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SHORT COMMUNICATION

Teratogenicity *In Vivo* Experiment of Antidiabetic Polyherbal (*Smallanthus sonchifolius, Syzygium poloyantum, Stevia rebaudiana, Camellia sinensis*) in Pregnant Mice

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Abstract

Background: Polyherbal extracts, composed of Yacon (*Smallanthus sonchifolius*), Bay leaves (*Syzygium polyantum*), Stevia (*Stevia rebaudiana*), and tea leaves (*Camellia sinensis*), are known for their anti-diabetic properties. To be developed as standardized herbal medicines, these formulations must meet safety standards, including teratogenicity testing. **Aim:** This study aims to assess the teratogenic effects of an ethanolic extract of this anti-diabetic polyherbal formulation in pregnant adult female mice. **Methods:** An *in vivo* study was conducted using pregnant female mice. The polyherbal extract, suspended in 0.1% Na-CMC, was administered at doses of 100 mg/kg BW, 200 mg/kg BW, and 400 mg/kg BW. The extract was given during the organogenesis phase, from day 6 to day 15 of gestation. The mice were sacrificed on day 17 to evaluate fetal development. **Results:** Results indicated a dose-dependent delay in fetal bone ossification with increasing extract doses. However, there were no significant effects on physical fetal development, nor were there abnormalities observed in the soft tissues or skeletal structures of the fetuses. **Conclusion:** The administration of the polyherbal extract in pregnant female mice caused a delay in fetal ossification but did not result in teratogenic effects, such as physical deformities or soft tissue abnormalities. Further studies are needed to assess long-term safety and potential developmental impacts at varying doses.

Keywords: Antidiabetic, Mice, Natural Product, Polyherbal, Teratogenic

1. Introduction

Previous studies on polyherbal extracts consisting of Yacon (*S. sonchifolius*), bay leaves (*S. polyantum*), stevia (*S. rebaudiana*), and tea leaves (*C. sinensis*) showed antidiabetic effects both *in vitro* and *in vivo*[1]. Preclinical trials on mice showed that the administration was safe for long-term use and to develop polyherbal as standardized herbal medicines for antidiabetics, it is necessary to conduct special toxicity studies, including teratogenic tests[2]. Teratology studies intended to look at a wide range of potential *in utero* effects for the conceptus, including as deformities, death, impaired function, and developmental delays. Additionally, exposure at sensitive times may interfere with normal development and have immediate impacts, or it may later impair normal physiological or behavioral functioning[3].

In the global healthcare market, various plant products considered to be of therapeutic importance

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are accepted regardless of their toxicity information[4]. Conversely, a number of the phytochemical components found in therapeutic plants have the potential to cause birth defects, and the majority of these compounds are often employed in daily life. Therefore, it is necessary to conduct a teratogenic test on polyherbal preparations as an antidiabetic. Since some people with gestational diabetes need to take diabetes medication during pregnancy, polyherbal may be recommended. Teratogenicity testing is required for these polyherbal to determine their safety for pregnant women especially the development of the fetus.

2. Materials and Methods

2.1 Materials

Five-randomized groups of Swiss Webster strain mice were divided into control (0,1% CMC-Na) and three experimental groups: 100, 200, and 400 mg/kg BW of polyherbal extracts that formed into a suspension of 0,1% Na-CMC. The maximum dosage was a limit dose from the teratogenicity test, while the lowest dose was obtained from the conversion of therapeutic dose in humans. Testing at lower dosages is not required if the maximum level of embryotoxicity or teratogenicity is not seen. Additionally, the medication was given orally once daily between days 6 and 16 of gestation, during which the organogenesis phase of the mouse's gestation was being observed.

Fetus fixation was conducted using a 10% formalin buffer solution, and the skeleton was stained using KOH, distilled water, alizarin red s, and glycerin. Meanwhile, formalin buffer solution, tap water, 70%, 80%, 90% ethanol solution, absolute ethanol I, absolute ethanol II, xylene I, xylene II, xylene III, liquid paraffin, hematoxylin-eosin, and entellan® were used for histopathology preparations.

2.2 Methods

2.2.1 Pre-treatment

There was a total of 27 Swiss Webster female mice (*Mus musculus*), weighing 20–25 grams and aged 12–14 weeks, 5 of which were male. After being acquired from Educational Veterinary Hospital, IPB, the mice underwent seven days of adaptation prior to mating with the males. Moreover, a microscope inspection of vaginal smears revealed the estrous cycle. One male and three female mice in the proestrus to estrus cycle were mated, and the animals were then housed in a cage for the night. It was indicated as the 0^{th} gestation day when sperm was found in the vaginal smears the next morning. Mice that were unwell or not pregnant were omitted from the body weight data, which was collected up until the 20^{th} day of gestation[5]. The study protocols and animal care procedures were approved by the Animal Ethics Committee, Faculty of Veterinary Medicine, Bogor Agricultural University (018/KEH/SKE/XI/2020).

2.2.2 Teratogenic Effect Test

The organogenesis phase was when the therapy was administered (6^{th}–17^{th} gestation day), and on the 20^{th} day, the pregnant mice had a cesarean section to separate the fetuses. In addition, records were kept on the quantity of viable fetuses, resorption, and morphological anomalies[6].

2.2.3 Skeletal Preparation

Before the fetus was skinned and its organs removed, it was fixed with ethanol for one week. The fetus was fixed with a 0.5% potassium hydroxide solution for at least 24 hours, and then with 1% hydrogen peroxide for two to three hours. Moreover, it was submerged in an alizarin solution for at least 24 hours after being bathed in distilled water for 10 minutes. Every week, it was progressively immersed in a solution containing 5, 20, 40, and 80 % glycerol[5].

3. Results and Discussion

3.1 Material Observation

At the time of the cesarean delivery, all of the pregnant mice were still alive. In the control and treatment groups, the mean mother weight gain during pregnancy was 25.33±1.02 and 25.67 ± 0.94 g and 26±1.04, respectively (Table 1 and Table 2).

3.2 Influence of Polyherbal on Fetus General Appearance and Congenital Parameter Status

Observations were made of the axial skeleton which includes the skull (cranium), spine (vertebrae), ribs (costae), and sternum (sternebrae), while the appendicular includes the bones of the extremities.

Table 1. Maternal weight gain of mice groups

Group	Weight (g)			
	Day 0	Day 16	Before Delivery	After Delivery
Control	25.33 ±1.02	34.13± 0.17	28.33±0.05	32.33±0.17
100 mg/kgBW	26 ±1.04	38.63±0.1	29.33± 0.04	36.67± 0.08
200 mg/kg BW	25.67±0.94	34.27±0.16	29±0.03	32.67±0.15
400 mg/kgBW	26±0.04	37.53±0.05	29±0.03	36±0.05

Table 2. Body weight, tail length, and number of fetal whose mothers were treated with polyherbal

Group	The Average Number of Fetal	Average Fetal Body Weight (g)	Average Tail Length (cm)
Control	26	0.68	2.18
100 mg/kg BW	35	1.11	2.46
200 mg/kg BW	29	0.93	2.14
400 mg/kg BW	36	0.96	2.15

*Values are presented as mean ± standard error of the mean (SEM). P>0.05 not statistically different from the control group.

Table 3 showed the results of the examination of the number of fetal with skeletal abnormalities in each treatment group. In the groups receiving polyherbal treatment and control, there was no presence of polydactyl, wide eyes, or Middle Ear Disease (MED). As shown in Table 3, the fetus overall appearance was normal in all groups.

Observation results did not show any skeletal abnormalities in each group. Therefore, the administration of antidiabetic polyherbal during organogenesis did not cause abnormalities in the fetal skeleton of mice by each dose level to the control group.

Figure 1 shows that the percentage of fetuses with delayed ossification increased with the administered dose. However, there was a delayed ossification on the administration of 400 mg/kg polyherbal dose, at 44%.

Skeletal ossification was observed after Alizarin Red S staining, which binds to calcium contained in the ossified bone to show red color (Figure 2). Observations of the skeleton that had been stained with Alizarin Red S dye solution were then compared to the fetuses from the negative parent group, and the skeletal assessment was conducted using a loop.

Abnormalities in the spine, ribs, sternum, and extremities were caused by the administration of 100, 200, and 400 mg/kg BW polyherbal doses. This

Table 3. Effects of polyherbal on congenital parameters status and general appearance of fetal indicates absences of deformity

Group	Middle Ear Disease	Open Eye	Poly dactyl	General Appearance
Control	-	-	-	normal
100 mg/kgbw	-	-	-	normal
200 mg/kgbw	-	-	-	normal
400 mg/kgbw	-	-	-	normal

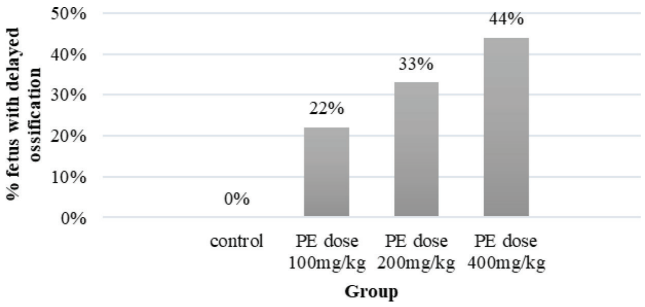


Figure 1. % Fetal with delayed ossification.

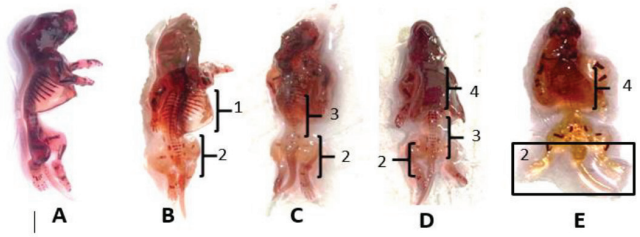


Figure 2. Comparison of fetal bones. Description**:(A).** Fetal bones that have undergone complete ossification; **(B), (C), (D), (E).** Fetal bones that have not fully ossified; Barriers to reinforcement (ossification) in: (1) costae; (2) extremities; (3) vertebrae; (4) Sternebrae.

is because the bone was not stained by Alizarin Red S dye, indicating complete formation. Therefore, administration of antidiabetic polyherbal extracts showed delayed response to dose-related ossification.

The delay in the ossification process (bone formation) was due to the presence of teratogenic compounds found in polyherbal that entered through the placenta and caused delays in the transfer of nutrients from the mother to the fetus. Table 3 showed that the rate of curing delay caused by the effect of polyherbal exposure to antidiabetics increased with increasing dose.

3.3 Effects of Extract on Histology of Fetus Liver and Kidney

The histological observations of the liver and kidneys of the fetus in mice can be seen in Table 4.

Table 4. Histological structure of the fetal liver and mice kidney

Organ	Group	Variable					
		CS	AS	AP	PN	KR	KL
Liver	Control	-	-	-	-	-	-
	Dose 100 mg/kg BW	-	-	-	-	-	-
	Dose 200 mg/kg BW	-	-	-	-	-	-
	Dose 400 mg/kg BW	-	-	-	-	-	-
Kidney	Control	-	-	-	-	-	-
	Dose 100 mg/kg BW	-	-	-	-	-	-
	Dose 200 mg/kg BW	-	-	-	-	-	-
	Dose 400 mg/kg BW	-	-	-	-	-	-

Description:- : negative; + : positive; CS : Cloudy swelling; PN : Pyknosis; AS : Apoptosis; KR : Karyorrhexis; AP : Autolysis Post-mortal; KL : Karyolitic

Histological observations in the control and the group given 100, 200, and 400 mg/kg BW polyherbal doses had the normal appearance of liver and kidney cell composition. (Figure 3) It was characterized by the absence of cloudy swelling, namely enlargement of cell size due to accumulation of fluid in the cloudy cytoplasm; occurrence of apoptosis, which is the shrinking of cells; Post-mortal autolysis i.e. concurrent cell death and indistinct boundaries; Pyknosis is condensation material and chromatin, the nucleus darkens and shrinks; Karyorrhexis i.e nuclear fragmentation; and Karyolysis, which is the dissolution of the nucleus into smaller basophilic fragments/granules (Table 4).

The medications that the mother take aid in supplying the fetus with the nutrients it needs to grow and develop by passing through the placenta in the same direction. Nevertheless, medications that are absorbed by the embryo may result in miscarriage, deformity, or even death[7]. In this study, the incidence of mouse parent reproduction was examined in order to detect exterior defects using morphometry. The number of living fetuses, intrauterine mortality (dead fetuses and resorption), weight and length measurements, and observation of anomalies such as bleeding and deformities in specific fetal body parts were used in this investigation.

Acute toxicity preclinical studies administration of polyherbal extracts at doses up to 5000 mg/kg in did

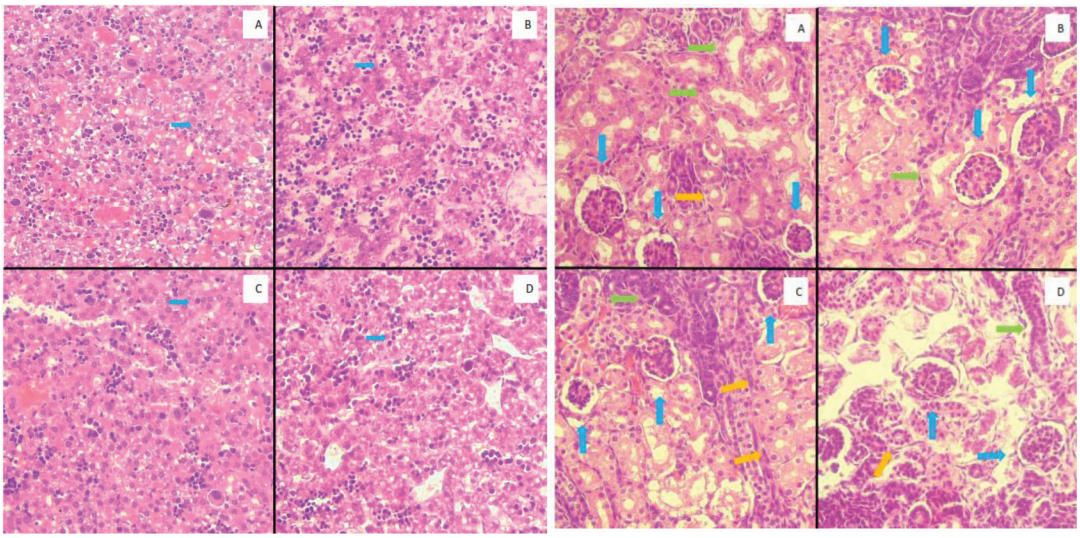


Figure 3. Histopathological structure of the fetal liver (left) and kidney (right) of mice at 100 × magnification. Description**:(A).** Negative control group; **(B).** Treatment dose of 100 mg/kg BW polyherbal; **(C).** Treatment dose 200 mg/kg BW polyherbal; **(D).** Treatment dose 400 mg/kg BW polyherbal.

not show any mortality or toxicity from polyherbal extracts[1]. It has been analyzed that LD_{50} of each herbal, 5000 mg/kg is considered safe[8,9]. Meanwhile, flavonoid compounds, tannins, saponins, steroids, alkaloids, terpenoids are contained in the extract based on phytochemical screening shows the activity as antidiabetic[2].

Organogenesis may cause pregnant mice's body weights to gradually grow. All groups decreased body weights following parturition serve as evidence for this. Furthermore, the absence of a statistically significant variation in the mice's body weight between the treatment and control groups implies that the extract could not have anti-nutritive components that could lead to reduced body weight during pregnancy, lack of appetite, and malabsorption of nutrients. From the results, the polyherbal extract showed no fetal deformities in all administration groups and normal liver and kidney cell features but experienced a dose-dependent delay in ossification. The polyherbal extract consists of Yacon leaves (*S. sonchifolius*), bay leaves (*S. polyantum*), stevia (*S. rebaudiana*), and tea leaves (*C. sinensis*), and the safety data for each ingredient are as follows.

Yodyingyuad and Bunyawong studied the toxicity of stevioside (*S. rebaudiana*) on the growth and reproduction performance of male and female hamsters. The growth and fertility in both sexes in the research fed by stevioside resulted normal condition. The duration of the pregnancy, the number of fetuses, the frequency of parturition, and the histological characteristics of the reproductive tissues were unaffected. In conclusion, there were no abnormalities seen in the growth or reproductive performance of hamsters fed stevioside at a dosage of up to 2.5 g/kg body weight/day[10]. Toxicity study of *S. polyanthum* showed the effect of giving its ethanolic extract at a dose of 0.5 mg/kg BW did not cause resorption and external malformations. However, at a dose of 5 mg/kg BW resorption was found (6.15%) and the fetal eyelids were open (1.63%). At doses of 50 and 500 mg/kg BW resorption (7.69%; 9.34%) and fetal hemorrhage (1.63%; 1.47%) were experienced. Statistically, ($P > 0.05$) the ethanol extract of the leaves of *S. polyanthum* with a dose of 0.5; 5; 50 and 500 mg/kg BW during the organogenesis period did not significantly affect the morphological development of the fetal mice. A study conducted by Nurdiana *et al.,* showed that exposure to extracts of *C. sinensis* in zebrafish (*Danio rerio*) embryos caused cardiac malformations, reduced body length, and hypopigmentation in zebrafish larvae[11]. This study can be used in determining the safety of green tea consumption during the pre-conception period or early pregnancy. Furthermore, a study conducted by Sulastri *et al.,* with docking analysis to predict the toxicity of bioactive compounds from Yacon leaves (*S. sonchifolius*), bay leaves (*S. polyantum*), stevia (*S. rebaudiana*), and tea leaves (*C. sinensis*) using software Protox II showed moderate toxicity with no teratogenic effect[12].

4. Conclusion

This shows the administration of antidiabetic polyherbal extracts with caution in pregnant women. The polyherbal extract had a dose-dependent delay in ossification but no fetal malformations in any of the treatment groups, along with normal liver and kidney cell characteristics. Although, each plant has been docking researched before to have no teratogenic potential. The safety of administering polyherbal remedies to diabetic pregnant women should be further investigated.

5. Acknowledgments

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6. References

1. Abdillah S, Lestari AG, Monika P, Sumaryono W, Hisyam K. Preliminary analysis of the antidiabetic activity of indonesian polyherbal formulation. J Nat Remedies. 2021; 21(3):219–223. https://doi.org/10.18311/jnr/2021/25960
2. Abdillah S, Nafisa S, Dewi QS. Acute and sub-acute toxicity studies on an antidiabetic polyherbal formulation in mice. Trop J Pharm Res. 2021; 20(6):1171–1178. https://doi.org/10.4314/tjpr.v20i6.11
3. Sharwan G, Jain P, Pandey R, Shukla SS. Toxicity profile of traditional herbal medicine. J Ayu Herb Med. 2015; 1(3):81–90.

4. Seukep AJ, Noumedem JAK, Djeussi DE, Kuete V. Genotoxicity and teratogenicity of african medicinal plants. Toxicological Survey of African Medicinal Plants. Elsevier. 2014; p. 235–275. https://doi.org/10.1016/b978-0-12-800018-2.00009-1
5. Badan Pengawas Obat dan Makanan. Peraturan BPOM No 17 Tahun 2019 [Internet]. BPOM RI Jakarta, Indonesia; 2019. Available from: https://peraturan.bpk.go.id/Details/285011/peraturan-bpom-no-17-tahun-2019
6. Organisation for Economic Co-operation and Development (OECD). Reproduction or Developmental Toxicity Screening Test. Guideline for the Testing of Chemicals. OECD. 2015. https://doi.org/10.1787/9789264242692-en
7. Elefant E, Hanin C, Cohen D. Chapter 26 - Pregnant women, prescription, and fetal risk. Handb Clin Neurol. 2020; 173:377-389. https://doi.org/10.1016/b978-0-444-64150-2.00027-7
8. Yaghoubi M, Mousavi Z, Rastegar T, Amin G. Safety assessment of *Arctium lappa* L. fruit extract in female wistar rats: Acute and repeated oral toxicity studies. Res J Pharmacogn. 2019; 6(2):39–48.
9. Choi J, Ryu SJ, Kim KJ, Kim HM, Chung HC, Lee BY. Single, 14-day, and 13-week repeated dose toxicity studies of daily oral *Gelidium elegans* extract administration to rats. Mol. 2018; 23(1):1-19. https://doi.org/10.3390/molecules23010217
10. Orellana-Paucar AM. Steviol glycosides from *Stevia rebaudiana*: An updated overview of their sweetening activity, pharmacological properties, and safety aspects. Mol. 2023;v28(3):1258. https://doi.org/10.3390/molecules28031258
11. Nurdiana N, Subandi R, Mukhamad N, Arsana WIW, Siswanto S, Sabatina W, *et al*. The teratogenic effects of the ethanolic extract of green tea (*Camellia sinensis*) in the larval stage of zebrafish (*Danio rerio*). Med Plants - Int J Phytomed Relat Ind. 2017; 9(4):259-267. https://doi.org/10.5958/0975-6892.2017.00041.7
12. Sulastri L, Simanjuntak P, Sumaryono W, Syamsudin. Prediction of toxicity and inhibition activities of a-glucosidase enzyme of the chemical compounds isolated from Indonesian medicinal plants using molegro virtual docking. Int J Chem Sci. 2020; 4(2):1–7.