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Research Article

Study Of Teratogenic Effects Of Chinese Spinach Extract (Amaranthus Tricolor L) In Wistar Rat

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ABSTRACT
Environmental factors such as the use of drugs that are teratogenic account for 16% of cases of congenital malformations. It has led people to use herbal plants in overcoming diseases experienced during the pregnancy process. Many plants have been used as traditional medicine, one of which is Chinese spinach leaves (Amaranthus tricolor L.). It has been widely used to treat dysentery, fever, and as an alternative to increasing blood flow. The purpose of this study was to determine the teratogenic effect of Chinese spinach leaves extract which was tested in vivo. The ethanol extract of these leaves is divided into three different doses, namely 50, 400, and 1000 mg given orally on the 6th to 15th day of pregnancy. On the 19th day, the female rats were dissected and the fetus was observed. The observations were made by observing abnormalities in the number of skeletons and organs in the fetus from the test and control groups and by observing the fetal body weight, the number of fetuses, and the amount of fetal resorption. There was no statistically significant difference between these three parameters in the test group ($p > 0.05$). The administration of Chinese spinach leaves extract at all test doses did not show any abnormalities in the fetal organs and did not cause abnormalities in the skeleton, so it could be concluded that Chinese spinach leaves extract did not cause teratogenicity.

Keywords: Chinese spinach, Amaranthus tricolor, Teratogenic.

INTRODUCTION
Teratogens are defined as agents or factors that cause defects in the developing embryo. Teratogenesis means the occurrence of defects in the fetus due to the presence of a substance that interferes with development and results in congenital defects[1]. The congenital defect in question refers to the form of a small defect with a change in function that is not normal[2].). For example cleavage of the roof of the mouth, hydrocephalus, behavioral variations, and hidden defects such as vaginal carcinoma in girls with a cholesterol diet during pregnancy[3].
Traditional medicines are medicines that are formulated and used from generation to generation in treating various cases of the disease. There have been many plants used as traditional medicine, one of which is the leaves of Spinach spinach (Amaranthus L.). These leaves are considered as one of the important medicinal plants in some parts of the world.
Infusion of 30% Chinese spinach leaves orally can increase serum iron, hemoglobin, and hematocrit levels in rabbits which makes anemia significantly[4]. However, its safety for use in pregnant women is unknown. Therefore, it is necessary to test the teratogenic effects by looking at the possibility of abnormalities in the fetus.
A teratogenic test is needed to see the toxicity of the test substance to rat fetuses by giving the test substance to its mother. The test substance was administered in the organogenesis phase. Surgery is performed just before delivery.

OBJECTIVES
This study aimed to examine the toxicity at the organ level, which may occur in fetuses of rats from mothers who were given Chinese spinach leaves extract (Amaranthus tricolor L.) in the organogenesis phase.

RESEARCH METHODS

Female animals that have tested positive for pregnancy are given a test preparation during the organogenesis period, namely on the 6th to the 15th day. On the 19th day, the animals are dissected and the number and weight of the fetus are observed, then the skeleton and soft tissue assessments are carried out. In the assessment of the skeleton, the back of the skeleton is first examined: the skull, spine, ribs are examined. The skeleton is then inverted to observe the front: the oral cavity, the bones surrounding the shoulders and hips, the front and hind limbs. The results of the examination of the structure, morphology, number, and position of the bones were recorded. In the soft tissue assessment, the observation on the part of the head between the upper and lower jaws was cut using a razor blade, and the palate was examined. In the upper head section, a transverse incision from the nose to the back is made with a maximum distance of 1 mm with emphasis on the nose, eyes, and lateral ventricles. Then the skin around the stomach is incised with a scalpel, the front is cut and removed, the organs in the abdominal cavity are carefully removed. The condition of the ureters, genital glands, and the bladder is checked. All abnormalities were recorded.

Materials and Tools

Materials

Chinese spinach leaf extract (Amaranthus tricolor L), CO2, solution for the skeleton and soft tissue observation, Bouin solution, KOH 0.5%, KOH 1%, ethanol 90%, Glycerin 5%, Glycerin 20%, Glycerin 40%, Glycerin 80% and 1% Hydrogen peroxide, and aquadest.

Tools

Scale, microscope, animal house, surgical instruments, slide, cover glass, pipette, tissue, oral needle, Petri dish.

ANIMAL TESTED

Male and female Wistar rats, with a bodyweight of approximately 200 grams, were obtained from the Palembang Rat Center and acclimatized at the Animal Laboratory, Aisyah University of Pringsewu.

EXPERIMENT PROCEDURE

Rats were randomly divided into four groups, namely: one control group given aquadest and three groups given Chinese spinach leaves extract at doses of 50, 400, and 1000 mg/kg body weight. Each group consisted of 10 female rats. The drug is given orally once a day on the 6th - 15th day of pregnancy. The period of administration of this drug is in the middle of the organogenesis phase of the rat pregnancy.

Early Pregnancy Determination

Male and female rats are placed in one cage with a ratio of one male to four females. Starting one day after being put in the same cage, vaginal smears were examined for female rats. The rats' vaginal opening was rinsed with a sufficient amount of 0.9% NaCl using the tip of a pipette. The rinse water is then dripped onto the slide while blending. After drying, the smears were stained with 0.1% methylene blue. The smear is then examined under a microscope at 100 times magnification. The zero-day of pregnancy was determined by observing the vaginal plug or the presence of sperm in the vaginal smear, as can be seen in Figure 1. There were ten pregnant animals in each group.

Surgery and Observation

On the 19th day of pregnancy, the rat was dissected for fetal retrieval. Each fetus is then dried and weighed. A normal fetus just removed from the uterus is shown in Figure 2.

Preparation of Preserved Skeleton

Preparation: The fetus is fixed in 90% ethanol solution for at least 1-2 weeks. After drying the fetus is skinned perfectly. The eyes and lumps of fat at the nape of the neck are removed, and the trachea is cut. The groin and armpits were slashed so they didn't stick to the body. The abdominal wall is torn off and the sex of the fetus is checked. After skinning and removing its internal organs, the fetus is returned to the fixation bottle.

Purification: After the fetus is skinned and its internal organs removed, the ethanol fixation solution is replaced with 0.5% KOH. Cleansing lasts for one day. Occasionally the container is shaken to expel air from the chest cavity.

Bleaching: After complete purification, the KOH solution is discarded, the fetus is rinsed with water and the remaining fat is removed. The water is then replaced with a 1% hydrogen peroxide solution. The fetus is left in it for 2-3 hours, rocking occasionally. Bleaching is considered complete if the inside of the bones is white.

Staining: After complete bleaching, the fetus is rinsed and soaked in water for +10 minutes. The water is then replaced with a solution of alizarin dye. The fetus is left in it for no more than 24 hours. The stain is considered perfect when the outline is visible.

Final cleaning: After complete staining, the alizarin dye solution is discarded, the fetus is rinsed with water several times and then gradually immersed in glycerol solution of 5%, 20%, 40%, 80%, and finally pure glycerol for 1 week each.

Outline assessment: Assessment is made after the preparation has been immersed in 80% glycerol for at least 1 week. At first, the back of the skeleton is examined: the skull, spine, and ribs are

examined. The skeleton is then inverted for observation of the front: the skeleton of the oral cavity, the bones surrounding the shoulders and hips, the front and hind limbs. The results of the examination of the structure, morphology, number, and position of the bones were recorded.

Soft Tissue Assessment

After 1-2 weeks of fixation in Bouin solution, the fetuses from one parent are transferred to a beaker filled with water. The fetus is then dried with a tissue, and cut in a certain pattern. The head between the upper and lower jaws is cut with a razor blade, and the palate is examined. In the upper head section, a transverse incision from the nose to the back is made with a maximum distance of 1 mm with emphasis on the nose, eyes, and lateral ventricles. Furthermore, the skin around the stomach is incised with a scalpel, the front is cut and removed, the organs in the abdominal cavity are carefully removed. The condition of the ureters, genital glands, and the bladder is checked. All abnormalities were recorded.[5]

RESULTS OF EXPERIMENT AND DISCUSSION

From every ten female rats per group that was mated within four months. It was produced ten pregnant rats in the control group, ten pregnant rats in the 50 mg/kg test substance group of BW, 20 rats in the 400 mg/kg dose group of BW, and 10 individuals in the 1000 mg/kg dose group of BW.

The results of observations on fetal weight did not show any significant differences between treatments (Table 1).

Table 1. Number of Live Fetuses per mother rat and body weight

Dose	Live Fetus		Undeveloped fetus		Fetus Weight Average (g)
	Total	%	Total	%	
Control	102	100	0	0	2,51±0,1
50 mg / kg of body weight	93	100	0	0	2.52±0,1
400 mg/kg of body weight	87	100	0	0	2,6±0,1
1000 mg/kg of body weight	78	97,4%	0	0	2,56±0,1

In the test groups, 50 mg/kg of BW and 400 mg/kg of BW showed a live fetus 100% comparable to the control group. In the group with a dose of 1000 mg/kg of BW, 2 fetuses that died after surgery weighed 2.05 grams and 2.12 grams with an average percent of life in the 1000 mg/kg of BW test group, namely 97.4%.

The results of observations on live fetuses did not show any abnormalities in all observed fetuses, fetuses in the test group were comparable to fetuses in the control group, the lowest weight was in the group dose 1000 mg/kg BW, namely 2.05 g (Figure 1).

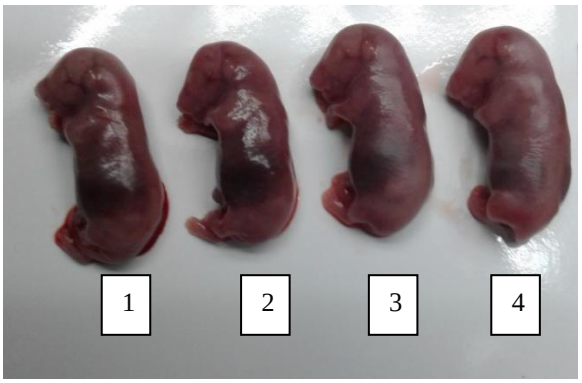

Fig.1:Full photo from the beginning
Fig.2: Fetus rats on day 19 of pregnancy
Notes: 1. Control group fetus, 2. Fetus group with a dose of 50 mg/kg of BW, 3. Fetus group with a dose of 400 mg/kg of BW, 4. Fetus group with a dose of 1000 mg/kg of 1000 mg/kg of BW.

Table 2 Percentage of Live Fetal Vertebral Abnormalities

Dose	Number of Pregnant Rats	Total number of fetuses	The number of fetuses was observed	% Incidence of vertebral abnormalities			
				C	T	L	S
Control	10	102	50	0	0	0	0
50 mg/kg of BW	10	93	45	0	0	0	0
400 mg/kg of BW	10	87	42	0	0	0	0
1000 mg/kg of BW	10	78	38	0	0	0	0

C : Cervix, T : Thorax, L : Lumbar, S : Sakrokaudal

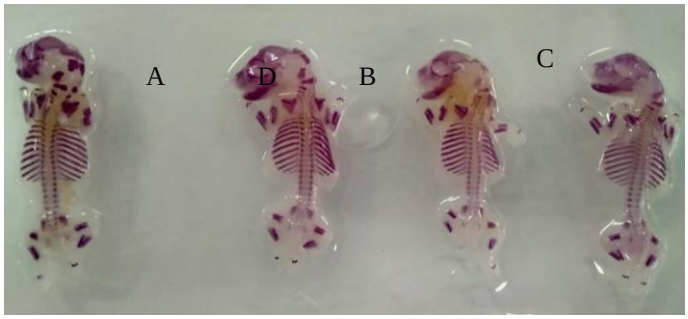

Fig.3: Fetus Skeleton

Figure 3 Skeleton of a Live Fetus in A: control group, B: dose 50
mg / kg of BW, C: 400 mg / kg of BW, D: 1000 mg / kg of BW

In all groups, there were no vertebral abnormalities (Table 2) and the number of normal skeletons (Figure 2) The normal structure and number of skeletons were: 7 cervical vertebrae, 13 thoracic vertebrae, 6 lumbar vertebrae, and 4 sacral vertebrae, which

compose the spine. ; Forelimb bone consists of 5 distal vertebrae, 4 proximal vertebrae, 4 metacarpal vertebrae; The vertebral column consists of 5 distal vertebrae, 4 proximal vertebrae, and 5 metatarsal vertebrae.

The treatments of Chinese spinach leaves extract (Amaranthus tricolor L.) on days 6-15 of pregnancy in this study did not cause defects in the fetus, but with the presence of two fetuses who died at a dose of 1000 mg/kg of WB. It must be careful with mothers who want pregnancy.

CONCLUSIONS

Chinese spinach leaves extract (Amaranthus tricolor L) does not cause defects in the fetal organs and skeleton so that it can be stated that it is not teratogenic.

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