

Assessment of Teratogenic Effects of Sappan Wood (*Caesalpinia Sappan* L.) Extract In Rats (*Rattus novergicus*)

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ARTICLE INFO

Article history:

Received March 24, 2022

Received in revised form May 16, 2023

Accepted June 27, 2023

KEYWORDS:

sappan wood,
teratogenic,
rat,
fetuses

ABSTRACT

Treatment using plant materials has been widely researched and observed to assist in curing a disease. Medicinal products derived from herbal plants are proven useful as drugs must follow guidelines not to cause acute or chronic toxicity. The purpose of this study was to investigate the teratogenic potential of an ethanolic sappan wood extract on the growth of unborn rats. Six groups of pregnant rats are created: Aquadest was administered to the Control group, whereas the Treatment groups received 100, 200, 300, 400, or 500 mg.kg⁻¹ BW of an ethanolic extract of Sappan wood. On the twenty-first day of gestation, pregnant rats were CO₂-euthanized and delivered vaginally. Rats' body weight during pregnancy was observed, along with fetal growth measurements, viscoelastic examination, and skeletal development. Wistar rats' reproductive systems, fetal body weight, body length, and tail length were all unaffected by ethanol extract of Sappan wood at doses from 100 mg/kg to 500 mg/kg. In fetus rat from the control group and the five dosage groups, no skeletal development had taken place and there were no obvious external abnormalities. Even at the maximum dose of 500 mg, sappan wood extract demonstrated no teratogenic effects on the development of fetal rats.kg⁻¹ BW.

1. Introduction

Plants are one of the potential sources of drugs (Mulyani *et al.* 2020). The use of plant materials in medical research to seek treatments for certain illnesses has increased in Indonesia. However, the plant materials must undergo many requirements such as safety, benefits, and standardization to be developed as potential candidate drugs (Raharjo *et al.* 2017). It is important to test the potential toxicity of the plant materials to ascertain their safety use. Teratogenic testing toxicity is helpful in anticipating damage to the fetus brought on by plant materials that are consumed during pregnancy. Some pharmaceuticals taken by pregnant women may pierce the placenta and undergo biotransformation into a highly reactive molecule, necessitating caution

in their usage. As a result, about 2-3% of them are thought to be caused by taking the drug (Inarah Fajriaty *et al.* 2019).

Caesalpinia sappan wood is a plant commonly used in the traditional treatment of tuberculosis, diarrhea, skin infection, and anemia in Thai. *Caesalpinia sappan* L., also known as sappan wood contains flavonoids and phenols such as brazilin and brazilin (Dapson and Bain 2015). Brazilin acts as an iron chelator that reduces hepatic and serum iron levels (Maskoen *et al.* 2016), total iron-binding capacity, and plasma transferrin saturation (Syamsunarno *et al.* 2021). The toxicological profile of this plant has been determined by several investigations. According to toxicological research on sappan wood extract, this plant is not toxic to the male Wistar rats employed in the experiments and has no negative effects on hematological, biochemical, or immunological parameters. Sireeratawong *et al.* (2010) at previous research showed that administered orally sappan

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wood extract did not toxic at various doses of 250 to 5,000 mg/kg BW. Administration of brazilin to ICR mice did not cause toxicity to the male reproduction, but increased the risk of fetal death when given to females before gestation. this is based on the finding that pre-mating doses of brazilin increased the spleen organ coefficients in father and male fetuses and brain organ coefficients in mother and female fetuses, indicating that brazilin in high doses had a toxic effect on the offspring. brazilin mechanism is reported to inhibit the expression of PPAR γ which affects placental development, trophobes differentiation, and regulates lipid metabolism, Additionally, it aids in the embryo's normal development (Yuan *et al.* 2016).

In addition, these plants are the potential for an iron chelating- agent. The works of Safitri *et al.* (2018) indicated that the ethanolic extract of *Caesalpinia sappan* wood contained 10% of brazilin and has the ability to act as chelating agent for iron in rats (*Rattus norvegicus* L.) with iron overload excess, and the recommended dose of sappan wood extract as chelation agent is 200 mg.kg⁻¹ bw.

The potential pharmacological effects and the abundance of active substances in the examination of *Caesalpinia sappan* wood's teratogenicity is required since it has the potential to be turned into a potent and safe medication. The effective dose of sappan wood extract is chosen based on its iron chelation treatment effectiveness in animal models. The highest dose was based on the LD 50 brazilin on pregnant rats, while the lowest dose was based on the effect of secang extract, which did not cause any toxicity symptoms. The aim of the current investigation was to find out whether *Caesalpinia sappan* wood extract was teratogenic in pregnant Wistar rats.

2. Materials and Methods

2.1. Materials

Caesalpinia sappan L. heartwood, female Wistar rats (*Rattus norvegicus*) aged 8-12 weeks, weighing 180-200 g were obtained from PT. Bio Farma, Indonesia. Sigma-Aldrich (St. Louis, Missouri, USA) was used to purchase all of the chemicals.

2.2. Plant Extraction

Sappan wood was obtained from the Wanagama area, Wonosari Central Java. Plant extraction was

done at Pharmaceutic Laboratory, Padjadjaran University. The extracted dried sappan wood is then pulverized. Using 96% ethanol for maceration. The finely chopped sappanwood was steeped in a 1:3 ratio of 96% ethanol for 48 hours. To create a thick extract of sappan wood, the macerate's products were concentrated using an evaporator at a temperature of 65°C. (Safitri *et al.* 2018). The extract was then diluted in distilled water to achieve verious doses 100, 200, 300, 400, and 500 mg.kg⁻¹ BW.

2.3. Ethical Consideration

The Padjadjaran University Research Ethics Commission has approved this study and assigned it the Ethical Clearance number 774/UN6.KEP/EC/2021.

2.4. Animal Experiment and Sample Collection

One hundred and twenty female rats were randomly assigned to one of six groups, each of which received sappan wood ethanol extract at doses of 100, 200, 300, 400, or 500 mg.kg⁻¹ body weight daily. The negative control group was given simply distilled water. (OECD 2011). Female rats in the estrus cycle were mated with male rats at a ratio of 1:1 and maintained overnight in a cage after being acclimated for seven days. Day 0 of gestation was defined as the day when sperms were discovered in vaginal smears or confirmed by a vaginal plug on the following day. The weight of rats during pregnancy was recorded until day 20 of gestation (Mulyani *et al.* 2020).

2.5. Teratogenic Examination

The teratogenicity examination was conducted in accordance with the guidelines of the Organization for Economic Co-operation and Development (OECD No. 414) (OECD 2011). Sappan wood extract was given orally during the organogenesis period starting on day 6 until day 15 of gestation (Malini *et al.* 2017). The pregnant rats were 20 days along euthanized using CO₂, and a cesarean section was performed to deliver fetuses (Vieira *et al.* 2020). Recorded data included the number of implantation sites, live fetuses, and resorption. The live fetus' identity was determined. Bodyweight and length, as well as tail length, were determined to check for any appearance abnormality (OECD 2011). Then, half of the live fetuses of each pregnant rat were fixed in Bouin solution (75% picric acid, 25% formaldehyde, and 5% acetic acid) for viscous examination, while the other half was fixed

in 95% (v/v) ethanol for three weeks and stained with alizarin red for the skeletal analysis (Chen *et al.* 2017).

2.6. Skeletal Preparation

The fetuses were fixed with ethanol for three weeks, after which the skin and internal organs were removed. The fetuses were then immersed in 1% KOH for 24 h. Fetuses were stained with 1% alizarin red S for 24 hours after they become hyaline, before they gradually soaked in transparent solution ratio (KOH; glycerol; and little addition of 1% H₂O₂) of 3:1, 1:1, and 1:3 for each subsequent week (Câmara *et al.* 2017). Then a light microscope was used to examine at each specimen with white background. The percentage of hypoplasia of the metacarpal, phalanx, pelvis, spine, and sternum according to reported to indices of skeletal development in fetuses (Deng *et al.* 2014).

2.7. Data Statistical Analysis

The mean and standard deviation (SD) of the data were showed. Statistical Package for the Social Sciences Version 25.0 program was used to analyzed the data. (IBM Corp., Armonk, NY, United States). First, it was determined whether the data were normal; if so, analysis of variance (ANOVA) was used for the overall comparison. The Kruskal-Wallis test was applied when the data were not normal, and the Mann-Whitney test was then employed for pairwise comparisons between the various dosages and the

control group. A p-value of 0.05 or less was regarded as significant.

3. Results

3.1. Sappan Wood Extract Effects on Gravid Parameters

The body weight during pregnancy in rats receiving the extract at various doses was similar to the negative control (Figure 1). The extract also did not affect the reproductive performance of the pregnant rats, in terms of size of litter, implantation sites, live and dead fetuses, and a resorbed fetus (Table 1). No fetus was found dead. Each fetus in each group still responded when touched, indicating that it was still alive. The results suggest that the extract is taken up to 500 mg.kg⁻¹ body weight was relatively safe in pregnant rats.

3.2. Sappan Wood Extract Effect on Fetal Development

The fetal parameters of rats who received sappan wood extracts up to 500 mg.kg⁻¹ body weight had no significant effect. In comparison to the control, there were no differences in the ratio of sex ratio fetuses, fetal body weight and length, or tail length. (P>0.05) (Table 2). No morphological abnormalities were found in the rat fetuses treated with sappan wood extract at all doses (Figure 2).

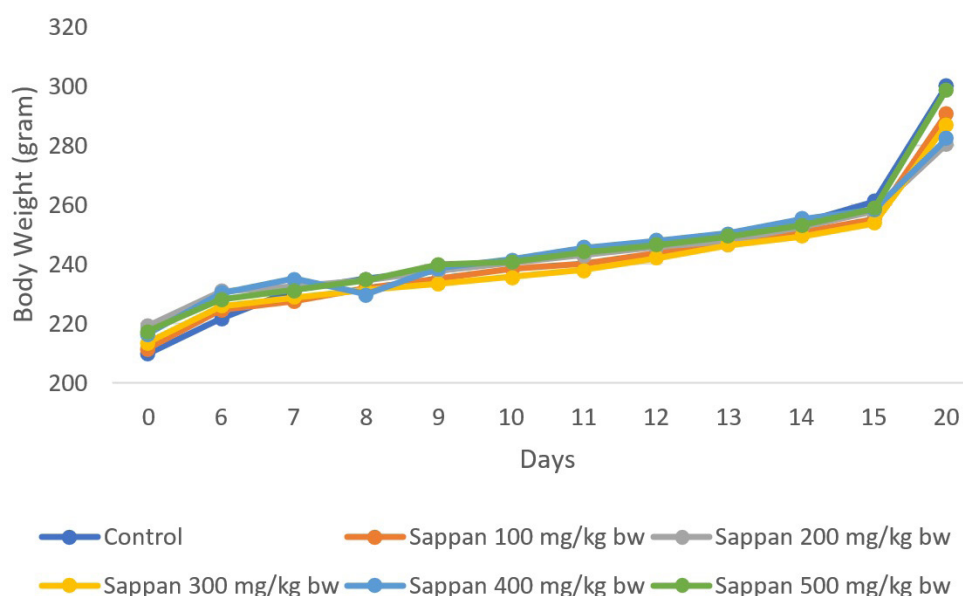


Figure 1. Bodyweight of pregnant rats during gestation. Points represent mean ± SD (n = 20)

Table 1. Reproductive performance in rat pregnant after giving sappan wood extract

Reproductive performance	Control group	Sappan wood extract (mg/kg BW) group				
		100	200	300	400	500
Number of pregnant rats	20	20	20	20	20	20
Number of implantation sites	9.3±1.05	9.1±0.99	9.22±0.97	9.00±0.70	9.3±1.41	9.27±3.69
Number of fetuses	8.7±1.41	8.8± 0.78	8.7±0.66	8.8±0.78	8.8±1.61	8.7±2.81
% Live fetuses	100	100	100	100	100	100
% Fetal resorption	0	0	0	0	0	0
% Fetal death	0	0	0	0	0	0

Table 2. Effects of sappan wood extract on rat body weight, tail length, and fetal sex

Groups	Male/ female ratio	Fetal body weight (g)		Fetal body length (cm)		Length of the tail (cm)	
		$\bar{x} \pm SD$	p-value	$\bar{x} \pm SD$	p-value	$\bar{x} \pm SD$	p-value
Control	0.703	4.57±0.67	/	3.43±0.50	/	1.09±0.16	/
Sappan 100 mg/kg bw	1.158	4.56±0.94	0.374	3.42±0.71	0.368	1.1±0.22	0.352
Sappan 200 mg/kg bw	1.086	4.60±1.04	0.991	3.45±0.78	0.958	1.1±0.22	0.936
Sappan 300 mg/kg bw	1.116	4.62±1.24	0.974	3.47±0.92	0.99	1.1±0.30	0.911
Sappan 400 mg/kg bw	1.087	4.59±0.88	0.893	3.44±0.66	0.945	1.11±0.21	0.923
Sappan 500 mg/kg bw	0.812	4.58±0.96	0.249	3.43±0.72	0.251	1.01±0.23	0.265

Data are expressed as mean ± SD (n = 505)

3.3. Sappan Wood Extract Effect on Skeletal Formation

The effects of the extract on the skeletal development of fetuses are shown (Figure 3). No visible occurrences of sternum hypoplasia, pelvis hypoplasia, metacarpal hypoplasia, spine hypoplasia, and phalanx hypoplasia of fetal rats in the control group and groups treated with sappan wood extract.

4. Discussion

The extract also did not affect the reproductive performance of the pregnant rats, in terms of size of litter, the number of implantation sites, dead and live fetuses as well as a resorbed fetus (Table 1). No fetus was found dead. Each fetus in each group still responded when touched, indicating that it was still alive. The results suggest that the extract is taken up to 500 mg.kg⁻¹ body weight was relatively safe in pregnant rats. The plant has been reported, which is consistent with our findings a significant increase in the ratio of the resorbed fetus in the group that received the highest dose (20 mg.kg⁻¹) of brazilein, which was four times higher than the pharmacological effective dose. It was reported that the high dose extract received in this research contained about 6.28 mg/g brazilein. The body weight during pregnancy in rats didn't change and was not significant different from the negative control group. The pregnant rat weight can also be

a representation of the quantity or weight of the resulting fetus when compared to other groups, with the resulting fetus being smaller or higher. The mildest manifestation of teratogenic chemical effects is weight loss. In human consumption, Secang wood has been scientifically proven to have numerous medicinal characteristics, and it is frequently drunk by the community as a nutritious beverage. It also contains potent antioxidant extracts with stronger antioxidative indices than commercial antioxidants (Arum *et al.* 2017). In pregnant women, sappan wood extract containing brazilien should be taken with caution. It may have a significant impact on embryo development and resorption when give to females before implantation (Yuan *et al.* 2016). This is especially important for pregnant women to avoid the possible risk of exposure to teratogens (Dillasamola *et al.* 2018).

There was no statistically significant difference between the sappan wood ethanol extract group and the control group with regard to the fetal parameter. From the rat fetal parameters in Table. 2 were not significantly affected by sappan wood extracts up to 500 mg.kg⁻¹ body weight. The ratio of male to female fetuses, their body weight and length, and their tail length were identical to those of the control group. However, sappan wood extract has no effect on embryo sexuality in comparison to the unfavorable controls. This finding is in dissaccord with previous data on mice fetal exposed with pure brazilin (Yuan

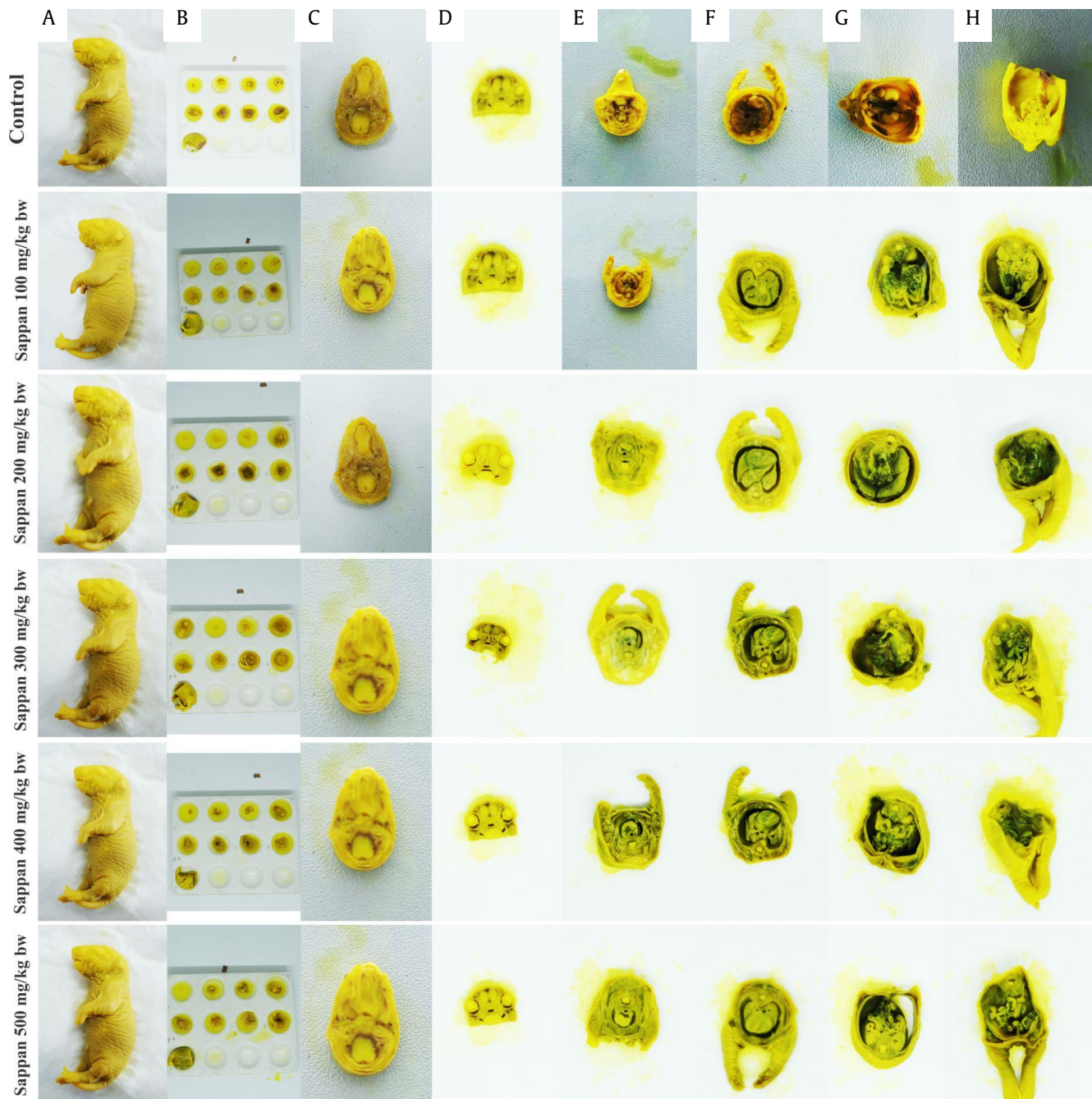


Figure 2. A 20-day-old rat fetus of the control and sappan groups fixed in Bouin solution, (A) whole fetus, (B) sectioned fetus, (C) average palate, (D) normal ventricles and eyes were visible in the coronal part of the brain, (E) a section of the neck showed normal of the esophagus, trachea, and thyroid, (F) a section of the chest showed normal intraventricular septum, (G) and (H) a section of the diaphragm and visceral organ showed normal include kidney

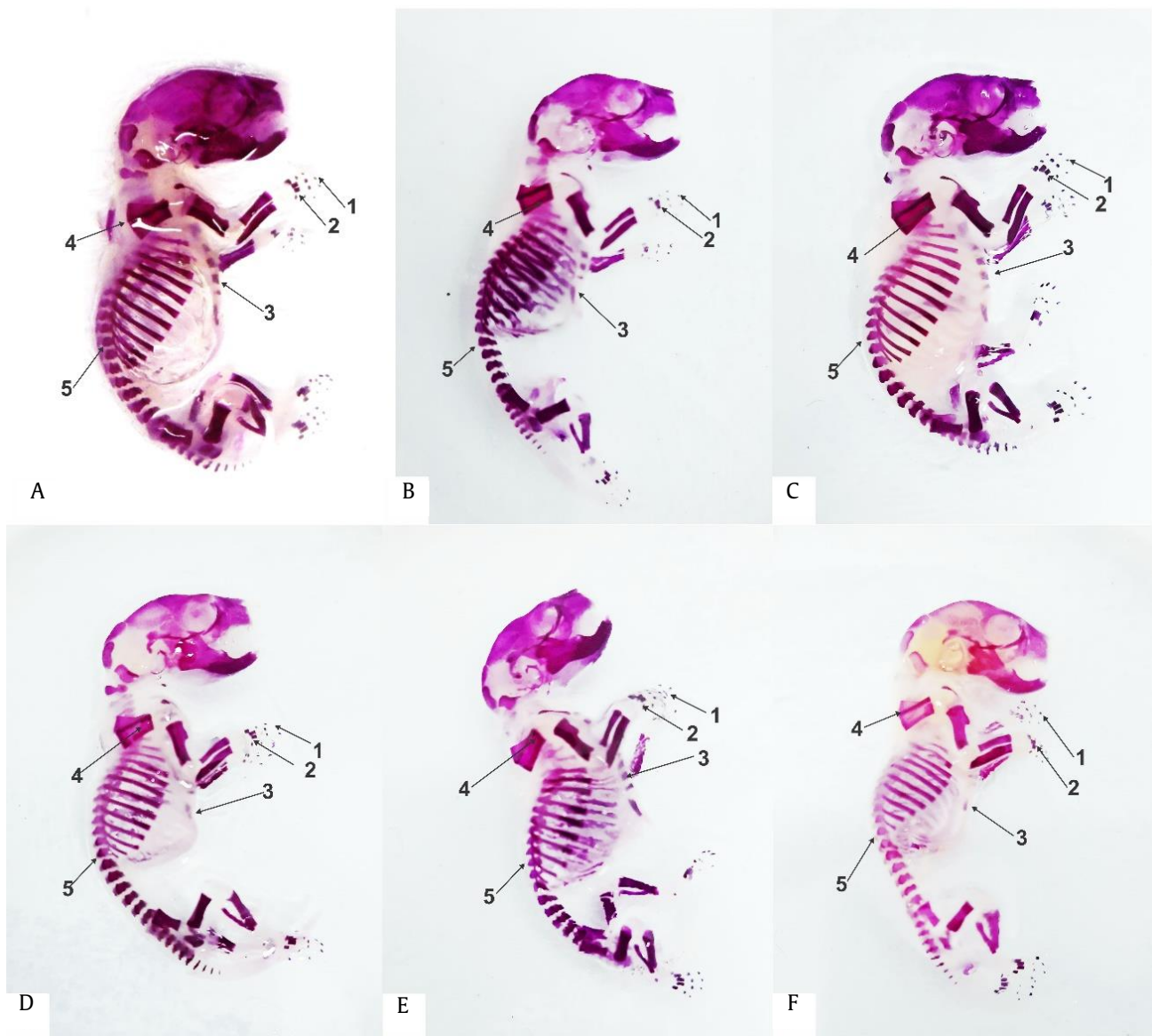


Figure 3. Alizarin red-stained rat fetuses on a 20th-day rat group; (A) control, (B) sappan 100 mg/kg bw, (C) sappan 200 mg/kg bw, (D) sappan 300 mg/kg bw, (E) sappan 400 mg/kg bw, (F) sappan 500 mg/kg bw. fetus showed no visible incidence of hypoplasia. (1) metacarpal, (2) phalanx, (3) pelvis, (4) spine, and (5) sternum

et al. 2016). Our findings, in this study, not effect morphological changes in the fetus because the brazilian compound was still below Ld50.

Visceral examination showed that no visible external abnormalities of brain, palate, chest organs the location of blood vessels heart, liver, stomach, spleen, kidneys, and reproductive organs were observed in the fetuses (Deng *et al.* 2014). The plant has been considered non-toxic in both acute and subacute investigations, which is consistent with our findings (Sireeratawon *et al.* 2010). Therefore, it maybe possible to conclude that sappan wood extract

did not pose any toxic effects on organogenesis when taken during the gestational period.

Noevident anomalies hypoplasia of the metacarpal, sternum, phalanx, spine, and sternum were found in all groups. This is because the compound in sappan wood extract does not inhibiting the synthesis of folic acid. Imbalance between the bone-forming activity of osteoclasts leads to osteoporosis, increased bone loss and reduced bone replacement (Inarah Fajriaty *et al.* 2019). Many of the bones in rodents, including rats, become ossified during the late fetal stage (Abebe *et al.* 2021). As a result, in teratogenicity, the

level of skeletal ossification is a crucial indicator of fetal maturity (Udoh *et al.* 2020). Normal ossification indicated by the incidence of hypoplasia or development of the sternum, leg bones, and toes was observed in all treated groups which were similar to that of the control. Observations on the skeleton fetus's development with Alizarin Red were used to test (Dillasamola *et al.* 2018). Alizarin binds calcium to the bone matrix, turning the fetus's bone a dark purple color (Wahab *et al.* 2020).

In conclusion, sappan wood extract at doses up to 500 mg.kg⁻¹ did not effect on fetal development, hence exhibiting no teratogenic effect in Wistar rats.

Acknowledgments

The research and publication were supported by the Indonesian Endowment Fund for Education under the "Rispro - LPDP" program, for which the authors are grateful.

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