ETHNOPHARMACOLOGY OF SUNGKAI LEAF (*Peronema canescens* Jack.) ON TERATOGENIC EFFECTS IN FEMALE WHITE MICE (*Mus musculus* L.)

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ABSTRACT: One of the ethnopharmacology of medicinal plants has been passed down from generation to generation by the Indonesian people, namely Sungkai leaves. Sungkai (*Peronema canescens* Jack.) is one of the efficacious plants used in traditional medicine in Indonesia. Sungkai leaves have several bioactive compounds that act as antimalarials, antiplasmodial, antibacterials, analgesics, and immunomodulators. This study aims to determine the teratogenic effect of administering the ethyl acetate fraction of sungkai leaves on mice during pregnancy. A total of 24 pregnant mice were divided into four groups: group 1 as a negative control (Na CMC 0.5%), groups 2, 3, and 4 were given fractions with doses of 28, 56, and 84 mg/kg BW, respectively. The fractions were given at the organogenesis phase from the 6th day to the 15th day of pregnancy. On the 18th day, the mice were dissected to observe maternal body weight, fetal weight, number of fetuses, morphological, visceral, and skeletal abnormalities. The research data were analyzed using one-way ANOVA and Duncan's. Based on the results of phytochemical screening, the ethyl acetate fraction of Sungkai leaves contains alkaloids, flavonoids, saponins, steroids, and phenols. The results showed that the administration of the ethyl acetate fraction of sungkai leaves during pregnancy affected the body weight of the mice (p<0.05). The administration of the ethyl acetate fraction of sungkai leaves did not significantly affect body weight and the number of fetuses (p>0.05). The results of fixation with Bouin's solution found no abnormalities in the clefts of the roof of the mouth. The fixation with alizarin solution found bone abnormalities in the caudal and phalanges.

***Keywords*:** Sungkai (*Peronema canescens* Jack.), ethyl acetate, teratogenic, fetus, ethnopharmacology

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INTRODUCTION

Ethnopharmacology has been carried out through the practical use of medicinal plants by people in Indonesia, especially in rural areas, since ancient times which are used until now (1). Indonesian traditional medicine is an Indonesian cultural heritage desired to be used in the health care system. Its use in the community has been used for treatment and health maintenance. It is passed down from generation to generation, survives sustainably, and is inseparable from people's lives.(18)

Indonesia is a developing country that has abundant natural resources. Plants that are spread throughout Indonesia have the potential to be used as medicine. Sungkai (*Peronema canescens* Jack.) is one of the efficacious plants used in traditional medicine in Indonesia (17). Sungkai is a plant with the Latin name (*Peronema canescens* Jack.), often known as jati sabrang, which belongs to the Lamiaceae family. Some people have widely used Sungkai leaves as traditional medicine. The Dayak tribe in East Kalimantan uses the young leaves of the sungkai plant as cold medicine, fever, worm medicine, and mouthwash to prevent toothache (2). Sungkai leaves also have several bioactive compounds that act as antimalarials, antiplasmodial, antibacterials, analgesics, and immunomodulators (3).

Sungkai leaves contain secondary metabolites of alkaloids, flavonoids, steroids, terpenoids, phenolics, and saponins (4). Flavonoid compounds in sungkai leaves play a significant role as color pigments, and flavonoids have antipyretic effects (5). Quercetin has been shown to exert a teratogenic effect in some animals that do not have a backbone. In addition, metabolites in flavonoids and saponins are cytotoxic, which can cause the death of several cells that make up normal cells. Flavonoids can cause teratogenicity in various ways; namely, they can be antiproliferative in the fetus, which works as estrogen receptor agonists and antagonists that inhibit chondrocyte cell proliferation producing pro-oxidant byproducts that can attack normal cells and can inhibit phosphodiesterase enzyme activity which causes an increase in cAMP concentrations and stunted growth acceleration occurs (6).

Preclinical testing is the initial stage of the development of a drug, one of them is the toxicity test. This study conducted a special toxicity test, namely the teratogen test using female white mice. Teratogenicity is the process of changing the shape of cells, tissues, and organs caused by physiological and biochemical changes. Teratogens can be in the form of ionizing radiation, chemicals (drugs, industrial chemicals), and viral infections that cause

changes in the shape and function of organs in fetal development during pregnancy (7). The stage of differentiation and early organogenesis is a period that is very quickly affected by teratogens. The initial mechanism of teratogenesis comes from pathogens in the form of aberrations in embryogenesis, such as cell death, failure of cell interactions, and inhibition of embryogenesis movement (8). The use of drugs or herbal products in pregnant women is often consumed in the first trimester of pregnancy. The use of drugs can cause accumulation in the fetus, while the fetus does not yet have a fully functioning metabolic system. Chemical compounds or active drug substances can enter the fetal blood circulation and affect the process of organ formation in the fetus (7). The use of traditional medicine in pregnant women must be supervised because few modern and traditional medicines cause teratogenic effects on the fetus. Traditional medicines used by pregnant women must pass the teratogenic test so that they are safe for consumption by pregnant women and do not cause side effects on the growth and development of the fetus.

Based on the explanation above, this study aims to provide safety data from the ethyl acetate fraction of Sungkai leaf (*Peronema canescens* Jack.) when consumed by pregnant women and to determine the effect of teratogens on mice during pregnancy. This research was conducted using in vivo analysis using female white mice as experimental animals.

RESEARCH METHODS

Materials

Fresh Sungkai (*Peronema canescens* Jack.) leaves were obtained from the yard of the Padang City Slaughterhouse, Aie Pacah, Kec. Koto Tangah, Padang City, 70% ethanol, n-hexane, ethyl acetate, 0.5% Na CMC, Aquadest, Alizarin solution, Bouin's solution, quercetin, phytochemical reagents, filter paper, TLC mobile phase (n-hexane and ethyl acetate), a standard diet of mice, 24 female white mice aged 2-3 months with a weight between 20-35 grams.

Extract Preparation

600 g of simplicia sungkai leaves were soaked in 70% ethanol for three days with three repetitions. Furthermore, it was filtered using a funnel lined with filter paper to obtain the maserate. The obtained maserate was then concentrated using a rotary evaporator at a temperature of 40-60°C to obtain a thick extract (9).

Preparation of the Fraction

The thick ethanol extract that has been obtained is then fractionated using a separating funnel. The first fractionation stage is ethanol fractionation with n-hexane until a clear-colored n-hexane is obtained. Furthermore, the ethanol residue was fractionated again with ethyl acetate until it

became clear. The ethyl acetate fraction is then concentrated using a rotary evaporator until a thick ethyl acetate fraction is obtained and then weighed (9).

Preparation of Test Animal

This study used 24 female white mice aged 2-3 months weighing between 20-35 grams. Mice were divided into four groups consisting of group 1 as a negative control (Na CMC 0.5%), group 2, group 3, and group 4 were given the ethyl acetate fraction of sungkai leaves with varying doses of 28 mg/kg BW, 56 mg/kg BW and 84 mg/kg BW (9). The treatment was given orally using a probe. The treatment was started on day 6 to day 15 of pregnancy. The weighing was carried out every day, and the weight gain of the mice was observed. On the 18th day, the mice were sacrificed by cervical dislocation, followed by a laparotomy for fetal examination (10).

Acclimatization of Test Animals and Determination of the Estrus Cycle

Acclimatization of test animals for teratogenic experiments was carried out for ten days. Test animals are included in the healthy category if their body weight is not more than 10% of the initial weight and has an estrus cycle period of 4-5 days (10).

Mating of Test Animals

Female mice undergoing the estrus phase were mated with a male-to-female ratio of 1: 4. Animal mating was carried out by inserting male animals into the female cage. The success of copulation can be seen directly in the mice's vagina, which is indicated vaginal plug, so that day is counted as day 0 of pregnancy. The pregnant mice were separated, and the unmarried mice were mixed again with male mice. After finding the female *Mus musculus* vaginal plug, they were separated into cages (10).

Laparotomy

On the 18th day, the mice were weighed, then sacrificed by neck dislocation (pulling the tail of the mice as hard as possible after the neck was pressed with a blunt object). After that, a laparotomy was performed (removing the fetuses of mice by slashing the abdomen of the mice) to remove the fetuses of mice by cutting the uterus and placenta on the fetus and then observed on the fetus. The number of fetuses was counted in each part of the uterus, fetuses that were still alive, and fetuses that had died. After that, the fetus was weighed to determine the average birth weight (10).

Fixation and Observation of Morphological Defects

After the mice fetuses were visually observed, then the fetuses were fixed. There are two fixative solutions, namely Bouin's solution to see visceral abnormalities such as the cleft palate and alizarin to see skeletal and bone abnormalities (skeletal). Bouin's solution contains 40% formalin, glacial acetic acid,

and saturated picric acid. Immersion of the fetus with Bouin's solution lasts 14 days until the fetus is yellow and becomes rigid. Visceral observations such as the cleft palate can be made in the mouth with a sharp knife and slash towards the back until the head is separated into two parts. Alizarine solution contains 1% KOH and six grams of red alizarin powder. Immersion of the fetus with alizarin solution is done to see skeletal and bone abnormalities in the fetus. The alizarin immersion only lasted for three days (10).

Data Analysis

The research data were analyzed using one-way Analysis of Variation (ANOVA) for the parameters of maternal weight, number of fetuses, and fetal weight. If the data obtained do not match or there is a significant difference, it is continued using the Duncan test.

RESULTS AND DISCUSSION

The study results show that the people of West Sumatra have carried out ethnopharmacology for generations and the result of the administration of the ethyl acetate fraction of sungkai leaves in the test group affected the body weight of the mice during pregnancy. The average body weight of mice during pregnancy in negative controls (Na CMC 0.5%), doses of 28 mg/kg BW, 56 mg/kg BW, and 84 mg/kg BW, respectively, were 34.68 g, 31 .38 g, 28.61 g, and 28.23 g. The data included in the statistical test was data on the difference in body weight of the mother mice on day 18 and day 0. Based on the data processing results using one-way ANOVA, the difference in body weight of the parent showed a p-value of 0.00 where the sig value ($p < 0.05$). Furthermore, Duncan's test was carried out, and the results showed that the treatment group differed significantly from the negative control group. The average difference in weight gain of pregnant mother mice was the largest in the negative control group and the smallest average in the dose group of 84 mg/kg BW

Day-	Negative Control	Dose 1	Dose 2	Dose 3
0	22,38	21,57	21,78	21,42
1	22,75	21,83	22,02	21,75
2	23,15	22,20	22,50	22,32
3	24,40	23,17	23,17	22,73
4	25,87	23,98	24,15	23,33
5	27,52	25,07	24,60	23,98

6	29,07	26,20	25,30	24,70
7	30,55	27,35	26,15	25,45
8	32,30	29,02	26,58	26,22
9	33,57	31,23	27,45	27,25
10	35,17	32,00	28,65	27,90
11	37,33	33,57	29,68	29,57
12	39,80	35,48	31,10	30,93
13	41,65	36,75	31,85	31,97
14	43,72	38,98	33,22	33,17
15	44,98	39,92	34,28	34,37
16	47,28	41,70	36,75	35,42
17	48,37	42,50	36,76	36,28
18	49,08	43,72	37,58	37,53
Average	34,68	31,38	28,61	28,23

Note: D1: The dose group is 28 mg/kg BW
D2: The dose group is 56 mg/kg BW
D3: The dose group is 84 mg/kg BW

The results of observations on the average number of fetuses in the negative control group (Na CMC 0.5%), doses of 28 mg/kg BW, 56 mg/kg BW, and 84 mg/kg BW, respectively, were 10.67, 9.00, 8.33, and 9.67 mice. Based on the data processing results using one-way ANOVA, the average number of fetuses showed a p-value of 0.445, so the administration of the ethyl acetate fraction of sungkai leaves did not significantly affect the average number of fetuses (p>0.05). The number of children per birth in mice is the number of live and dead children at birth. The number of children per birth is about 6-15 (11).

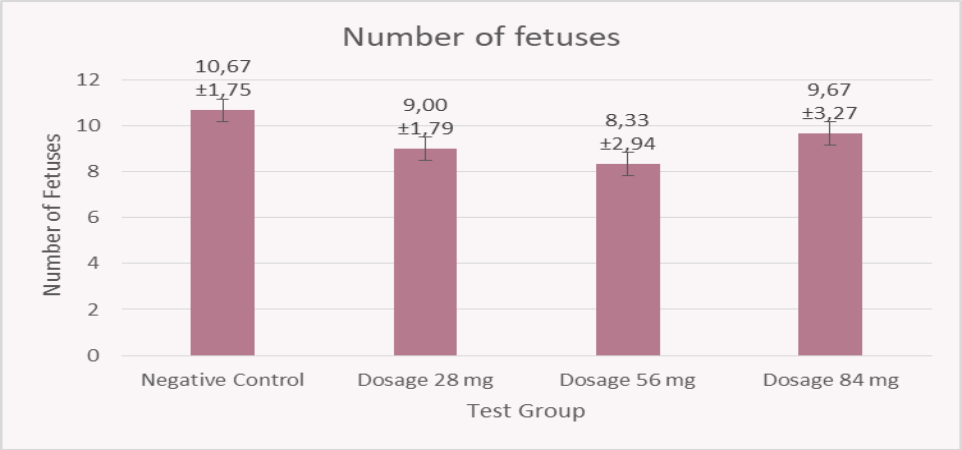

Figure 1. Graph of the number of fetuses

The results of observations on the average fetal weight in the negative control group (Na CMC 0.5%), doses of 28 mg/kg BW, 56 mg/kg BW, and 84 mg/kg BW were 1.32 g, respectively, 1.19 g, 1.13 g, and 1.12 g. The data processing results using one-way ANOVA, the average number of fetuses showed a p-value of 0.358, so the administration of the ethyl acetate fraction of sungkai leaves did not significantly affect the average fetal weight (p>0.05). The body weight of mice usually ranges from 0.5 to 1.5 g/head (11).

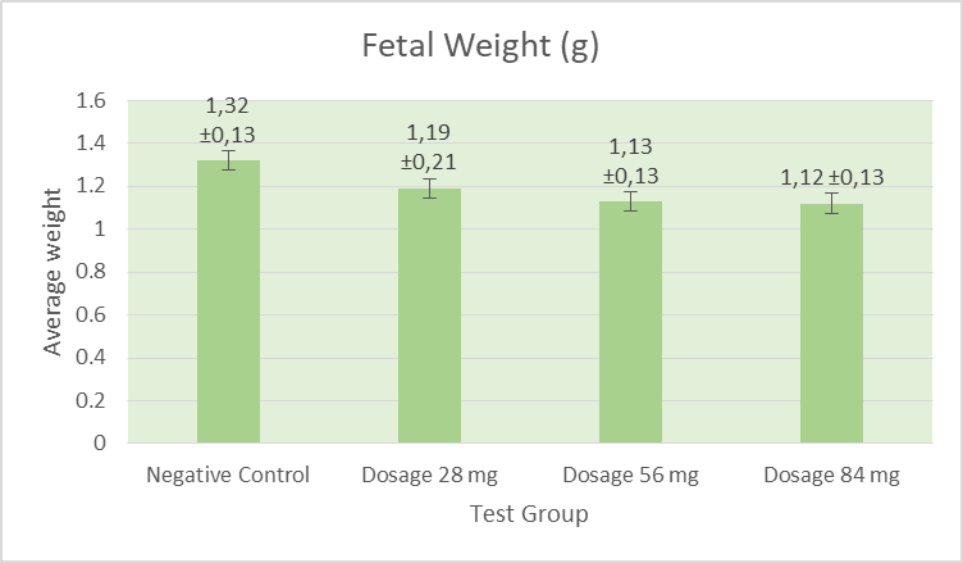

Figure 2. Fetal weight chart

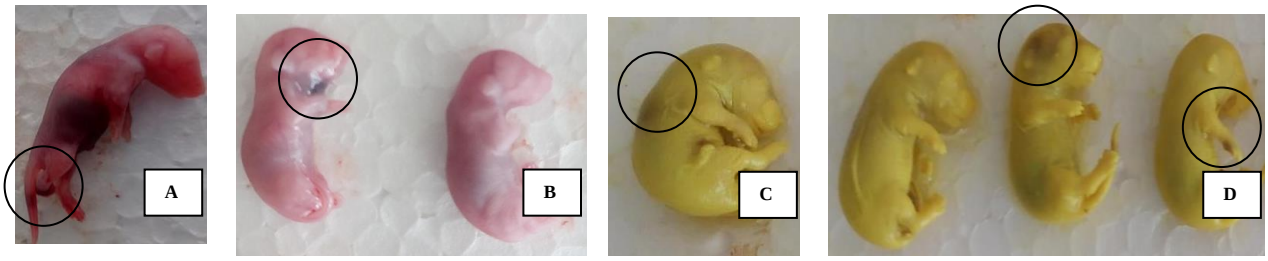

Figure 3. The fetus has a hemorrhage in the (A) legs and tail, (B) neck , (C) back, (D) head and legs

On morphological observation, several hemorrhagic fetuses were found. Hemorrhage is the discharge of blood from the cardiovascular system, accompanied by accumulation in body tissues. Foreign substances in tissues can change the osmotic pressure. The osmotic imbalance occurs due to pressure and fluid viscosity disturbances in different parts of the fetus, between blood plasma and extra capillary space. This causes blood vessels to burst, and hemorrhage

occurs. Hemorrhage tends to increase with increasing doses (12).

There were two dead fetuses in the 56 mg/kg BW dose group. Stillborn fetuses are characterized by the absence of fetal movement when expelled from the uterus. Some fetuses died, probably due to the influence of the test preparations. In the early stages of organogenesis, the test preparations directly inhibited growth, so implantation did not have time to grow, and the embryo died. In addition, fetal death occurs due to genetic factors from mother mice, where each mice has resistance to exposure to different test preparations (12).

Fetuses death can occur because the content in the form of flavonoids and saponins is cytotoxic, which can cause the death of several cells that make up normal cells. Flavonoids can cause teratogenicity in various ways, including being antiproliferative in the fetus, working as estrogen receptor agonists and antagonists that inhibit chondrocyte cell proliferation, producing pro-oxidant byproducts that can attack normal cells and inhibit phosphodiesterase enzyme activity, causing an increase in cAMP concentrations and accelerated growth hampered. The dead fetus is thought to have occurred because of the terpenoid content in the sungkai leaves, which is thought to have a teratogenic effect in the form of embryotoxic (poisoning the embryo) in genetically sensitive embryos. (6).

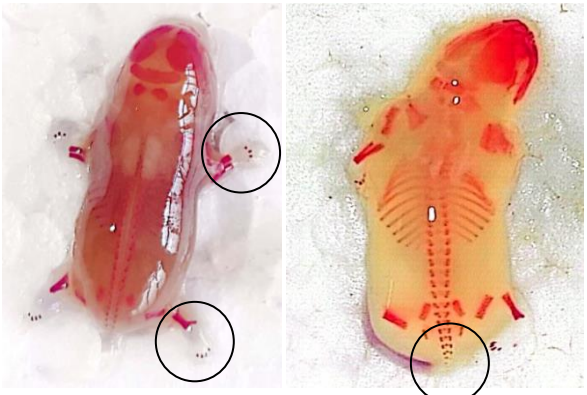

Figure 4. Skeletal abnormalities in the caudal, posterior phalanges, and anterior phalanges

Based on the results of immersion with Alizarin solution, several bone abnormalities were found. This is due to the steroid compounds in the ethyl acetate fraction of sungkai leaves. Steroids can interfere with bone formation by affecting calcium metabolism. The effect is to reduce calcium absorption in the gastrointestinal tract and increase calcium excretion through the kidneys (13). Chemical compounds such as terpenoids and steroids can cause malformations in the facial bones (craniofacial) of mice fetuses which include eye size reduction (microphthalmia) and hydrocephalus (14).

The results of alizarin fixation in the treatment group at doses of 28, 56, and 84 mg/kg BW showed defects in the upper and lower movable bones. Teratogen compounds during organogenesis can cause bone growth inhibition. Teratogenic compounds that enter through the placenta will inhibit the transfer and metabolism of nutrients from the mother to the fetus for the growth and development of fetal organs and minerals for bone formation (15).

Figure 5. Results of fixation of the fetus with Bouin's solution

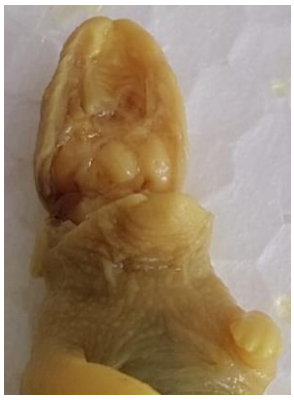

Figure 6. Cleft palate normal

The results of soaking the fetus with Bouin's solution lasted for 14 days until the fetus was yellow and became hard. The picric acid in Bouin's solution causes the fetus to turn yellow, making it easy to observe. The formaldehyde and acetic acid present in Bouin's solution will preserve the embryonic tissue (12). Visceral observations such as the palate (cleft palate) can be made in the mouth with a sharp knife and slash towards the back until the head is separated into two parts (10). Based on the immersion of Bouin's test results, all groups had no abnormalities in the cleft palate.

In this study, the negative control group did not experience any morphological, skeletal, or visceral defects. In the treatment group that was given the ethyl acetate fraction of sungkai leaves, not all had defects, and several fetuses did not experience any defects. This can occur based on the type or severity of the disability caused by the teratogenic agent and depending on the genetic susceptibility carried by the mother and fetus. For example, variations in the metabolism of a particular drug will determine what metabolites the fetus is exposed to and the duration of exposure. The genetic susceptibility of the fetus to specific teratogenic agents will affect the outcome of fetal defects (16).

Based on the results of research conducted in vivo, fetuses born to mothers who were given the ethyl acetate fraction of sungkai leaves during the organogenesis period, several mice fetuses had morphological and skeletal defects. The teratogenic effect of giving the ethyl acetate fraction of sungkai leaves in this study was in the form of hemorrhage on the fetus's head, neck, back, legs, and tail. The bone has not developed properly due to disruption of bone development, such as the caudal bone and posterior and anterior phalanges. Meanwhile, no mice fetuses showed cleft palate in the treatment group or the control group. Thus, these results indicate that the administration of the ethyl acetate fraction of sungkai leaves is still not safe for consumption by pregnant women.

CONCLUSION

1. The people of West Sumatra have carried out ethnopharmacology for generations.
2. There was a significant difference ($p < 0.05$) between the body weight of mice in the control group and the treatment group, given the ethyl acetate fraction of sungkai leaves. At the same time, there was no significant difference ($p > 0.05$) in body weight and the number of fetuses given the ethyl acetate fraction of sungkai leaves.
3. There are morphological abnormalities in the form of hemorrhage in the fetuses in the treatment group in the fetus's head, neck, back, legs, and tail. There are

skeletal abnormalities in the caudal bone, anterior phalanges, and posterior phalanges of the mice fetus.

SUGGESTION

For further research, it is recommended to conduct research on the potential for teratogens of sungkai leaf microscopically and histologically against other species of animals so that the complete toxicity of this plant and its safe dose can be determined.

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