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Teratogenic Toxicity of Ethanol Extract of Mahkota Dewa Fruit *Flesh (Phaleria macrocarpa (Scheff.) Boerl)*

Maelani Julianti, Yuandani*, Urip Harahap

Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Jl. Tri Dharma No. 5, Kampus USU, Medan, Sumatera Utara, Indonesia 20155

Abstract : Mahkota Dewa is known as one of the medicinal plants in Indonesia. It contains bioactive compounds and potentially has pharmacological activity. This study aimed to determine the possible toxic effects of Mahkota Dewa during the organogenesis period. The ethanol extract of Mahkota Dewa fruit flesh was given to rats. Group I (control 0.5% Na-CMC), groups II, III and IV as a treatment group (Mahkota Dewa at doses of 100, 500 and 1000 mg/kg bw, respectively). Group V as a control satellite (Na-CMC 0, 5%) and group VI as a satellite of the ethanol extract at a dose of 1000 mg/kg bw., Each groups were treated on day 6 to 15 of gestational. On the 19th day getation, the rats were dissected and observed the skeletal malformations. The data were analyzed using one-way ANOVA followed by a post-hoc tukey. The results toxicity showed that no fetuses had external malformations. On fetal skeletal appearance, all skeletal bone preparations were normal. Based on the study, the ethanolic extract of Mahkota Dewa Fruit Flesh did not cause a teratogenic effect on the fetus during the organogenesis period at doses of 100 mg/kg bw, 500 mg/kg bw, and 1000 mg/kg bw.

Keywords: Fruit mahkota dewa, Teratogenic, Toxicity

Abstrak : *Mahkota dewa dikenal sebagai salah satu tanaman obat di Indonesia. Tumbuhan ini memiliki sejumlah kandungan kimia yang memiliki aktivitas farmakologi.. Penelitian ini bertujuan untuk mengetahui efek toksik Buah Mahkota Dewa yang mungkin terjadiselama periode organogenesis. Ekstrak etanol Buah Mahkota Dewa diujikan terhadap tikus sebagai hewan coba uji teratogenik Kelompok I (kontrol Na-CMC 0,5%), kelompok II, III dan IV sebagai kelompok perlakuan (mahkota dewa dosis 100, 500 dan 1000 mg/kg bb), kelompok V sebagai satelit kontrol Na-CMC 0,5% dan kelompok VI sebagai satelit ekstrak etanol daging buah mahkota dewa dosis 1000 mg/kg bb. Sediaan uji diberikan setiap hari pada hari ke-6 sampai ke-15 kehamilan, pada hari ke-19 hewan dibedah lalu diamati malformasi internal dan malformasi skeletal. Data dianalisis menggunakan one-way ANOVA dilanjutkan dengan uji post-hoc tukey. Hasil uji teratogenik menunjukkan tidak ada fetus yang mengalami malformasi eksternal pada kelompok kontrol maupun kelompok uji. Pada pengamatan tampilan skeletal fetus, semua preparat tulang kerangka menunjukkan kondisi normal. Berdasarkan hasil penelitian ini,ekstrak etanol daging buah mahkota dewa tidak menimbulkan efek teratogenik terhadap fetus selama periode organogenesis baik pada dosis 100 mg/kg bb, 500 mg/kg bb, dan 1000 mg/kg bb*

Kata Kunci: *Buah mahkota dewa, teratogenik, toksisitas*

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*Corresponding author at: Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara

E-mail address: yuandani@usu.ac.id

1. Introduction

Mahkota Dewa fruit is known as simalakama fruit in Sumateraif we consume excessly, this fruit will cause unexpected effects, thrush, dizziness, nausea,vomiting, and other health problems. The safety of Mahkota Dewa plant in the treatment of various diseases is still debatable from mild to severe, such as dysentery, gout, rheumatism, skin diseases, hepatitis, diabetes, hyperlipidemia, atherosclerosis, high blood pressure, cancer is quite worrying, especially degenerative diseases. Meanwhile, the efficacy data of this plant; acute toxicity ; subchronic toxicity and chemical composition is not available [1].

Mahkota Dewa is known as one of the medicinal plants in Indonesia. Almost all parts of the plant contain chemicals that are useful as medicine. The leaves, for example, contain antihistamines, alkaloids, saponins and polyphenols (lignans); the peels contains alkaloids, saponins, and flavonoids; while the fruit contains alkaloids, tannins, flavonoids, phenols, saponins, lignans, essential oils, and sterols. Flavonoids are the most abundant compounds found in the flesh of Mahkota Dewa[2].

The use of the Mahkota Dewa in community to treat various diseases from mild to severe, especially degenerative diseases, without scientific evidence and information cause new problems. Therefore,, it is necessary to provide information based on research to the public about toxicity.[3]. Teratogenic study is a specific toxicity test designed to evaluate the specific effects of a compound on the fetus during pregnancy. The teratogenic test will assess the effect of ethanol extract of the Mahkota Dewa fruit flesh on the reproductive function, external malformations and skeletal malformations in the fetus. Teratogenic test is a test to obtain information on fetal abnormalities that occur due to administration of sample during the formation of fetal organs (organogenesis period). The information includes abnormalities of the outside of the fetus (morphology), tissue and fetal skeleton [4], [5].

Based on the popularity of the Mahkota Dewa plant as a medicine for various diseases and the large number of people who consume this plant, it is worried that it will bring out side effects when consumed in large quantities. Research on this plant is still rarely published, so it is necessary to do the teratogenic study in order to determine the safety of consuming the Mahkota Dewa plant.[3], [6].

2. Results and Discussion

Estrous Cycle Determination

The estrous cycle consists of proestrus, estrus, and diestrus. The ovulation occurs from proestrus to the estrus phase. The appearance of the rat estrus cycle through vaginal smears can be seen in Figure 1.

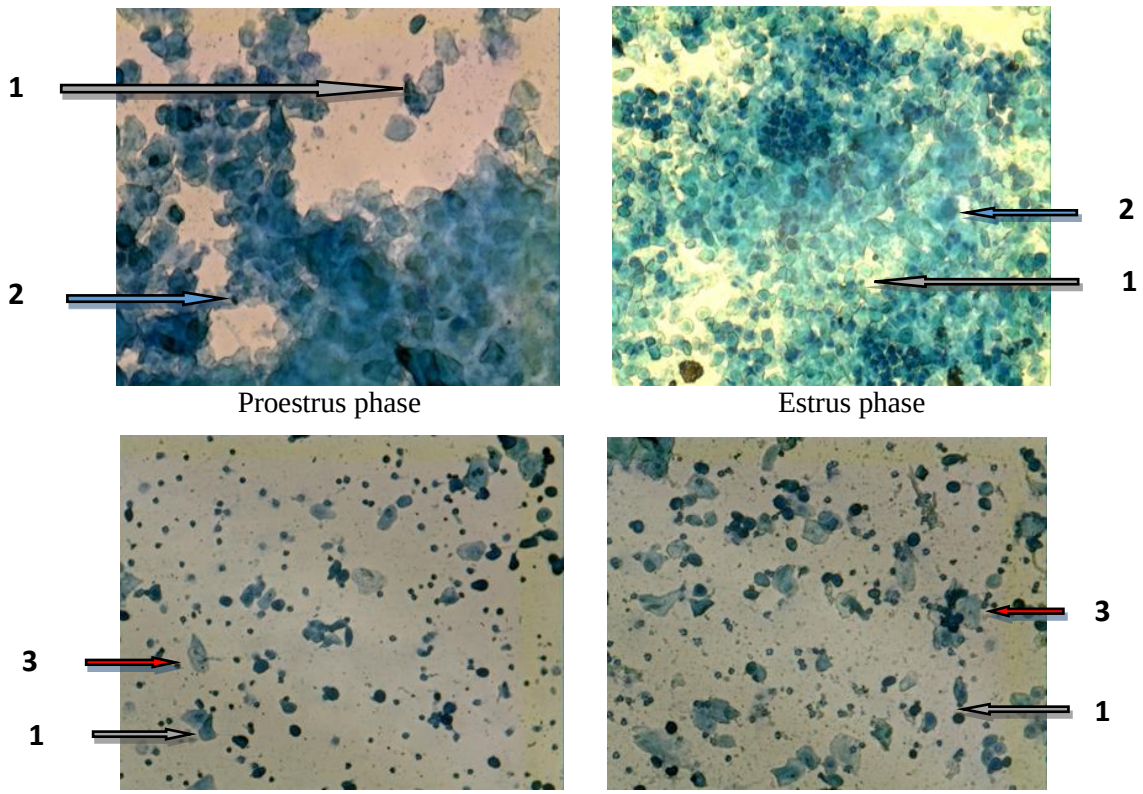

Figure 1. Vaginal swab in one estrous cycle with methylene blue dye.
Description: 1 (Horned epithelial cells); 2 (nucleated epithelial cells);
3 (Leukocyte cells).

3. Provision of Test Preparation During Organogenesis

Organogenesis is a critical period in rat pregnancy because there is a very intensive process of cell differentiation become organs and tissues. Therefore, the organogenesis is very susceptible to teratogenic substances [7]. The results of ratsbody weight during the organogenesis period was presented in Table 1.

Table 1. Rats body weight during organogenesis

Group	**Body weight (Mean ± SD)**			
	CMC Na 0,5 %	MDFEE 100 mg/kg bb*	MDFEE 500 mg/kg bb*	**MDFEE 1000 mg/kg bb***
Day 6	159.28 ± 3.37	155.50 ± 2.99	160.66 ± 4.97	**159.18 ± 4.69**
Day 7	160.98 ± 3.61	156.76 ± 2.92	162.02 ± 4.83	**160.86 ± 4.60**
Day 8	163.64 ± 3.74	158.86 ± 3.16	164.12 ± 4.52	**162.72 ± 4.55**
Day 9	166.14 ± 3.89	161.22 ± 3.68	166.42 ± 4.66	**164.76 ± 4.23**
Day 10	168.76 ± 2.87	163.30 ± 3.04	169.00 ± 4.49	**167.08 ± 4.23**
Day 11	171.46 ± 2.72	165.78 ± 2.90	170.94 ± 4.28	**169.02 ± 4.16**
Day 12	174.00 ± 3.33	168.80 ± 2.54	172.82 ± 3.81	**173.60 ± 3.97**
Day 13	176.72 ± 3.06	170.54 ± 2.97	175.12 ± 3.83	**173.60 ± 3.97**
Day 14	178.22 ± 2.00	173.12 ± 3.51	177.76 ± 3.75	**176.02 ± 3.04**
Day 15	181.42 ± 1.70	175.72 ± 3.88	180.34 ± 3.33	**177.96 ± 3.35**
Day 16	183.78 ± 2.22	178.02 ± 3.96	182.86 ± 3.85	**179.98 ± 3.39**
Day 17	185.94 ± 2.09	180.70 ± 2.85	185.38 ± 3.82	**182.38 ± 3.49**
Day 18	188.94 ± 2.25	183.10 ± 3.17	187.52 ± 3.97	**182.90 ± 3.64**
Day 19	**191.50 ± 2.17**	**186.00 ± 3.81**	**189.92 ± 3.93**	**183.98 ± 2.98**

SD = Standard deviation; * = there is no significant difference with the control group (p > 0,05); MDFEE : Mahkota Dewa Fruit Ethanol Extract

4. Observation of Fetal Reproductive Appearance and External Malformations

The gestation period pf rats is approximately 21 days. On Day 19 of pregnancy, the rats were sacrificed. The rats were dissected before parturition considering the tendency of the rats to directly eat her baby who was born with defects, died or almost died [8]. The results of observations of the number of live, dead and resorption fetuses were presented in Table 2.

Table 2. Number of live, dead and resorption fetuses

Group	Number of ratss	Number of Fetuses		
		Life	Death	Resorption
CMC-Na 0,5%	5	28 (100%)	0 (0%)	0 (0%)
MDFEE Dose 100 mg/kg bb	5	30 (100%)	0 (0%)	0 (0%)
MDFEE Dose 500 mg/kg bb	5	29 (100%)	0 (0%)	0 (0%)
MDFEE Dose 1000 mg/kg bb	5	33 (100%)	0 (0%)	0 (0%)

Fetal biometrics is quantitative data used to see the effect of the teratogenic toxicity. One of the fetal biometric data includes the number of deaths, the number of live fetuses, the number of death fetuses [9]. Fetuses that have been separated from the uterus and placenta are cleaned and dried with a tissue and then weighed . MDFEE did not have a significant effect on the number of fetuses, with the results of statistical analysis showed $p > 0.05$. The results of this study were in line with the procedure carried out that the administration of the test preparation is carried out during the organogenesis period (Days 6 to 15 of pregnancy), so the assessment was only aimed to know congenital defects in the fetus [10], not to assess the potential of MDFEE as a cause of abortion in pregnancy. Teratogens can react to the mother, placenta or to the embryo/fetus. In this study, there was no abnormality in the uterus either in the control group or the test group. Fetuses that have been assessed for their reproductive performance were then divided into 3 parts. Two-thirds fetuses from each mother were immersed in Bouin's solution for 3 days to observe the external malformations, the remaining 1/3 were immersed in 95% alcohol fixation solution for 2 weeks to prepare fetal skeletal observation before soaking in alizarin reds solution.

The results of this study, there were no fetuses that experienced external malformations in the control or the test group. The external malformations such as hydrocephalus, cleft palate, micromelia, spina bifida, anenchepaly, humpback body, limb defects, and visual observations on

the picture of the internal organs of the fetus also looked normal, both in terms of size and color of the organs. All fetuses were born with normal shape.

The results of the visual analysis of the external malformations of the fetus were provided in Table 3.

Table 3. Effect of the extract on the appearance of fetus external malformations

Parameters	Treatment group			
	CMC-Na 0,5%	MDFEE Dose 100 mg/kg bb	MDFEE Dose 500 mg/kg bb	MDFEE Dose 1000 mg/kg bb
Number of fetuses studied	14	15	15	19
Hydrocephalus	-	-	-	-
Cleft palate	-	-	-	-
Micromelia	-	-	-	-
Anencephaly	-	-	-	-
Spina bifida	-	-	-	-
Humpack body	-	-	-	-
Limbs defect	-	-	-	-
Liver	-	-	-	-
Heart	-	-	-	-
Kidney	-	-	-	-

5. Conclusion

The ethanol extract of the flesh of Mahkota Dewa (Phaleria macrocarpa (Scheff.) Boerl did not cause teratogenic effects on the fetus during the organogenesis period at doses of 100 mg/kg BW, 500 mg/kg BW, and 1000 mg/kg BW.

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