Jurnal Sains dan Teknologi Farmasi, Vol. 13, No. 2, 2008, halaman 86 - 88
Akreditasi DIKTI Depdiknas RI No. 49/DIKTI/Kep/2003

ISSN : 1410 – 0177

Teratogenic Assessment of *Phaleria macrocarpa* (Scheff.) Boerl. Extract *In Ovo*

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Diterima tanggal : 11 Juli 2008 disetujui : 12 September 2008

Abstract

Teratogenic activity of the aethanolic extract of *Phaleria macrocarpa* (mahkota dewa) fruit was observed by *in ovo* method on quail eggs. The extract was given by direct injection to the yolk at the 4th, 6th,8th and the 10th day of incubating with variety of doses of 25, 50, 100 and 200 mg/kgEW respectively. The experiment was conducted under aceptic conditions. The eggs were incubated at 38-39 °C for 14 days. In day 15th embryos were taken and then were immersed in red alizarin and the rest in Bouins solution. Results showed that the extract affected the weight of eggs and embryos. Injection of extract at 4th and 6th day caused lethal effects, wich was found in all variaty of dosage. The injection at day 8th for all variety of doses, only several organs growth completely. While the injection at day 10th with doses of 25, 50 and 100 mg/kgEW respectively showed dislocation of the intestines. At dosse 200 mg/kgEW dislocation of intestines and incomplete formation of feathers were observed.

Keywords : *Phaleria macrocarpa*, teratogenic, *in-ovo*

Introduction

Phaleria macrocarpa is a dense evergreen tree, growing well in tropical climates. The fruit was not much known as herbal medicine because it's a hard toxic. In fact, this plant contains a lot of benefits to treat various diseases. The fruits are better dried off for a few days before beeing consumed because its sap is very hot, when is eaten fresh, it causes blister in the mouth.

In Indonesia, fruits and leaves of *Phaleria macrocarpa* has been used to cure various health problems, including empirical treatment for cancer. From the fruits of *Phaleria macrocarpa*, icariside), phalerin, and mangiferin were isolated and Icariside showed a slow vasorelaxant activity against noradrenaline-induced contraction of isolated rat aorta (Shiori *et al.* 2008).

This study reported on the teratogenic effects of the extract that injected during organogenesis on Japanese quail (*Coturnix japonica*) eggs.

Methods

Fertilized chicken eggs (8-12 grams) were purchased from "Sentra Puyuh" Padang and kept refrigerated (14°C) until use. All stock solutions were made by dissolving drugs in 0.9% saline, and working dilutions for all drugs were prepared for these experiments using 0.9% saline in tubes and in 20°C until use. Eggs incubated in the incubator at a temperature devices 38-39 ° C (100 to 102 ° F) with the blunt side to the top (Jelinek, 1977; Karnofsky, 1965).

Samples of dried husks of *P. macrocarpa* was purchased from the PT. Karya Sari, Bogor. Seeds weighed 1 kg, chopped finely and macerated with ethanol. Maceration performed three times each for five days, then filtered. Concentrated filtrate obtained by using rotary evaporator. The dose of extracts used in this experiment were 25, 50, 100 and 200 mg / kgEW.

Embryos *in ovo* were divided into groups of 5 and preincubated in a rocking humidified incubator set at 39°C for 4 h. Embryos *in ovo* then were removed from incubator, and the first dose (50 µL in 0.5% CMC Na) was administered to each treatment group, which is a typical dose size for experiments wherein extract are delivered to avian embryos. Immediately after treatment of embryos, the eggs were resealed using a small amount of melted paraffin wax and returned to the incubator. Similar treatments were given at days of 4, 6, 8 and 10 after this time. On the 15th day the embryo was hatched by breaking the egg shell.

The embryos were washed with distilled water followed by alcohol and then dried and weighed. Furthermore any anomalies observed

morphologically and compared with the control group (Fu, 2000).

Some embryos from each group soaked in a solution of alizarin red for three days or until the embryos become transparent. Bone abnormalities observed and counted the number of defects. All the observations compared with control group (Kotwani and Metha, 1995).

Result and Discusion

Brown extract obtained from maceration was 103.2 grams, with 16.35% of water content. Decline in egg-weight between day 1 and the 15th consecutive incubation were 5.56, 7.22, 8.11 and 9.08% (treated on day 4); 6.17, 6.38, 7.12 and 11.06% (treated on day 6); 6.67, 6.99, 8.53, and 12.43% (treated on day 8); 6.19, 6.39, 7.26, and 8.25% (treated on day 10), respectively (Table 1).

Table 1. Persentage of weight egg decrease during baching

DOSES	GROUPS BASED ON DAY TREATED				CONTROL
	4	6	8	10	
25	5,56	6,17	6,67	6,19	5,640
50	7,22	6,38	6,99	6,39	
100	8,11	7,12	8,53	7,26	
200	9,08	11,06	12,43	8,25	

On this fourth day the embryo is very vulnerable to substances that are suspected as teratogens. While day-1 till 3 are not given the treated substance because exist of the nature of the embryo totipotensi that can repair tissue damaged.

From 5th until the 14th day of incubation, eggs turned twice a day. Screening eggs aims to flatten the heat during incubation. In addition to prevent the embryo not attached to one of the eggshell. When the eggs are not turned or changed its position so the yolk will be pushed to one of the main side or attached to the eggshell egg embryos die as a result.

Dosing on day 10 reduced mean of embryo weight from 4 to 3 grams. It sugested that the extract giving retarded the the normal growth of embryos. Anomalies were found at dose of 25 mg / kgEW when injected at the day 10 of incubation. Anomalies existed such dislocations of intestine in a single embryo, while at doses of 50 and 100 mg the dislocations were found in two embryos. It was clear that embryonic exposure to extract *P. macrocarpa* disrupted the normal development of the intestene. However, no mechanism could explained of how this extract affected the intestine. Dosage of 200 mg, also presented dislocations in two embryos and unperfect formation of hair follicles on three embryos (Figure 1). The embryos were found stilbirth. No anomalies were found in skeletal and visceral in this assesment.

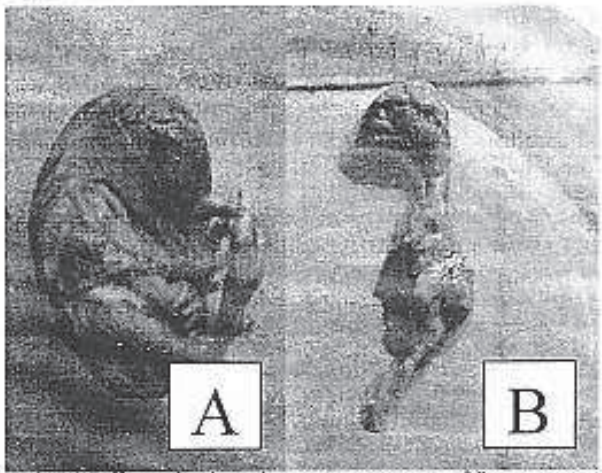

Figure 1. Photographs illustrating the effects of embryonic exposure to extract of *P. macrocarpa* with dose of 200 mg/kg EW. Embryo with intestine dismorphy (A), embryo hatched hairless and stillbirth (B).

Conclution

Our results suggested that ethanol extract of the fruit of *P. macrocarpa* caused more severe embryological defects on developing of intestine and also produced embryo hatched hairless and stillbirth.

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