Jurnal Ilmiah Biologi Eksperimen dan Keanekaragaman Hayati (J-BEKH)

Volume 10, Issue 2, November 2023

Size Shortening of Body Parts of Fetus Mice (*Mus musculus* L.) After Giving Bitter Melon Fruit Extract (*Momordica charantia* L.)

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Article History
Received: May 9, 2023
Accepted: November 15, 2023
Published: November 1, 2023

Abstract

Bitter melon (*Momordica charantia* L.) is a plant that has traditional medicinal properties. In bitter melon fruit, there are several toxic compounds, namely momordicoside K and L, which are thought to have cytotoxic properties. This study aims to determine the teratogenic effect of bitter melon fruit extract given to pregnant females on the length of the fetus' cranium and sternum. Bitter melon fruit extraction was carried out using the maceration method using 95% ethanol. By using a completely randomized design 20 male mice and 20 female mice were divided into 4 groups. Each group consisting of 5 pregnant female mice was given bitter melon extract orally starting from day 6 to day 17 of pregnancy once a day in the morning with a treatment dose of aquabidest as a control [A], [B] 22.5 mg/30 gr BW, [C] 30 mg/30 gr BW, and [D] 37.5 mg/30 gr BW. Next, the female mice were dissected, the fetus was removed and the length of the cranium and the length of the sternum were measured. The results showed that in general, the administration of bitter melon fruit extract caused a decrease in the average length of the cranium and sternum. The results of the further analysis with the Least Significant Difference (LSD) at the 5% level showed that administering doses of [B], [C], and [D] had a significant effect on reducing the length of the cranium and sternum. The most effective dose to reduce the length of the cranium and sternum of fetal mice is 37.5 mg/30 g BW.

Keywords: *Momordica charantia* L., *Mus musculus* L., teratogenic, toxic

How to Cite: S. Andriani, N. Nurcahyani, H. Busman, Sutiyarso, E. Kurniawaty, M. Sahroni, E. W. Puspa, "Size Shortening of Body Parts of Fetus Mice (*Mus musculus* L.) After Giving Bitter Melon Fruit Extract (*Momordica charantia* L.)," *Jurnal Ilmiah Biologi Eksperimen Dan Keanekaragaman Hayati (J-BEKH)*, vol. 10, no. 2. pp 16-20, 2023, doi:

INTRODUCTION

Plants contain chemicals that are utilized in various industries, such as food, cosmetics, agriculture, and medicine. Plants have been used to treat and prevent human diseases for many years [1]. Natural treatment using plants is an alternative to treatment using

ISSN 2338-4344
eISSN 2686-200X

chemical drugs because it is considered safer with lower toxicity levels than synthetic chemical drugs [2]. Herbal medicine from plant extracts is estimated to be used by about 80% of the human population in the world, especially in rural areas and developing countries [3]. The utilization of medicinal plants in Indonesia has been carried out since the royal period, as indicated by the presence of carvings of medicinal plants used by the community at the Borobudur temple around 720 AD. Other evidence can be found in the royal heritage books written around 1100-1200 AD [4]. Indonesia has around 950 plant species that have potential as herbal medicines [5]. This number will continue to grow as the exploration of medicinal plants in Indonesia increases. Bitter melon is one of the plants that grow in Indonesia and has the potential for utilization as a medicinal plant.

Bitter melon or *Momordica charantia* L. is a plant included in the Family Cucurbitaceae member, which is widely distributed in subtropical to tropical regions [6], including Indonesia. Bitter melon has been widely used as a food ingredient since thousands of years ago [6]. Besides being used as a food ingredient, bitter melon is also widely used as a medicinal plant. Bitter melon is used for the treatment of more than 30 types of diseases such as diabetes mellitus, tumors, cancer, kidney stones, fever, cholera scabies, anemia, diarrhea blood disease, bronchitis, gout, dysentery, rheumatism, ulcers, liver and spleen diseases [7][8][9]. Bitter melon has diverse bioactive compound content [10]. The phytocompounds of bitter melon include saponins, alkaloids, triterpenes, and steroids [6][8].

Bitter melon in the medical field has a negative impact; using bitter melon plant extracts has a toxicity effect at specific doses. Jia et al. [6] stated that testing bitter melon plant extracts in vitro and in vivo showed toxicity effects under certain conditions. This study aimed to examine the toxic effects 00of bitter melon plant extract on the growth of mice fetuses (*Mus musculus* L.).

METHODS

Extraction of Bitter Melon Fruit

The bitter melon pulp was cleaned from the seeds, washed, and put in the oven at 30-400 °C until dry and then finely ground. It was macerated within 24 hours with 96% ethanol while shaking and filtered with Whatman paper to separate the solvent from the solute. The solvent obtained is evaporated with a Rotary Evaporator until the crude extract (paste) remains and then dissolved in distilled water CMC. The treatment extract was made at the control (aquabides), 22.5 mg/30 gBW, 30 mg/30 gBW, and 37.5 mg/30 gBW.

Test Animals Care

There are 20 cages containing rice husks along with water bottles and feed containers provided for keeping test animals. Twenty female mice (*Mus musculus* L.) and twenty male mice aged ten weeks weighing 25-30 gr were acclimatized for a week and given pellet feed and drinking water.

Mice Mating

Twenty male and female mice were mated in cages. Each cage contains a pair of male and female mice. Copulation of mice occurs in the late afternoon because the copulation process occurs in the estrus phase, which starts from 16:00 to 22:00. Mice were determined to have copulation and 0 weeks pregnant when there is a vaginal plug or there is residual sperm in the vagina the next day
[12].

Extract Treatment

The test mice were grouped into four, each as follows: the control group was given 0.3 ml of aquabides, and three treated groups consecutively received bitter melon extract of 22.5 mg/30 g BW; 30 mg/30 g BW; and 37.5 mg/30 g BW respectively. The mice

were administered orally with a 0.3 ml/head/day dose. The treatment was given on days 6-17 of pregnancy (organogenesis phase). On day 17, mice on the container were anesthetized by chloroform and were dissected.

Research Parameters and Data Analysis

The data between treatments is processed statistically using the Analysis of Variance (ANOVA) test. If there is a statistical difference, a further test will be carried out with the post hoc LSD at the 5% level. Observation parameters were the malformation of fetus parts, namely cranium length and sternum length.

RESULTS AND DISCUSSION

The research results are consistent with the research objectives. The data on the head length of fetus mice treated with bitter melon extract is in Table 1.

Table 1. Head length of fetus mice treated with bitter melon extract

Treatment	Fetus Head Length (cm)	Sternal Length of the Fetus (cm)
Control	0,1985 ± 0,0342[a]	0,0789 ± 0,00502[a]
B	0,1208 ± 0,0004[b]	0,0603 ± 0,0030[b]
C	0,1165 ± 0,0011[b]	0,0517 ± 0,0014[c]
D	0,1277 ± 0,0484[c]	0,0600 ± 0,0000[b]

Note: Numbers followed by the same letter indicate no significant difference in the BNT test α≤5%.
B: 22.5 mg/30 gr BW, 30 mg/30 gr BW, 37.5 mg/30 gr BW.

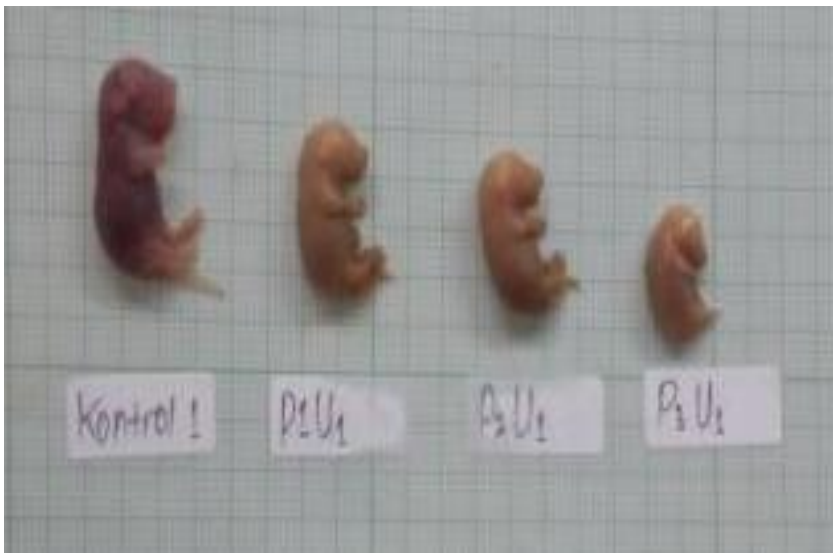

Figure 1. Fetus of mice, A: Control, B: 22.5 mg/30 gr BW, 30 mg/30 gr BW, 37.5 mg/30 gr BW.

The treatment of bitter melon extract significantly affected the results of the BNT test α≤5% (Table 1) on the characteristics of head length and sternum length of the fetus of mice. The control treatment without the provision of bitter melon plant extract showed a more excellent value of the fetus's head length and sternal length than mice treated with bitter melon plant extract. These results indicate a toxic effect of bitter melon plant extract on the growth of mice embryos. This is supported by the research by Thiagarajan et al. [13], which shows that the treatment of bitter melon plant extract has a toxic effect on zebrafish embryos. Treatment with bitter melon plant extract also causes death, delays hatching time, and inhibits the development of zebrafish embryos [14]. Khan et al. [15] stated that the administration of bitter melon seed and fruit extracts causes growth anomalies in the fetus, so its application needs to be considered to avoid disturbances in the embryo.

Different concentrations of bitter melon extract in mice impact mice embryos differently. Treatment with higher concentrations has positively correlated with decreased head and sternum length of mice fetuses (Table 1 and Figure 1). These results are due to the higher concentrations that increase the toxicity effect of bitter melon extract. It is in line with the results of the study by Perumal et al. [16] which shows that the toxic effect of bitter melon plant extract depends on the dose given and the length of time. The higher the extract concentration and the longer the treatment time will increase the toxicity effect, disrupting embryonic growth. The research results by Santos et al. [14] also showed that increasing the concentration of bitter melon plant extract resulted in a decrease in hatching percentage and increased mortality in zebrafish embryos.

In this study, the extraction of bitter melon fruit used ethanol. The difference in extraction methods used can provide different extract results. Besides that, the extraction method can also affect the toxicity of the resulting extract. The results of research by Thiagarajan et al. [13] stated that the cold aqueous extraction method has a lower toxicity level than the hot aqueous extraction method.

CONCLUSIONS

The research concluded that administering bitter melon extract to pregnant mice disrupted fetal growth as seen by reducing the length of the fetal cranium and sternum. Increasing the concentration of bitter melon extract was positively correlated with decreasing the length of the cranium and sternum of fetal mice.

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