

The Teratogenic Effect of The Mindi (*Melia azedarach* L) Leaves Ethanol Extract on Mice (*Mus musculus*) Fetus

Adisti Erlina Sutomo,¹ Trully D Sitorus,² Adhi Pribadi³

¹Faculty of Medicine Universitas Padjadjaran, ²Departement of Pharmacology and Therapy, Faculty of Medicine Universitas Padjadjaran, ³Departement of Obstetric and Gynecology Faculty of Medicine Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital Bandung

Abstract

Background: Mindi leaves (*Melia azedarach* L.) were used by Indonesians as a traditional medicine for pregnant women because it was considered to be safe. Mindi leaves contain several active compounds and one of them is suspected as a teratogen and can disrupt fetus growth in gestation. This research aims to know about the teratogenic effect of ethanol extract of Mindi leaves by using mice.

Methods: This was a laboratory experimental study using 27 pregnant female mice (*Mus musculus*) of Swiss Webster strain which were randomly assigned to 3 groups (n=9) controlled (Carboxymethyl cellulose 1% for day 1–18 of pregnancy), group I (mindi leaves ethanol extract 3.22 mg+Carboxymethyl cellulose 1% day 1–5 of pregnancy), and group II (mindi leaves ethanol extract 3.22 mg+Carboxymethyl cellulose 1% day 6–18 of pregnancy). Observation was done to see total amount of fetus, live normal fetus count, length and weight of fetus, abnormal fetus count consisting of dead fetus count with normal and abnormal external morphology, and resorbed fetus count. This research was done from October to November 2012 in Pharmacological laboratory of Faculty of Medicine Universitas Padjadjaran. Data analysis utilized unpaired t-test.

Results: The result showed a significant difference ($p < 0.05$), seen from live normal fetus count and abnormal fetus count consisting of dead fetus count with normal and abnormal external morphology, and resorbed fetus count.

Conclusions: Administration of Mindi leaves extract during pregnancy of mices can cause teratogenic effect. [AMJ.2015;2(1):221–25]

Keywords: Flavonoid, mindi leaves, teratogenic effect

Introduction

Consuming teratogenic substances during pregnancy may cause a miscarriage and birth defects if it is taken during organogenesis. It tends to have impacts that affect the function of certain organs or system such as the enzymatic system if it is taken a few weeks before childbirth. The impact will be visible in the neonatal period.¹ Herbal medicine as alternative medicine has been widely used by the public. Adverse effects that can be caused by pharmacological drugs has encouraged people to choose traditional medicine which are considered more natural, safer, easy to get, and the price tends to be more affordable.² World Health Organization (WHO) stated that 80% of people in developing countries have used herbal medicine and in 2000 estimated

that worldwide sales of herbal medicines was around U.S. \$60 billion.²

Basically, all substances both natural and chemical ingredients are likely to cause harmful effects; hence pre-testing on animal to determine the safety of the substance is needed. Safety test is a part of the preclinical requirements to assess the safety of a drug or substances to be used as supplement or food.³ Toxic effect is a reaction that can lead to serious health problems since it disturbs physiological and biochemical body function. This reaction usually happens due to overuse of drugs or chemical substances.⁴ It is not yet confirmed officially that Mindi leaves can be used as an alternative for the treatment of high blood pressure without causing certain side effects, especially when it is taken during pregnancy This is due to the presence of some

Correspondence: Adisti Erlina Sutomo, Faculty of Medicine, Universitas Padjadjaran, Jalan Raya Bandung-Sumedang Km.21, Jatinangor, Sumedang, Indonesia, Phone: +62 81912745124 Email: iniadisti@yahoo.com

toxic compounds speculated to be contained in Mindi leaves.

According to Werler et al., physiologically toxic compounds can cause vasoconstriction of the vessels which connects the uterus to the placenta. This constriction of the vessels will lead to a decrease of nutritional supply that the embryo needs. As already known, all embryonic requirements must go through the placenta.⁵ The researchers are interested in conducting research teratogenic effects using Mindi leaves due to the fact that many people, including pregnant women, use Mindi plants, especially the leaves, for antihypertensive remedy because they think it is safer than generic drugs.

Methods

A total of 27 Swiss Webster female mice (*Mus musculus*), 20–25 grams, 12–14 weeks, including 12 male mice, were used in this study. All mice were obtained from Inter-University Research Center, ITB (PPAU-ITB). Before receiving treatment, the mice were adapted for seven days and then mated with male mice. The next day, if a vaginal plug was found, the day was designated as gestation day one. Mice which were sick and otherwise not pregnant would be excluded. The research protocols and animal care procedure were approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Padjadjaran, Bandung.

Fresh Mindi leaves are obtained from Subang then be processed into extract using ethanol. The leaves used were fresh Mindi leaves which had been newly plucked from the plant. Preparation of ethanol extract of Mindi leaves in this study used cold maceration method, whereas the solvent used was ethanol 70%.⁹ The resulting extract were in paste form, then the resulting extract (paste form) was weighed as needed, and was dissolved

with CMC 1% solvent, the goal was to dilute the extract.

After adaptation period, female and male mice were put into the same cage, so that the female could be impregnated. Then, male mice and female mice were being separated and undergoes vaginal plug in order to test for pregnancy. If the female mice were confirmed pregnant, the day was counted as day-1 gestational period. Aside from that, physical condition was also examined as inclusion and exclusion criteria that taken as consideration.

On day 18 of gestation the mice were terminated by cervical dislocation and then the abdomen and uterus were surgically removed. Fetuses were removed from the membranes which wrapped them and then washed with the solution of physiological NaCl 0.9% and 70% alcohol. Observations were made of live normal fetus count and abnormal fetus count consisting of dead fetus count with normal and abnormal external morphology, and resorpted fetus count. Fetus with abnormality would then be compared with normal mice embryology.

Statistics of this study used quantitative data that had been collected (not paired or unpaired, consisting of two groups), after that the normality of variance was tested first, followed with the parametric test of unpaired t-test (average difference test sample) and non-parametric test with Mann Whitney test if the data were not normally distributed. Significance was determined by p-value of <0.05.

Results

The results revealed that there were significant differences ($p < 0.05$) between the control group against group I and group II seen from the number of normal fetus (alive), the number of abnormal fetuses consisting of the number of dead fetuses with the normal external morphology and abnormal

Table 1 The Group Division

Group	Number of Female Mice	Dosage (mg/kg BB)	Substance Given	Method of Intervention	Time of Intervention
Control	9	-	CMC 1%	Oral	Day 1–18
I	9	3.22 mg	Mindi leaves extract 3.22 mg+CMC 1%	Oral	Day 1–5
II	9	3.22 mg	Mindi leaves extract 3.22 mg+CMC 1%	Oral	Day 6–18

CMC: Carboxymethyl cellulose

Table 2 Number of Normal Fetus (alive)

Group	Mean Normal Fetus (alive)±SD
Control	8.78±2.28
I	1.11±2.20
II	2.44±3.81

Control Group=CMC 1% 2 mL, for 18 days, Group I= Implantation, given Mindi leaves ethanol extract 3.22 mg+CMC 1% 2 mL at days 1-5, II= Organogenesis, Mindi leaves extract 3.22 mg+CMC 1% 2 mL days 6-18

external morphology, and the number of fetus resorption.

Macroscopical observation of abnormal fetus consisted of fetus resorption, dead fetuses with normal morphology, and dead fetuses with abnormal morphology. From this study dead fetuses with abnormal morphology were found with hemorrhage, exencephaly, and abdominal wall defect. Table 3 showed an increase in the number of abnormal fetus in group I and group II compared to the control group. The number of abnormal fetus in the control group is much less when compared to group I and group II. Figure 1 is the result of observation was found one fetus with abdominal wall defect on mice included in group I and found one fetus with exencephaly on mice from group II. In addition, some fetuses were found with hemorrhage. Observation of fetus resorption seen in figure 1 as a yellow blob reddish or reddish-black inside the uterus of mice.¹⁰ Table 4 showed an increase in the number of fetal resorption in group I and group II compared to the control group. No fetal resorption was found in the control group.

Discussion

Mindi leaves (*Melia azedarach* L.) were

empirically being used as traditional medicine to treat several diseases among Indonesian Community. There were many active components in Mindi Leaves such as alkaloid, flavonoid, saponin, steroid and kaemferol. Based on the study, alkaloid and flavonoid were considered having teratogen effect as they can poison the embryo and also inhibit the contraction of smooth muscle on female mice.

Fetus total number consists of the all fetus, whether normal (alive) or abnormal. This study showed an increase in the total number of fetuses in each group. Group II generated the highest total number of fetus compared to the control group and the group I. The difference in the total number of fetuses between the control group and the group I with group II control group was not too large. There was a decrease the number of normal fetus (alive) in group I and group II compared to the control group. The number of normal fetus (alive) in group II greater than the group I. In group I there was more abnormal fetus than the normal number of fetus (alive). At day 1 to day 5 of pregnancy, there was a process of zygote fission and attachment of blastocyst.⁶ A decrease of average length on group I and II compared to control was observed. This decrease was the lightest teratogenic effect and an indication for a disruption in growth of fetus. Inhibition of growth occurred when teratogenic agents affected cellular interaction, proliferation, and associated with inhibition of nucleic acid synthesis.⁷ The decline of nutritional supply from the mother to fetus caused the fetus to receive inadequate nutrition in its growth period, and made possible the disruption in the length growth of the fetus.⁵ Mice of the group I and group II had a lighter average weight compared to control. Fetus weight was an important parameter to know the effect of teratogenic agent on fetus growth. The average body weight of normal

Table 3 Average number of Abnormal Fetus

Group	Mean Number of Abnormal Fetus±SD
Control	0.11±0.33
I	7.89±1.54
II	7.33±4.15

Control Group= CMC 1% 2 mL, for 18 days Group I= Implantation, given Mindi leaves ethanol extract 3.22 mg+CMC 1% 2 mL at days 1-5, II= Organogenesis, Mindi leaves extract 3.22 mg+CMC 1% 2 mL days 6-18

Table 4 Average Number of Fetus Resorption

Group	Mean Number of Abnormal Fetus±SD
Control	0.11±0.33
I	7.89±1.54
II	7.33±4.15

Control Group= CMC 1% 2 mL, for 18 days Group I= Implantation, given Mindi leaves ethanol extract 3.22 mg+CMC 1% 2 mL at days 1-5, II= Organogenesis, Mindi leaves extract 3.22 mg+CMC 1% 2 mL days 6-18

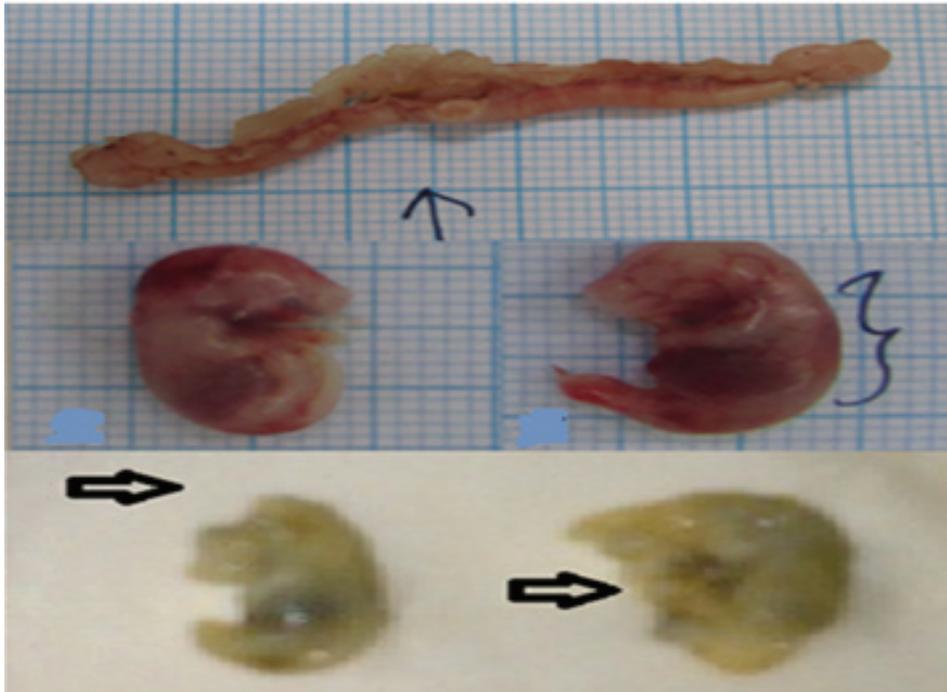


Figure 1 Fetus Resorption, Fetus with Cephalic and Whole Body Hemorrhage, and Fetus with Exencephaly and Abdominal Wall Defec

fetus at day 18 of gestation was 1.4 gram (Wilson and Warkany, 1965).⁷

The average weight of fetus in the control group, group I, and group II were all under the normal value. Another possibility than the teratogenic effect of ethanol extract of Mindi leaves is Intra-Uterine Growth Retardation (IUGR). Retarded fetal growth is an event in intra-uterine growth in which the fetal weight is below the normal value for the age of gestation. Retarded fetal growth is closely related to the health of the mother and number of carried fetus.

There is an increased number of abnormal fetuses in group I and group II compared to the control group. Abnormal fetuses observed in the study were dead fetuses with normal external morphology, fetuses death with abnormal external morphology. Fetuses dead with external morphological abnormalities found in this study were fetuses with bleeding, exencephaly, and abdominal wall defect. The gestation period between days 6 through to 15 is the period of organogenesis. Exposure to teratogens agents during this time more frequently causes congenital anomalies than spontaneous abortion or resorption of the embryo.⁶ Compounds found in the Mindi leaves

suspected to have embryotoxic potential is a flavonoid which can inhibit cell proliferation and cause DNA damage.⁸

Hemorrhage was found on the head, back, legs, and body. Hemorrhage is a discharge of blood from the cardiovascular system, followed by accumulation in tissues (Price and Wilson, 1984). Mindi leaves extract which has been given repeatedly lead to increase concentration in blood, causing an osmotic imbalance. Osmotic imbalance may be caused by disturbances of pressure and viscosity of the liquid in different parts of the embryo. Pressure disturbance occurred between extraembryonic and intraembryonic fluid, causing the blood vessels to rupture and bleed (Wilson, 1073).⁷ In addition to bleeding, fetuses exencephaly found in group II. Fetuses with exencephaly are caused by degeneration of neuroepithelial cells and proliferation of mesenchymal cells was disrupted in the head. The number of mesenchymal cells was reduced, thus the neural ectoderm cannot fold to form the neural tube and resulted in exencephaly. Abdominal wall defects was found in group II, in which there had been inhibited growth of mesenchymal cells, due to the disruption of cellular proliferation.⁵

The research concluded that the administration of Mindi leaves ethanol extract (*Melia Azedarach*.L) during gestation on mice caused teratogenic effect.

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