

The Effect of Sappan Wood Extract (*Caesalpinia sappan* L.) on Fetal and Placenta Histopathology of White Rat

(EFEK PEMBERIAN EKSTRAK KAYU SECANG (*CAESALIPINIA SAPPAN* L.)
TERHADAP HISTOPATOLOGI FETUS DAN PLASENTA TIKUS PUIH)

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ABSTRACT

Histomorphological assessment of the placenta and fetus was more effective in assessing fetal development on a research scale for determined an active substance during the gestation period in experimental animals. The placenta and fetus connect in the development process. This study aimed to analyze the effect of giving ethanolic extract of sappanwood on white rats' placenta and fetal organs, which were examined histologically at 20 days pregnant rats. The pregnant rats were divided into six groups: The negative group was given aquadest, and treatment groups were given an ethanolic Sappan wood extract 100;200;300;400;500 mg/kg BW. Euthanized with CO₂ and cesarian section was performed on pregnant rats on the 20th gestational day. Observation to record fetal body weight, body length, mean placental weight, and the histology of the placental area. Histomorphometry was used to measure the area of the fetal placental region. The group with sappan wood extract had no statistically significant difference in fetal body weight, fetal body length, fetal tail length, the weight placenta, and histomorphometry of the placenta compared to the control group ($p > 0.05$); this showed that the ethanolic extract of sappan wood does not have a toxic effect on the development of the placenta, which can interfere with fetal development during pregnancy. Sappan wood extract had a nontoxic effect on the placenta and fetal rat development on histological examination, even at the highest dose of 500 mg.kg⁻¹ bw.

Keywords: sappan wood extract, histology, fetal, placenta

ABSTRAK

Penilaian histomorfologi plasenta dan janin lebih efektif dalam menilai perkembangan janin pada skala penelitian untuk menguji toksisitas zat aktif selama periode kehamilan pada hewan percobaan. Plasenta dan janin terhubung satu sama lain dalam proses perkembangan selama kehamilan. Penelitian ini bertujuan untuk menganalisis pengaruh pemberian ekstrak etanol kayu secang terhadap perkembangan plasenta tikus putih dan organ janin yang diperiksa secara histologis pada tikus usia kebuntingan 20 hari. Tikus bunting dibagi menjadi enam kelompok: Kelompok negatif diberi aquadest, dan kelompok perlakuan diberi ekstrak etanol kayu Sappan tindakan 100;200;300;400;500 mg/kg bb. Euthanasia dengan

CO₂ dan dilakukan pembedahan pada tikus bunting pada hari ke-20. Penilaian dilakukan terhadap berat badan fetus, panjang badan, rerata berat plasenta, dan histologi area plasenta. Histomorfometri digunakan untuk mengukur luas daerah plasenta. Hasil: Kelompok yang diberi ekstrak kayu secang tidak memiliki perbedaan bermakna secara statistik berat badan janin, panjang badan janin, panjang ekor janin, berat plasenta, dan histomorfometri plasenta dibandingkan dengan kelompok kontrol ($p > 0,05$); Hal ini menunjukkan bahwa ekstrak kayu secang tidak memiliki efek toksik terhadap perkembangan plasenta sehingga dapat mengganggu perkembangan janin selama kehamilan. Kesimpulan: Ekstrak kayu secang memiliki efek nontoksik terhadap plasenta dan perkembangan janin tikus pada pemeriksaan histologis, bahkan pada dosis tertinggi 500 mg.kg bb

Kata kunci: ekstrak kayu secang, histologi, fetus, plasenta

INTRODUCTION

The increasing development of research using plant materials in Indonesia to be used as raw materials in the manufacture of drugs, but still few are used in health facilities because they have to pass many requirements such as safety, benefits, and standardization (Shaikh *et al.*, 2019). There are very important when using plant materials for making drugs to test the compounds' toxicity. Toxicity testing, a teratogenic test, will be beneficial in predicting damage to the fetus caused by the compounds contained (Mulyani *et al.*, 2020). Sappan wood (*Caesalpinia sappan* L.) contains flavonoids and phenols such as brazilin which can reduce and suppress the formation of free radicals and act as iron chelators, reduce iron levels in liver tissue, and reduce iron concentrations in serum, TIBC, and Tf saturation (Safitri *et al.*, 2018). Sappan wood extract contains five active compounds that function as antioxidants, including brazilin, a catechol group in its compound structure, indicating that brazilin can serve as an iron chelator both *in vitro* and *in vivo* effect (Syamsunarno *et al.*, 2021).

The increasing research development uses plant materials in Indonesia as raw materials to manufacture drugs. Still, few are used in health facilities because they must pass many requirements (Mulyani *et al.*, 2020). Safety, benefits, and standarization are essential when using plant materials to test the compounds safety for making medicine.

Examining the placenta and fetal development is an important parameter in evaluating the safety of sappan wood extract during pregnancy (Furukawa *et al.*, 2019). The placenta plays a pivotal role in fetal growth even though it is a temporary organ during pregnancy. It is the interface between the dam and developing embryo or fetus and is a multifunctional organ serving the liver, lung,

gut, kidney, and endocrine or exocrine glands (Cline *et al.*, 2014). About 2-3% of them are purportedly caused by taking the drug because some pharmaceuticals taken by pregnant women might pierce the placenta and undergo biotransformation into a highly reactive molecule, necessitating caution in their usage (Fajriaty *et al.*, 2019).

This study aimed to analyze the effect of giving ethanol extract of sappan wood on white rats's placenta and fetal organs, which were examined histologically at 20 days pregnant rats.

RESEARCH METHODS

Materials

The materials are Sappan wood (*C. sappan* L) selected for this study and were from Central Java, Indonesia. The other materials are thirty rats (*Rattus norvegicus* L) female aged 8-12 weeks, weighing 180-200 g, aquades, Bouin fixative solution, NaCl 0,9 %, CO₂ Alcohol (70 %, 96 %, and absolute), xylene, paraffin, dye (hematoxylin & Eosin) and entelan.

Plant Extraction

Sappan wood is obtained from the Wanagama, Wonosari Central Java. The dried sappan bark is then crushed and extracted. Extraction was carried out by maceration using 96% ethanol. The finely chopped sappan wood was soaked in 96% ethanol for 48 hours in a 1:3 ratio. The results of the macerate were then concentrated using an evaporator at a temperature of 65°C to obtain a thick extract of sappan wood. The extract was then dissolved with distilled water to obtain 100, 200, 300, 400, and 500 mg/kg BW doses.

Experimental Animal and Sample Collection

The study was conducted experimentally using a randomized post-test-only controlled group design with five doses and one negative

control. After being acclimated to the laboratory for seven days, female rats in the estrus cycle were mated with male rats at 1:1 and kept overnight in a cage. Sixty pregnant rats were grouped into; a negative control where only distilled water and treatments group given sappan wood extract 100; 200; 300; 400; 500 mg/kg⁻¹ daily with ten rats each from GD 6th until GD 15th. At the GD 20th. The data developing fetuses on mean fetal body weight, body length, mean placental weight, and the histology of the placental area was displayed in a measurement results table (Furukawa *et al.*, 2011). The placenta was analyzed quantitatively by measuring the entire placental area, labyrinth zone area, and basal zone area using ImageJ (Furukawa *et al.*, 2019).

Histological Preparation

After fixation, the placenta was cut transversely from the center. At the same time, the serial sectioning scheme of the whole fetus fetal sections to visualize most of the organs according to histological parameters (Kamar, 2018). The histological examination provides consistency in cell morphology and tissue structures in which most tissues can be analyzed. The fixed placenta and fetus were then sectioned with a thickness of 1 mm (Charest *et al.*, 2018). The tissue pieces are inserted into the tissue cassette for the processing process. Histological preparations of the fetus and placenta were stained with Hematoxylin-Eosin. Histological data of the fetus was obtained by observing the light microscope and taking digital camera images for documentation.

Ethical Consideration

This research has been registered with the Research ethics Commission of Padjadjaran University with the Ethical Clearance number 774/UN6.KEP/EC/2021.

Data Analysis

A measurement results table displayed the data on the table, mean fetal weight, mean placental weight, and the placental area. Histological preparations of the fetuses of Wistar strain white rats were observed qualitatively to see the histological structure of the fetus. The placenta was analyzed by measuring the entire placental area, labyrinth zone area, and basal zone area used ImageJ image management computer software ver.1.5.3. Data were reported as mean ± standard deviation (SD). The data

were analyzed using Statistical Package for the Social Sciences version 25.0 (IBM Corp., Armonk, NY, United States) program. The normality of the data was tested firstly; if it was not, Analysis of variance (ANOVA) was used for the overall comparison. When data is not normal, the wase Kruskal-Wallis test was used, and then the Mann-Whitney method was adopted for pairwise comparisons among the different dosages with control. A p-value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

Data regarding fetal and placental weight are presented in Table 1. Placental weight in the group treated with sappan wood extract was not statistically significant different compared to the control group and also the mean fetal weight. These results indicate that the administration of sappan wood ethanol extract during gestation did not affect the placenta or fetus. The results of measurements of the area of the placenta that was carried out in the labyrinthine zone, basal zone, and all parts of the placental surface histologically are presented in Table 2. The placentas of rats at 20 days of gestation in all treatment groups were composed of three parts, as shown in Figure 1 showed normal morphology of the placenta. Placenta’s labyrinth and basal zones are formed from the growth of the embryo’s trophoctoderm. In contrast, the mother’s endometrium develops the decidua and the material gland. The exchange of mother and fetus material occurs through the labyrinth zone, which has a lot of vascularity limited by the presence of the placental wall—

Table 1. Weight of placenta and fetus at 20th days of gestation

Groups	Placenta (g)(x ± SD)	Fetus (g) (x ± SD)
Negative Control	0,58 ± 0,038 ^a	3,49 ± 0,24 ^a
SWE 100 mg.kg BW	0,56 ± 0,051 ^a	3,46 ± 0,31 ^a
SWE 200 mg.kg BW	0,56 ± 0,044 ^a	3,46 ± 0,28 ^a
SWE 300 mg.kg BW	0,57 ± 0,042 ^a	3,46 ± 0,26 ^a
SWE 400 mg.kg BW	0,57 ± 0,044 ^a	3,49 ± 0,27 ^a
SWE 500 mg.kg BW	0,56 ± 0,046 ^a	3,45 ± 0,28 ^a

Note: N=180, SD = standar deviation; SWE=sappan wood extract

Table 2. Histomorphometry of placenta at 20th days of gestation (mean ± SD)

Groups	Whole placenta region (mm ²)		Labyrinth zone (mm ²)		Basal zone (mm ²)	
	$\bar{x} \pm SD$	P value	$\bar{x} \pm SD$	P value	$\bar{x} \pm SD$	P value
Negative Control	30,11 ± 2,04 ^a		21,37 ± 1,44 ^a		5,81 ± 0,52 ^a	
SWE 100 mg.kg BW	29,88 ± 2,67 ^a		21,21 ± 1,9 ^a		5,77 ± 0,51 ^a	
SWE 200 mg.kg BW	29,88 ± 2,35 ^a	0,991	21,22 ± 1,67 ^a	0,872	5,76 ± 0,45 ^a	0,148
SWE 300 mg.kg BW	29,84 ± 2,21 ^a		21,18 ± 1,57 ^a		5,76 ± 0,43 ^a	
SWE 400 mg.kg BW	30,17 ± 2,39 ^a		21,42 ± 1,69 ^a		5,82 ± 0,46 ^a	
SWE 500 mg.kg BW	29,8 ± 2,49 ^a		21,15 ± 1,77 ^a		5,75 ± 0,48 ^a	

Note: N=180, SD = standar deviation; SWE= sappan wood extract

composed of three layers of trophoblast cells: one layer of cytotrophoblast cells and two syncytiotrophoblast cells.

The group with sappan wood ethanol extract had no statistically significant difference compared to the control group. Sappan wood extracts up to 500 mg.kg body weight did not significantly affect rat fetal parameters (Table 3). The fetal male to female ratio, fetal body weight and length, and tail length were not different from the control. Observations were made descriptively on every vital organ of the fetus under a light microscope. Overall, there was no difference in the histological structure of the fetus's eye, heart, liver, kidney, and spleen in the group given the ethanol extract of the sappan wood and in the control group (Figure 2).

In mid-gestational age in rats, the placenta is fully functional and continues to grow to support fetal growth until the last day of gestation. During pregnancy, the weight of the placenta remained stable. The average weight of the fetuses and their placentas analyzed at the 20th day of gestation is presented in Table 1. The weight of the placenta and the weight of the fetus in the treatment group that was given sappan wood extract at a dose of 100-500 mg/kg body weight compared to the control group did not differ statistically significantly with a p-value > 0.05. These results indicate that administering sappan wood ethanol extract during pregnancy does not affect the placenta and fetus. Indicators of the pathological state of the newborn can be seen based on the weight of the placenta (Fowden *et al.*, 2009). The weight of the placenta in pathological conditions in the fetus will be different from the weight of the placenta in normal birth conditions. Placental weight is a measure that reflects many aspects of placental

growth and the increased surface area for the vascular exchange of nutrients. Thus, the growth of the placenta, which begins early in pregnancy, is a determinant of its transfer capacity, which supports the development of the fetus in achieving growth (Furukawa *et al.*, 2019).

In Table 2 was showed the histomorphometric data from the placenta. The results of this study indicate that the administration of sappan wood extract during pregnancy did not cause toxicity in the development of the placenta. The results of measuring the area of the placenta, the labyrinth zone, and the basal zone in each treatment group given a dose of 100-500 mg/kg body weight of sappan wood extract were not significantly different compared to the control group with a consecutive p-value 0,991, 0,872, and 0,148. Using medicine during pregnancy that are potentially toxic can change the size and structure of the developing placenta. These changes can be interpreted as indicators of physiological changes in the placenta (Furukawa *et al.*, 2019). This shows that the ethanolic extract of sappan wood has a non-toxic effect on placental development so that it can not interfere with fetal development during pregnancy.

The placenta proliferate quickly through the mother's bloodstream during pregnancy (Paul *et al.*, 2020). The placenta plays many roles during pregnancy, such as transport of nutrients, metabolic activities of drugs and endocrine, and protection of the fetus. Therefore, the placenta becomes a target that is very vulnerable to side effects caused by the use of drugs or chemicals during pregnancy. Stress conditions and functional injuries that occur in the placenta caused by the induction of the use of drugs during pregnancy can result in abnormal growth and development of the fetus

so that it can cause resorption or be teratogenic to the fetus. In their research, Furukawa *et al* (2011) explained that a macropathological decrease in the placenta's weight or the placenta's area could be observed if a smaller than normal placenta is found. The placenta's condition is caused by inhibiting mitosis, apoptosis, degeneration, and necrosis of trophoblast cells induced by direct injury to the placenta or by non-specific effects related to the mother's environment causing inhibition of placental development.

Sappan wood extracts up to 500 mg/kg body weight did not significantly affect rat fetal parameters. The fetal body weight, body length and the length of the tail were not different from the control ($P > 0.05$) (Table 3). There are no different diversities of body length and tail length

fetuses among all groups, illustrating that sappan wood extract has no effect on the sexuality of embryos compared with the negative controls.

At the age of 20 days of gestation, each organ in the fetus has an immature cell structure; no inflammatory cells were found that lead to cell damage. Many blood vessels have been formed, and no areas of necrosis were found. The description of organs examined is described as follows:

Eye. Histological areas of the eye can be seen in figure 3 A. At the age of the fetus, on the 20th day of gestation, the eyelids were in the area of the epithelial bridge, and the eye as a whole was still undeveloped. The corneal epithelium consists of a single layer of squamous to oval epithelial cells, while the corneal stroma is hypercellular and contains many keratocytes

Table 3. Effects sappan wood extract effect on rat fetal development, body weight, and tail length.

Groups	% Live fetuses	% Fetal resorption	% Fetal death	Fetal body length (cm)	Length of the tail (cm)
Negative Control	100	0	0	3,25 ± 0,27 ^a	1,01 ± 0,11 ^a
Sappan 100 mg/kg bw	100	0	0	3,35 ± 0,22 ^a	1,09 ± 0,08 ^a
Sappan 200 mg/kg bw	100	0	0	3,10 ± 0,21 ^a	1,00 ± 0,12 ^a
Sappan 300 mg/kg bw	100	0	0	3,32 ± 0,18 ^a	1,13 ± 0,17 ^a
Sappan 400 mg/kg bw	100	0	0	3,35 ± 0,19 ^a	1,22 ± 0,17 ^a
Sappan 500 mg/kg bw	100	0	0	3,11 ± 0,22 ^a	1,02 ± 0,16 ^a

Note: N=180, SD = standar deviation

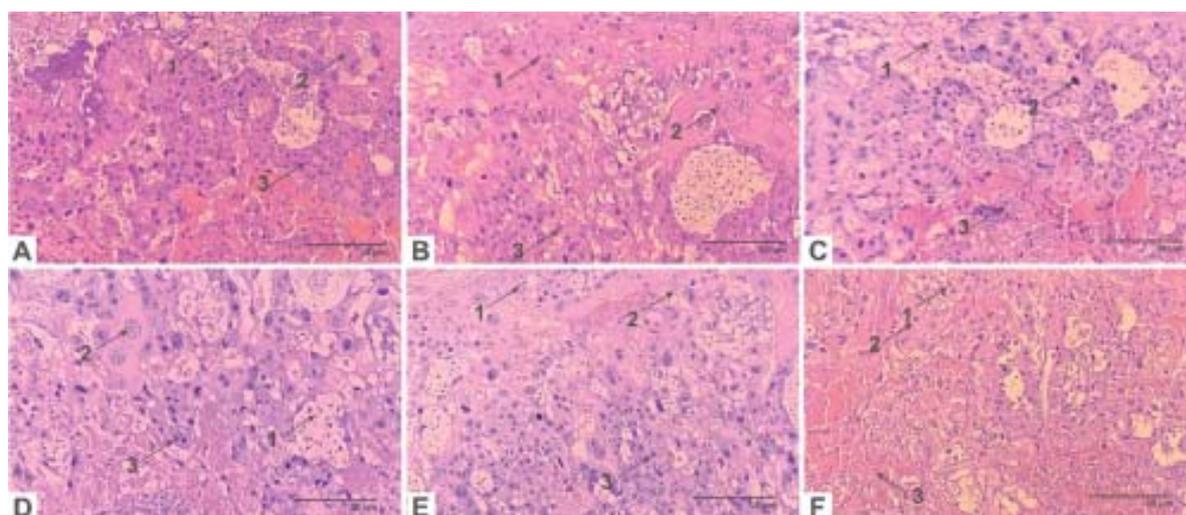


Figure 1. A photograph of the 20-day gestational placenta that had been removed from the rat's uterus with Hematoxylin-Eosin staining showed a normal morphology placenta. (A) negative control group, (B) SWE 100 mg.kg BW, (C) SWE 200 mg.kg BW, (D) SWE 300 mg.kg BW, (E) SWE 400 mg.kg BW, (F) SWE 500 mg.kg BW. (1) Natural Killer Cell, (2) spongiotrophoblast cells, and (3) Trophoblast cells

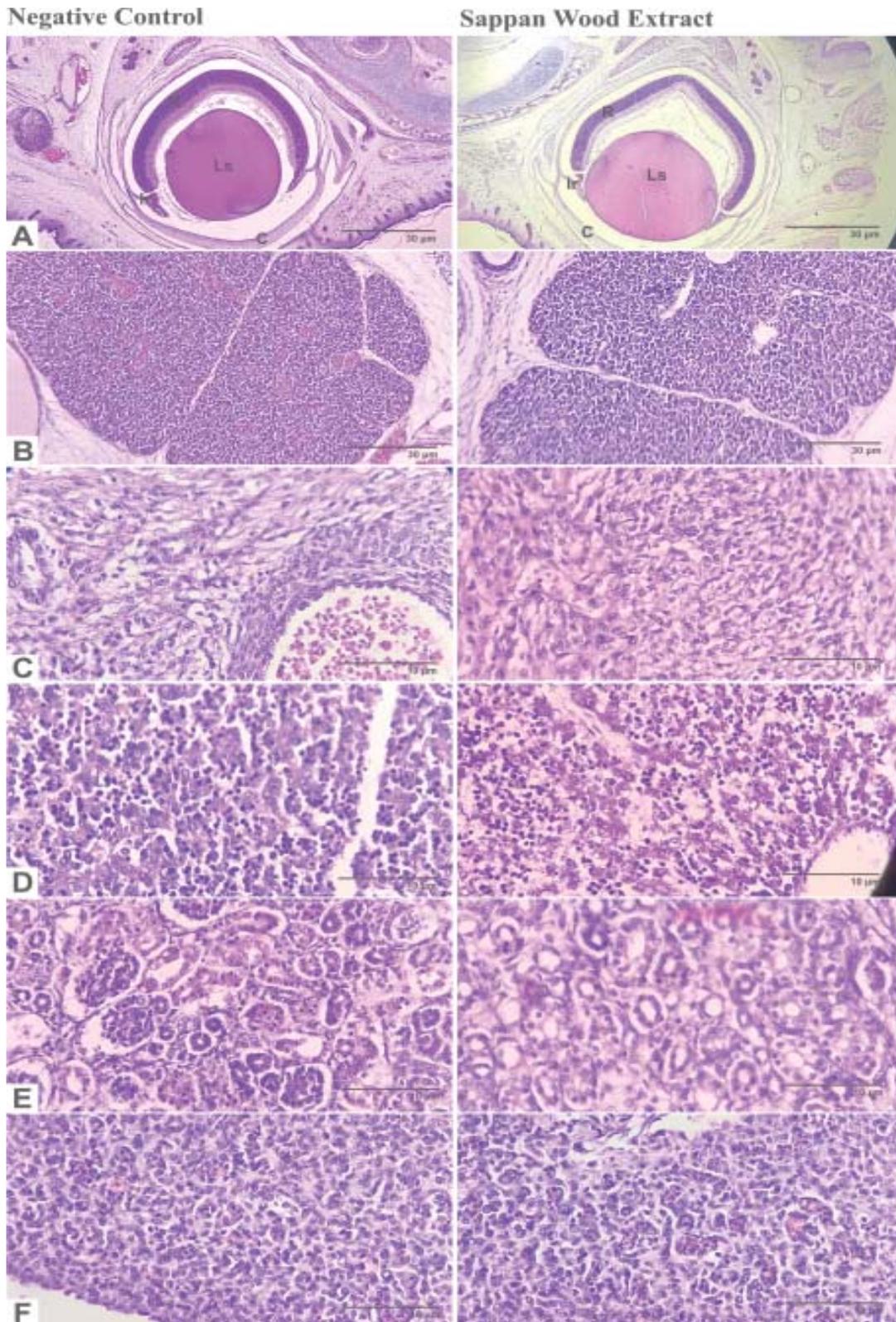


Figure 2. Photographs of the 20-day gestational rat fetuses in negative control and treatment groups showed normal morphology regions around the eye (A). Ls = Lens; R = Retina; Ir = Iris; C = Cornea. Photograph of the thymus (B), Heart (C), Liver (D), Kidney (E), and Spleen (F).

(Vrolyk *et al.*, 2018). At this fetal age, the lens is composed of many secondary fiber cells, which results in a high density of nuclei at the tip of the lens. The core of the lens fiber cell is observed through the entire width of the lens. The capillarity of the hyaloid vascular system is readily apparent. The iris is short and composed of two layers of immature epithelial cells. The retina is still immature and consists of an inner nerve fiber layer, the ganglion cell layer showing hypercellularity. The optic nerve consists of randomly arranged glial cells and unmyelinated nerve fibers (Kumar *et al.*, 2020).

Thymus. The thymus gland is seen in figure 3 B. On observation, the thymus of the fetus is more significant than its size when it enters adulthood due to the involution process. The thymus gland consists of lobes with more cortex and less medulla (Parker *et al.*, 2015).

Heart. Cardiac organs are seen in figure 3C. The myocardium is still developmental, consisting of young cardiac muscle cells separated by sinusoids; endothelial cells have begun to form in the apex area. The myocardium is hypercellular, with cardiomyocytes having a high cytoplasm ratio to the nucleus. Many active cell division processes such as mitosis are still visible. The aortic wall is still hypercellular, with many immature smooth muscle cells being observed and lined with large endothelial cells (Lehtoranta *et al.*, 2013).

Liver At 20 days of gestation, the liver is seen almost filling the abdominal cavity in the fetus. The boundaries of each lobe are clear so that the liver in the fetus can be seen in figure 3D. Hepatocyte cells are seen to be immature and irregular, separated by sinusoids but still in a normal state (Shimono *et al.*, 2016). No areas of necrosis were found, and areas of blood vessels and epithelial precursor cells were seen. At this fetal age, the process of hematopoiesis was still found, such as the discovery of young megakaryocytes scattered in the liver parenchyma area.

Kidney. Fetal kidneys were in figure 3E. No necrosis and inflammatory cells in the kidneys led to the damage. The process of nephrogenesis has begun to be seen even though it is still in an immature cell state (Wahab *et al.*, 2020). There are still many cell division processes in the tubular and glomerular areas (Hashemi *et al.*, 2019). The renal tubules are

lined with immature cuboidal epithelial cells, and the medulla is almost indistinguishable. The fetal spleen is seen in figure 3D. The splenic parenchyma area comprises a randomly distributed layer of erythrocytes fused with various hematopoietic precursor cells (Kämmerer *et al.*, 2020).

Histological morphology of rat fetuses given sappan wood extract during pregnancy showed no difference in fetal histology structure for each organ observed in the group given sappan wood and the negative control group. This is in line with the condition of the placenta that is structurally not abnormal in this study. The state of the placenta will affect abnormalities in the fetus if it experiences toxicity or abnormalities during pregnancy. Furukawa *et al.* (2019) found that the placenta plays a vital role in fetal growth even though it is a temporary organ during pregnancy. The placenta is a carrier of nutrition and protection for the developing fetus. It serves as a protective barrier protecting the fetus against chemicals that can cause secondary injury to the fetus. Therefore, ethanolic extract of sappan wood at doses of 100 mg.kg⁻¹ until 500 mg.kg⁻¹ did not have a toxic effect on Wistar rats seen from placenta and fetus development.

CONCLUSION

Ethanolic extract of Sappan wood at doses of 100-500 mg.kg⁻¹ did not give a teratogenic effect on Wistar rats based on finding on histology of fetal and placenta structure.

SUGGESTION

Researchers suggest further research in this study, such as molecular tests on the placenta.

ACKNOWLEDGMENT

The authors thank the Indonesian Endowment Fund for Education/*Lembaga Pengelola Dana Pendidikan* (LPDP) for funding the research and publication through the scheme of "Rispro – LPDP"

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