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Research Article

THE IMPACT OF *CASSYTHA FILIFORMIS* BUTANOL FRACTION TO THE PREGNANCY AND FETAL DEVELOPMENT ON MICE

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ABSTRACT

The impact of *Cassytha filiformis* butanol fraction to the pregnancy and fetal development had been conducted. The fertilized mice were treated with butanol fraction of *C. filiformis* at doses of 2.5; 5; 10; and 20 mg/kg of body weight (BW) orally for 5 consecutive days during first, second, and third periods of pregnancy. Parent BW was monitored and the fetal number, BW, death and/or resorptive site and defect were measured. ANOVA followed by Duncan multiple range test (significance at $p<0.05$) was performed to analyze data. The parent weight was reduced according to fraction dose and the period of pregnancy and the interaction of those factors ($p<0.01$). Only one mouse treated during the first period became pregnant with less fetus number ($p<0.05$) but all of treated during the second and third period but death, resorptive site and underdeveloped fetus were found. These indicated the butanol fraction of *C. filiformis* produced infertility and slowed pregnancy development and produce fetal defect on mice.

Keywords: *Cassytha filiformis*, Pregnancy development, Fetal number, Death, Fetal defect.

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INTRODUCTION

The world is witnessing an unprecedented growth in the usage of herbal products. India, for example, is a mother hub for natural herb-based science [1]. Since 2007, Indonesian government has released a regulation for the traditional medicine policy to develop and increase their quality, safety, and efficacy [2]. Unfortunately, the number of marketed herbal products still limited. To support the government policy, we intended to elaborate our natural resources to get some valuable herbal medicines.

Cassytha filiformis is among Indonesian traditional medicine that needs scientific approved to increase its grade to be a phytopharmaca. This Lauraceae family is a parasite on a variety of plants, such mango (*Mangifera indica*), clove (*Eugenia aromatica*), nutmeg (*Myristica fragrans*), avocado (*Persea americana*), and others [3]. It contains bioactive compounds, such as alkaloids, phenol, saponin, flavonoids, terpenoids, and tannin [4,5].

Conventionally, *C. filiformia* is used to treat jellyfish bites, to induce childbirth, anticancer [3]. Scientific researches described that this plant produced antitrypanosomal activity [5], and antibacterial [6], while our previous study indicated that this plant produced an anti-hypertension effect [7,8] anticoagulant [9], antipyretic, and analgesic activities [10]. In addition, antidiabetes [11,12], vasorelaxant [13], antioxidant, and hepatoprotection [14] also have been reported.

Besides some advantages, all herbal drugs are not safe; some may be poisonous or may cause allergenic reactions [15] or effect to one with gestation, both to the mother and the fetus [16]. *C. filiformis* is slightly toxic and produced delayed toxicity [17], reversible toxic to the liver [18], but both ethyl acetate and butanol fractions are irreversibly toxic to the kidney [19].

These research objectives are to evaluate the impact of *C. filiformis* butanol fraction to the development of pregnancy and fetus on mice. This is done to develop safe herbal medicines as required by the WHO 2014–2023 policy [20].

METHODS

The fraction was made from the *C. filiformis* herbs that were collected from the area of Padang city (identification was made by Andalas University Herbarium, Padang, Indonesia No. 0912/K-ID/ANDA/2011).

The study protocol was approved by the Andalas Animal Ethics Committee (approval number: 323/KEP/FK/2018). A total of 36 pregnant female white mice (indicated by vaginal plug) were divided into four groups consisting of one control group and three other groups treated with *C. filiformis* fraction orally at doses of 2.5, 5, and 10 mg/kg of body weight (BW) for 5 days during the first, second, and third trimesters of pregnancy. On day 18, the animals were killed and the laparotomy was made to measure the fetal number, weight, and fetal defect. The fetal defect was measure after they were fixed in Bouin and red alizarin solutions (Taylor *et al*., 2005). The parent BW was observed during the experiment. Data of parents' BW were analyzed by three-way ANOVA while others were analyzed by two-way ANOVA. These analyses were followed by Duncan's multiple range test with a 95% confidence interval.

RESULTS AND DISCUSSION

Results

Parent BW was significantly ($p<0.001$) affected by the period of treatment, dose of fraction and the duration of pregnancy and the interaction of those factors (Fig. 1).

The average change of BW of pregnant mice treated with the fraction during the first period was lower, compared to those treated during the second and third periods of gestation. The reduction of the BW was also dose-dependent ($p<0.01$). In this situation, the development of pregnancy of mice treated with the butanol fraction of *C. filiformis* at 2.5 mg/kg BW during the period 1 and 5 mg/kg during the period 2 of gestation was faster than the other treated group at the same period. The development of pregnancy during the third period of gestation was almost equal in all group. In addition, all mice showed some progress in weight development day by day, during 18 days of the experiment. The average percentage changes in the parent BW of control group, and the butanol fraction treated group during the first, second, and third periods of pregnancy, as seen in Fig. 1.

Butanol fraction of *C. filiformis* dose and the period of treatment significantly influence the number of the fetus of mice ($p<0.01$), but not the interaction of these factors ($p>0.1$). In general, the number of the fetus in the treated animal was less than those of the control group. In addition, there was no significant difference in the number of the fetus between mice treated with the different doses ($p>0.1$). The average number of the fetus of mice treated with butanol fraction of *C. filiformis* during period 1 was greater compared to those treated during the periods 2 and 3, but the number of the fetus of mice on average between periods 2 and 3 was not significantly different. The average number of the fetus of control mice remained higher than all treated animals (Fig. 2 and Table 1).

Fetal BW of *C. filiformis* butanol fraction treated animal was significantly affected by dose, the period of treatment and interaction of these two factors ($p<0.001$). The average fetal BW on mice treated with butanol fraction was higher compared to those of control group, and the average of fetal BW treated during period 1 of gestation was less than those treated during period 2 and 3. In this situation, fetal BW of animal treated at a dose of 2.5 mg/kg of butanol fraction of *C. filiformis* during period 1 and 3 was lower compared to those treated during period 2 (Fig. 3 and Table 2).

There was no fetal defect found on all treated mice, except resorptive sites was found on the mice treated with 5 mg/kg of fraction and one dead fetus at a dose of 2.5 mg/kg and retarded fetus of the mother treated during the second period of gestation.

DISCUSSION

These experimental study objectives are to determine the impact of the butanol fraction of *C. filiformis* to mice during gestation period at the effective therapeutic repeated doses. The treatment was made such of gestation to determine whether it produces an adverse effect in that different gestational situation.

Before being treated, mice were acclimatized for 10 days to familiarize the animals with the experimental conditions and avoid the stress that could affect the final results of the study [21,22]. The nullipara female mice were used to obtain more fetus number [23]. Those animals have regular estrus cycles, to assured animal pregnancy when they are mated [24].

As noted everywhere, certain drug can produce an adverse effect on individuals during pregnancy, both to the mother and the fetus [25]. This study provides scientific information about the impact of *C. filiformis* butanol fraction to the pregnant mice, both to the parent and the fetus.

The observation showed that most of the matted mice treated with the butanol fraction of *C. filiformis* in the first period of pregnancy were not

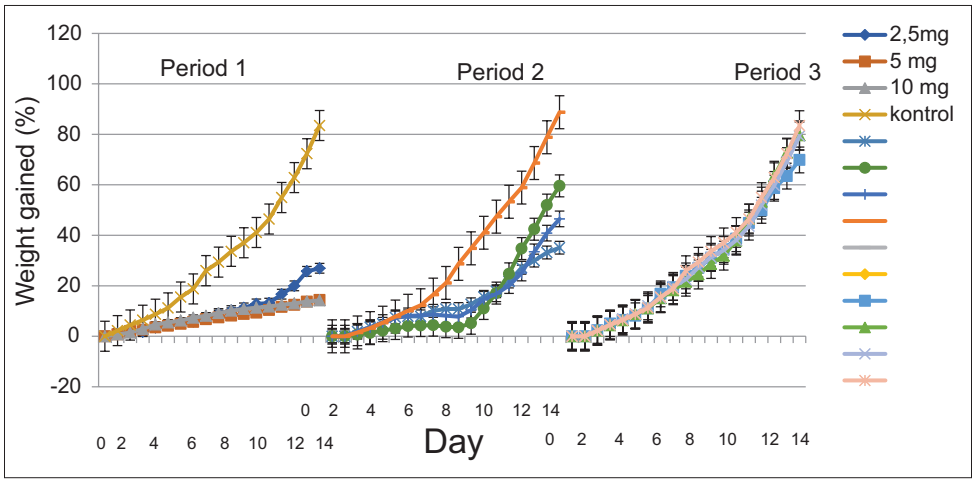

Fig. 1: The impact of butanol fraction of *Cassytha filiformis* to parent body weight on mice

Table 1: Fetal number of mice treated with butanol fraction of *Cassytha filiformis*

Doses mg/kg	Average fetal number of mice treated during periods of pregnancy			Average
	1	2	3	
Control	16.000±0.943	15.000±0.943	14.000±0.943	15.000±0.544[b]
2.5	14.000±1.633	8.000±0.943	10.000±0.943	10.667±0.703[a]
5		11.667±0.943	10.667±0.943	11.167±0.667[a]
10		10.667±0.943	10.000±0.943	10.333±0.667[a]
Average	15.000±0.943[b]	11.333±0.471[a]	11.167±0.471[a]	

Data are expressed as mean ± SEM. Average data with different superscript are significantly different ($P<0.05$).

Table 2: Fetal body weight of *Cassytha filiformis* butanol fraction treated mice

Doses (mg/kg)	Average fetal body weight (gram) of mice treated during periods			Average
	1	2	3	
Control	0.736±0.012	0.741±0.012	0.834±0.013	0.770±0.007[a]
2.5	0.839±0.022	1.213±0.017	1.147±0.015	0.996±0.011[c]
5		0.812±0.014	0.951±0.015	0.883±0.010[b]
10		0.800±0.015	0.904±0.015	0.868±0.010[b]
Average	0.787±0.013[a]	0.892±0.007[b]	0.915±0.007[c]	

Data are expressed as mean ± SEM. Average data with different superscript are significantly different ($P<0.05$).

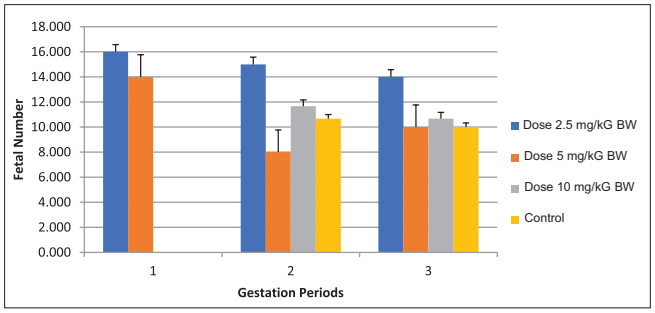

Fig. 2: The impact of butanol fraction of *Cassytha filiformis* to the fetal number on mice

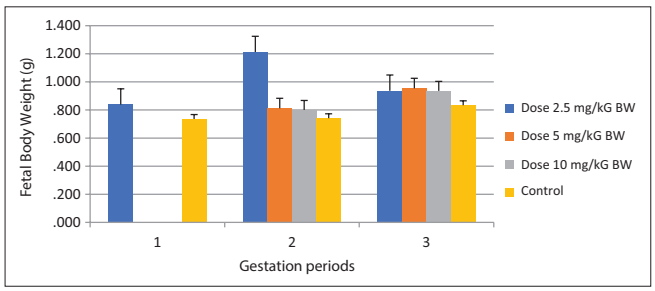

Fig. 3: The impact of butanol fraction of *Cassytha filiformis* to fetal weight on mice

pregnant, except one of three mothers those treated with the lowest dose (2.5 mg/kg) which was where pregnant with 14 fetuses.

Mammalian fetal development passes through three main phases: Blastocyst formation, organogenesis, histogenesis, and maturation of function [26]. According to Sadler [27], during the first trimester of pregnancy, the morula phase, zygote is continuously divided, which produce two groups of cells. One cell group will become embryo's body, and the other becomes complementary cells, which include the trophoblast, periblast, and auxiliary cells. These cells protect the embryo and make contact between the embryo and the parent. Many teratogens have the ability to inhibit this division and kill embryo, which was involved in blastocyst formation. However, most of the time the embryo survives; its subsequent development does not generally seem to be compromised [28,29]. That is why, when a cytotoxic compound is given during the first period of pregnancy, it will affect cell division and the morula phase formation, and thus disrupted the formed fetus as seen in this study, since *C. filiformis* contain cytotoxic compounds [4].

On the other hands, all animals treated with the fraction during the second trimester of pregnancy were pregnant, but with slower development. It is indicated by a slower increase of the parent BW, especially those treated with a higher dose (10 mg/kg BW) of the fraction. The similar situation also shown by the animal treated with a lower dose of *C. filiformis* during the first period of pregnancy.

Our previous study showed that the butanol fraction of *C. filiformis* caused a decrease of food intake associated with central nervous system depressant and muscle relaxant effects on mice and thus decrease the mice BW [17]. When the compound is given to the pregnant individual, such as mice in this study, similar response may be produced. When the mother feed intake is lower, the fuel needs of the fetus will also unfulfilled, and thus, fetal development will become slower. If both mother and fetus BW gain progress are slower, they will lead to a lower average parent BW, as seen in this study. This is in agree with [29] that poor maternal nutritional intake after the periconceptional period of pregnancy can negatively impact fetal genetic growth trajectory and can result in fetal growth restriction. Similarly, Vonnahme *et al*. [30] also describe that maternal undernutrition on the vascularity of nutrient transferring tissue during different stages of pregnancy effect pregnancy development.

Furthermore, there was no bone abnormality seen under the red alizarine neither with Bouin's solution on the embryo fixation. The eyes, ears, feet, toes, tail, and the cleft palate seemed anatomically complete and normal. Unfortunately, the fetus of the mice treated with the 2.5 mg/kg BW during the second period of gestation grew slowly, and one of them found died. A similar result also found in three of fetus where the parent was treated with 5 mg/kg BW of the fraction. In addition, there was a resorptive site on the mice treated during the second period of gestation.

Many chemicals have the ability to penetrate animal tissue and developing fetus, negatively impacting the reproductive health of human [25,31]. According to several authors [28,31,32], fetuses are very susceptible to teratogenic compounds during the second trimester, also called the critical period of pregnancy. During this period (organogenesis phase), the eyes, brain, heart skeletal, urogenital, and other organs are formed. If the fetus defects, this will lead to fetal death and form a red mass (fetal resorption) because of no more totipotential properties of the cells [28]. That is why, when fetal damage occurred during this period, it could not be repaired and neither further developed. This condition is one of teratogenic indication of an agent [25,28,33], as also seen this study.

Treating the mice during the third period of pregnancy produce interesting results. All animals in the treated groups were pregnant. Even though the number of the fetus in the treated animal was decreased significantly compared to control animals, the pregnancy developed faster, especially those treated with 10 mg/kg BW butanol fraction. In this situation, mice treated with butanol fraction of *C. filiformis* produced as many as 10 fetuses, compared to those by control mice of 14. According to McLaren and Michief [34], the number of fetus affects the increase in BW of mice. If the number of the fetus is higher, the parent BW will also be higher.

The dose-response relationship found in this study is not linear. Calabrese and Baldwin [35] described that the nature of the dose of the response generated is not something linear that allows a U-shaped dose-response relationship, often called biphasic or hormesis. This effect is often found in the fields of pharmacology and toxicology [36]. The effect is may due to several factors, such as antagonistic effects of the mixture compounds available in the fraction, compounds that have different target and mechanism of action as well as their pharmacokinetic profiles, etc., that may need to further investigation.

From the above explanation, it can be concluded that the butanol fraction of *C. filiformis* produced infertility and deliberate pregnancy development and fetal defect on mice.

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