade 2012; Latha et al. 2013). That cronology can cause group of saponin compound in SFEE and has effect of cytotoxic and can lysis the hemoglobine (Podolak et al. 2010).

The effect of estrogenic from SFEE depend on dose given. The biggest dose from SFEE namely 100 mg/kg BW can cause decrease the size in weight of rats when compared with group of KN and group of SFEE with dose 50 mg/kg BW. The previous research shows that the estrogenic compound decrease weight of uterine when it is used in high dose (Mackic and Ahmetovic 2012). The changing of uterine weight in rats is allegedly can happen because there is no balance in level of hormone in the body that is caused by group of saponin compound (Pal et al. 2013). The decrease of uterine weight has impact in fewer of number of implantation place and number of live fetuses that shown on observation.

Antifertility effect from ethanol extract has proven in animal testing. There is group of saponin compound in a lot of amount in SFEE that has responsibility in antifertility effect in rats. The results were in coherence with (Dande et al. 2014), who reported that triterpenoidal saponins shown antifertility effect caused of their structural similarities with steroid receptor. In this research SFEE can show 2 effects, namely pre implantation loss and post implantation loss, the effect depend on high dose. The mechanism is more specific than antifertility effect from SFEE has not yet known, therefore, it is necessary to do next research about measurement of hormone levels that influence on reproduction system in female rats before and after giving extract.

### ACKNOWLEDGEMENTS

This research was funded by Hibah PEKERTI 2016 by Ministry of Research, Technology and Higher Education of Indonesia.

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