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Original Research Article

Teratogenic Effect of Ethanol Extract of Yellow Root Stem (*Coscinium fenestratum* (Gaertn.) Colebr) on Mice

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ABSTRACT

The yellow root (*Coscinium Fenestratum* (Gaertn.) Colebr) has several bioactive compounds with antimicrobial, inhibitors of intestinal parasites, antidiarrhea, anti-inflammatory, anti-hypertension, anti-tumour, hepatoprotective, anti-cancer and anti-malarial properties. This study evaluated the phytoconstituents and the teratogenic effect of yellow root stem ethanol extract in mice during pregnancy. A total of 24 pregnant female mice divided into 4 groups were used for this study. Group 1 served as negative control (NaCMC 1 %), and groups 2, 3, and 4, respectively, were given extracts at doses of 200, 400, and 800 mg/kg bw. The extract was administered in the organogenesis phase (6th to 15th day of pregnancy). The mice were weighed during the experimentation period and sacrificed on the 18th day. The fetus was removed, and the number of fetuses was counted and weighed. The morphological, visceral and skeletal abnormalities were examined after staining with Bouin's solution. The phytochemical screening revealed that the ethanol extract of yellow root stems contains alkaloids, saponins and terpenoids. The results showed that the administration of yellow root stem extract during pregnancy affected the weight of the mice ($p<0.05$). The plant extract did not affect the number and weight of the fetus significantly ($p>0.05$). Morphological examination of the fetus fixed with Bouin's solution did not show disability in the gap of the sky of the mouth, but there were signs of bleeding in the head and stomach. Similarly, the staining results revealed abnormalities in the phalanx and caudal bone. The study concluded that the use of the yellow stem extract may impact teratogenic deformities in experimental animals.

***Keywords*:** yellow roots (*Coscinium Fenestratum* (Gaertn.) Coleb), ethanol, teratogenic, fetus

Introduction

Indonesia is a country that is famous for its abundant biodiversity. Most of the existing plants have been used as medicines for various diseases since the time of our ancestors.[1] Indonesia is a country with a large bio-diversity ranking below Brazil. It has been estimated that of the over 30,000 plant species in Indonesia, 7500 species can be used as medicine, with only about 2000 medicinal plants currently identified. In Indonesian traditional medicine, 1200 of these medicinal plants have been utilised, while about 300 species are harnessed commercially by industries.[2]

Coscinium fenestratum is known as a traditional medicine popularly used in local and rural communities for various purposes (food, medicine, etc.). This plant is said to be a storehouse of food and chemicals with many benefits, such as medicine for various diseases. The ability to formulate drugs and herbs in most communities is a hereditary legacy that has been strongly attached to the community.[2]

Teratogens are substances or anything (chemicals, pollutants, drugs, viruses and physical substances) that can cause changes in the function and shape of organs in fetal development during pregnancy. These compounds have the potential to cause congenital disabilities when used during pregnancy, especially when taken during the process of organogenesis. Teratogenicity can be caused by several factors, such as environmental factors (use of drugs, radiation, infection, hormone or vitamin deficiency) and genetic factors (aberrations and mutations). There are a number of materials that may cause teratogenic effects in humans or animals, such as ionising radiation, cytomegalovirus infection, rubella virus, herpes virus, syphilis toxoplasmosis, metabolic imbalances, for example, due to alcohol consumption during pregnancy and folic acid deficiency.[3]

Humans come into contact with chemicals (therapeutic drugs, metabolic products, food, and environmental compounds) from the zygote stage. If the exposure continues during embryonic and fetal development, these chemical loads can be largely transferred from the mother to the developing embryo. Precise genetic and physiological regulation is required in animal development, and susceptibility is high in the early stages. If this regulation is altered, exposure to teratogenic chemicals can result in embryo-fetal toxicity and will later lead to developmental delays, physical malformations, behavioural changes and death.[4] Drugs used in pregnant women can cause problems if not considered properly, which may affect both the mother and the growing fetus. The frequency of repeated use of herbal medicines during pregnancy can also cause accumulation in the fetus, while the fetus does not yet have a perfectly functioning metabolic system. Active substances or chemical compounds of drugs can enter the circulatory system in the fetus, affect the formation process of fetal organs, and this can later have a teratogenic effect. Herbal medicines used in pregnant women may have the potential to cause teratogenic effects on the fetus since they may also contain chemicals with teratogenicity effects if used at certain doses.

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Therefore, teratogen testing is needed on herbal medicines used in traditional medicine and for the market.[3]
This research was conducted to investigate the teratogenic effects of the yellow root plant, a plant widely used in medicine and food, in pregnant Wistar mice. The study aimed to highlight the safety of the yellow root plant use during pregnancy in animals and humans.

Material and Methods

Equipment and Chemicals
Rotary evaporator (Buchi®), maceration container (brown bottle), funnel, animal scale, analytical balance (Ohaus®), animal drum, animal water dish, measuring cup (Pyrex®), test tubes, mortar and pestle, dropper pipette, vial, aluminium foil, scissors, blades, racks and test tubes, sonde, syringes, capillary tubes, microtubes, micropipettes, micropipette tips, oven (Memmert®), furnace, waterbath, gel clot activator tubes, fetus soaking container, lumping, colouring container, standard mice food, test animals, yellow root plants (*Coscinium fenestratum* (Gaertn.) Colebr)
The chemicals used included 70% ethanol, 2N HCl, Mayer reagent, Draggendorf reagent, Wagner reagent, distilled water, 10% NaOH, distilled water, 0.5% NaCMC, 0.05N ammonia, chloroform, 2N H_2SO_4, alizarin solution, and Bouin solution.

Animals
Thirty-six (36) female mice aged 2-3 months with a body weight of 20-30 g were used. Healthy animals which have never been used for the experiment. The animals were acclimatised and showed normal behaviour and no change in body weight of 10%. Ethical approval for the test animals was obtained from the Faculty of Pharmacy Ethics Committee of Universitas Andalas, with approval number 36/UN.16.10.D.KEPK-FF/2023.

Plant Materials and Collection
The yellow roots used as samples were collected in the Palupuah District, Agam Regency, West Sumatra Province.

Preparation of the Extract
Fresh plant material (8 kg) was dried, and 300 g was ground to a fine powder. This sample was subsequently macerated with 70% ethanol at a ratio of 1:10, sample-to-solvent, in a dark-coloured glass container for 6 hours. The filtrate was dried using a rotary evaporator at 45°C under reduced pressure.

Characterisation of the Extract
The characterisation of the extract included nonspecific, specific, and chemical testing as follows: a) nonspecific testing measured the loss of weight on drying, total ash content, b) specific testing included organoleptic tests (i.e. shape, colour, taste, odour), parameter identify (i.e. names identified and compounds contained); and c) chemical testing determined the phytochemical components (e.g. alkaloids, saponins, and terpenoids).[4]

Administration of Yellow roots plant Ethanol Extract Suspension
The body weight of the mice was weighed on day 0 of pregnancy, during administration of the extract, and before autopsy. The extract was administered to pregnant mice for 10 consecutive days, starting from day 6 to day 15 of pregnancy. During the treatment period, animals were allowed access to food and water *ad libitum.* The animals were observed every day during the test period. The presence or absence of death, dying, changes in behaviour, and symptoms of toxicity were noted.

Preparation of Fetus for Morphological Examination
The harvested fetuses were fixed by immersion in Bouin's and or Alizarin solution. The Bouin's solution contains 40% formalin, glacial acetic acid, and saturated picric acid. While the Alizarin solution consists of red alizarin 6 mg/L and KOH 1%.[5] There are after the fixed fetuses are observed for any morphological changes.

Statistical Analysis
The data were analysed by one-way ANOVA. Subsequently, the significant results were analysed by Duncan's multiple range test (p<0.05) using IBM SPSS Statistics V24.

Results and Discussion

Fetal Morphology Observations
Morphology is an observation parameter to be able to see any abnormalities on the outside of the fetus's body, such as completeness of hands, completeness of legs, haemorrhage, bent body and dwarfism, which can be done macroscopically. This observation of fetus morphology is the most important part of conducting teratogenic tests. This experimental observation can be done using Bouin's solution to see the morphological state of the fetus. Bouin's solution makes the fetus hard and also impacts the yellow colour. Observation of fetus morphology can be done visually by comparing the control group with the treated group.[5] Several other fetus morphological parameters that could be observed include completeness of the external organs of the total fetus, which includes body length, body weight, and morphological abnormalities (changes in nose, jaw, mouth, eyes, brain, forelimbs, hindlimbs, fur, tail skin and mucosal layer), all of which are observed whether they look normal or not. Externally deformed fetuses look like dwarfs, usually determined when the average body weight of the fetus does not reach 2/3 of the average body weight of a normal fetus or control group.[6]
Based on the results of observations from day 0 of pregnancy until day 9 of pregnancy, there was no significant increase or decrease in the weight of the mice, except a mild weight gain. The occurrence of this weight gain could have been influenced by several factors, such as the development of the fetus mice, the increasing volume of amniotic fluid, the amniotic membrane and also the placenta. And the most influential is the increase in the weight of the fetus. The average number of fetuses of negative control, group 1 and group 2 were the largest. The average number of fetuses of the smallest mice was in group 3, which received 800 mg/kg yellow root extract. In the negative control group, treatment groups 1, 2, and 3, there was no significant effect on the average number of mice fetuses. The result of the body weights of the foetuses is shown in Figure 1.
Weight loss in mice fetuses is one of the effects of the presence of foreign bodies that can interfere during pregnancy. The lack of fetal body weight is an indication of growth retardation of the fetus. The number of mice born per birth is 6-15, with an average fetal body weight of 0.5-1.5 g. The average body weight (0.7-1.3 g) of the fetus from this experiment was within the normal range. There was a slight difference in the fetal body weight after the extract administration. At 800 mg/kg BW, the fetal body weight decreases slightly. The highest gain in average fetal body weight was found in the control group. The lowest average weight of the fetuses was observed in group 3. However, this decrease was still within the normal range. The newborn fetuses generally had no hair, undeveloped legs, ear holes that were still closed, and a short tail.[7]
Haemorrhage was observed in several mice fetuses, especially at the dose of 800 mg/kg BW extract. Under normal circumstances, the embryo develops in amniotic fluid, which is said to be isotonic with body fluids.

Figure 1: A complete and well-formed fetus

Figure 2: Appearance of Hemorrhage in the fetus

Figure 3: Absence of cleft palate in fetus mice

The introduction of foreign bodies into the body can affect the osmosis of the embryo, leading to disturbances in the pressure and viscosity of fluids in different embryos, between blood plasma and extra-capillary spaces or extra- and intra-embryonic fluids. These differences can cause blood vessels to rupture and haemorrhage to occur.[8]

In the negative control group, the fetus that had been soaked with Bouin's solution showed no haemorrhage. Haemorrhage was observed in the treated group, especially at a dose of 800 mg/kg BW (Figure 2). This was observed in the head and abdomen of the fetus. All the fetuses were alive.

Fetuses soaked in Bouin's solution are also used to see abnormalities or defects that occur in the visceral of mice fetuses, such as the presence or absence of a gap in the palate of the fetus mice (Figure 3), often called cleft palate. A cleft palate is a congenital defect or abnormality in the palate of the mouth. This incident usually occurs in several humans and animals such as dogs, horses, mice, cats, sheep, cows or pigs. This cleft palate is one of the defects that can occur during fetal or embryonic life as a result of incomplete development of anatomical structures related to craniofacial development.[9]

Observation of the cleft palate is done by cutting the head using a scalpel, then cutting starting from the mouth and slicing towards the back until the top and bottom of the head are divided into two parts. And then, the roof of the mouth is observed for the presence or absence of a cleft palate. In this study, it was observed that neither the control group nor the treated group showed any cleft palate, an indication that the ethanol extract of the yellow root stem does not cause cleft palate or disability in the cleft palate of the mouth of the fetus mice.[10]

Observation of ossification in mice fetuses can be done first by immersing the fetus into a red alizarin solution, which colours the bones of the mice fetus for easy observation and study. Observation using the alizarin solution is difficult to do because specimens in the alizarin solution are very vulnerable to hard objects when taken or moved to other observation sites. Observing this skeletal abnormality requires high accuracy and caution. Damage that occurs due to impact outside or when moving the specimen can also be seen as a defect, resulting in inaccurate results.[9]

In this experiment, the fetuses were immersed in the Alizarin solution for 3 days, under close observation, to avoid fetal destruction, which may occur over prolonged immersion in the solution. The prolonged stay of the fetus in the staining reagent solution may lead to fetal damage, and the reinforcement in the fetus may not appear, making structural observation difficult. The fetus's bone structure could be observed descriptively with a comparison between the treatment and control groups.

The mice fetus obtained in the negative control group showed the completeness of the reinforcement. Figure 4 explains what parts are present in the fetus of mice. As can be identified, the skull bones include a pair of parietal bones (P), a pair of frontal bones (F), one exoccipital bone (Eo), one interparietal bone (Ip), and supraoccipital (So). The vertebral bones are seven cervical bones (Cv), thirteen thoracic bones (Tv), four sacral bones (Sv), six lumbar bones (Lv), two caudal bones (Cuv), six sternum bones (St), ten ribs (Ri), This is because the ribs are divided into 7 pairs of true ribs (costae verae), 2 pairs of floating ribs (costae fluctuantes), and 3 pairs of false ribs (costae spuriae), for observation of the fetus it is difficult to see in detail.[11] Then, the facial skeleton includes one premaxilla (Pre-M), one nasal bone (N), and one mandibular (Mn). The upper limb bones include two pairs of radius (R), two pairs of ulna (U), one pair of humerus (H), clavicle (C), four metacarpus (Mc), scapula (S), and there are also eight anterior phalanges (Pha). Finally, the lower limb bones consist of a pair of femur bones (Fe), a pair of fibula bones (Fi), a pair of tibia bones (Ti), and eight posterior phalanges (Php).

From visual observation of the control compared to the treatment groups, there were differences in bones in the tail, toes and hands of the fetuses at a dose of 800 mg/KgBW. While at doses of 200 and 400 mg/kg BW, there were no significant differences ($p>0.05$) in bone when compared to the control group.

Statistically, there was a significant decrease in the body weight of the mother mice treated with the yellow root extract at a dose of 800 mg/kg BW ($p<0.05$). However, there was a slight growth that occurred in the treatment group fetuses at a dose of 800 mg/kg BW, with no visible internodes on each finger and toe and also no caudal bones. Thus, these results indicate that ethanol extract from yellow root stems could be unsafe for consumption during pregnancy. Other observations made include changes in the physical appearance of the mother mice prior to the administration of the yellow root extract and after. At the time of pregnancy, before the extract was administered, the fur of the mother mice looked neat, soft and smooth. However, post-extract administration, the fur of the mother mice stood straight. The observations seen are in accordance with the literature, which reported that at doses of 800, 900 and 1000 mg/kg BW, differences in the fur of the mother mice were observed, with fur becoming straight and standing.[11]

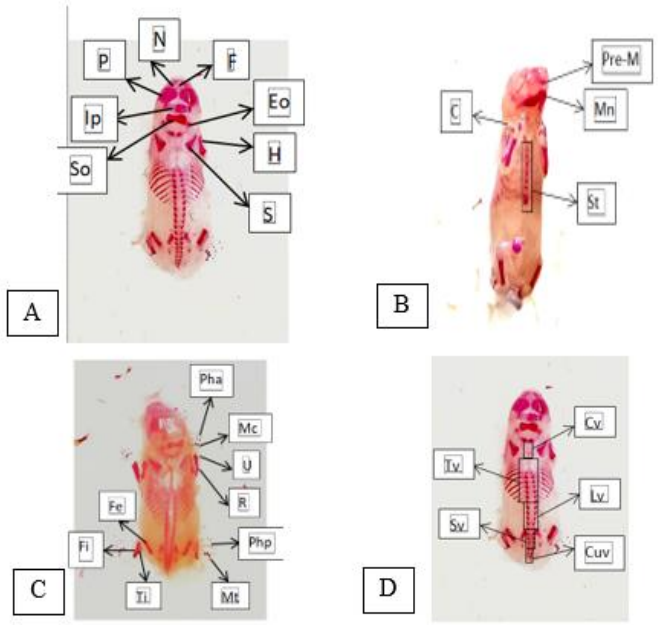

Figure 4: Bone identification (A) Skull bones, upper limbs (B) Skull bones, vertebral (C) Upper and lower limb bones (D) Vertebral bones

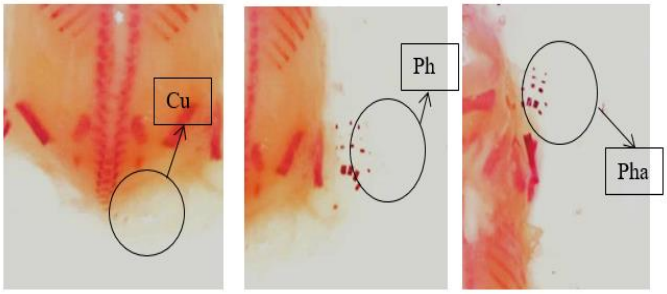

Figure 5: Observation results of the negative control group

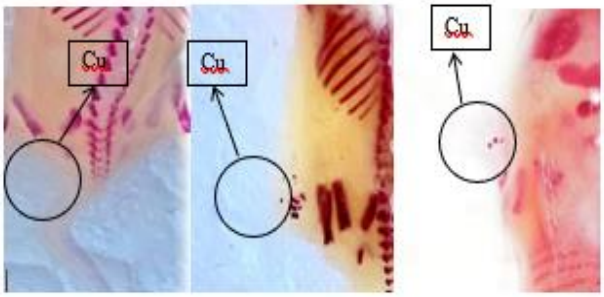

Figure 6: Observation results of disability that occurred in group 3 (dose 800 mg/kg BW)

It further indicates the non-toxic profile of the extract on foetal development when orally administered at 100 mg/kg pre-conception. Intrauterine growth restriction (IUGR) is the single largest contributing factor to perinatal mortality in non-anomalous foetuses.Suboptimal intrauterine growth affects up to 10% of pregnancies and confers an increased risk of perinatal morbidity and mortality. The perinatal outcome of IUGR fetuses is largely dependent on the severity of growth restriction.[12]

Conclusion

Extracts of yellow root stems have become a significant part of Indonesian traditional medicine practice and are currently marketed as an herbal remedy for various diseases. The results study showed that the extract of *Coscinium fenestratum* may cause congenital abnormalities at an oral dose of 800 mg/kg bw and beyond. There is, therefore, a need to weigh its benefits and risks before usage, especially in pregnant women.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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