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METHANOL PLANT EXTRACT OF RUMPUT TEKI (*CYPERUS ROTUNDUS* L.) CAUSING FETAL SKELETON RETARDMENT IN MICE

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ABSTRACT
Rumput teki (*Cyperus rotundus* L.) is a weed plant possesses biological and pharmacological activities and commonly used in treatment of various disease in many folk remedy systems. However, scientific studies on the safety and biological side effects of this plant use is still limited. This study aimed to investigate the possible effects of crude extract of nut grass (*Cyperus rotundus* L.) on the skeletal development of mice (*Mus musculus* L) during organogenesis period. Pregnant females (n=24) divided into four groups consisted of six mice each. Group 1 treated orally with distilled water (as control), whereas group 2, 3 and 4 were consecutively given extract at the dose of 45 mg/40g BW, 90 mg/40g BW and 135 mg/40g BW. After being treated for 12 days (day 6 to day 17), on the day 18 the fetuses were taken by caecarean section. The fetus count, fetal weight and length, and fetal tibial epiphyseal cartilage zones thickness namely the zone of reserve, proliferation, maturation, and calcification were assessed as the study parameters. The results showed the number, weight and length of fetus, the thickness of reserve, proliferation and maturation zones significantly lower by the higher dose of extract. In contrast, the thickness of calcified cartilage zone was increased by the increase of the dosage. Thus, it can be concluded that methanolic plant extract of rumput teki causing fetal skeleton retardation in mice.

KEYWORDS: *Teki, nut grass, Cyperus rotundus, epiphyseal cartilage, fetal retardation, chondrocyte, calcification.*

INTRODUCTION
Rumput teki is the Indonesian name of nut grass (*Cyperus rotundus* L.). In many folk medicine system, mainly in Africa and Asia, this weed plant commonly used in treatment of various diseases including nausea and vomiting, dyspepsia, colic, flatulence, diarrhoea, dysentery, intestinal parasites, fever, malaria, cough, bronchitis, renal and vesical calculi, urinary tenesmus, skin diseases, wounds, amenorrhoea, dysmenorrhoea, deficient lactation, loss of memory, insect bites, food poisoning, indigestion, nausea, dysuria, bronchitis, infertility, cervical cancer, menstrual disorders[1] antiobesity and mosquito repellent.[2] In Indonesia, the nut grass was used to treat poor appetite, diarrhea, dysentery, fevers, parasites, gastritis, indigestion, and sluggish liver symptoms.[3] Phytochemicals that is believed to make nut grass possessing medicinal and pharmacological effects including cyperone, cyperene, cyperotundone, cyperol, B-selinene, B-cariyophyllene, valerenal, sugeonyl acetate, a copaene, pathchoulene, isocyperol, sugeonol, sugetriol, isokobusone, and sitosterol.[4]

Laboratory tests carried out in mice and rats to investigate possible side effects of the nut grass herbs, so far, showed results unnecessarily to be feared. The in-vitro studies on antiamoebic activity and cytotoxicity (MTT assay) of ethanol extract of *C. rotundus* L. showed that the use of this plant for remedy purposes was safe.[5] Even at the dose of 5000 mg/kg C rotundus did not produce signs of toxicity, behavioral changes, mortality and differences on gross appearance of internal organs. However, at the dose of 1000 mg/kg the test animals showed slightly decreased in motor activity.[6]

Given the nut grass extract possesses antiobesity and insect repellent activities, proven to decrease motor activity as mentioned above and showed cytotoxic effects[7], it is very likely that long-term use of the herbs extract can have negative effects. Studies carried out by Indonesian researches showed that plant extract of nut grass affects reproduction system including fertilization impairment.[8]

Much of the skeleton formation during embryonic and fetal development initiates as a cartilaginous scaffold, which is progressively resorbed and replaced by new bone.[9] In the context of long bone such as tibial bone, longitudinal growth occurs at the growth plates,where cartilage proliferates in the epiphyseal areas, before subsequently undergoing mineralization to form primary new bone. That is why the thickness of epiphyseal cell layers is used as the essential parameter of bone formation or skeletal development.[10]

There are many factors that are known to affect skeletal development in either human and animals, especially during the epiphyseal development. Various endocrine, paracrine, and autocrine agents such as growth, thyroid and sex hormones, beta-catenin, bone morphogenetic proteins, insulin-like growth factor, iodothyronine deiodinase, leptin, nitric oxide, and vitamin D are known to be associated to chondrocyte proliferation and differentiation.[11] Thus it can be hypothesized that all external disturbances to epiphyseal cartilage growth regulatory factors, either directly or indirectly, will affects bone formation and development.

In order to investigate any possible detrimental effects of phytochemicals extracted from *C. rotundus* herbs on fetal bone formation and development this study was carried out using the pregnant female mice as the subjects.

METHODS

Plant Samples and Extraction

Plant samples of rumput teki (*C. rotundus* L.) were collected from suburb area Bandar Lampung. Taxonomic verification of the plant was carried out by botanist at the Botany Taboratory of the Faculty of Mathematics and Sciences, University of Lampung, Indonesia. The plant extraction protocol is as follows. Fresh tubers of the grass which has been previously washed were sun dried. The simplicia were ground to be a powder form and macerated using methanol solvent and concentrated using rotary eavporator at the temperature of 35^{o}C, rotation of 60 rpm for 60 minutes.

Animals and Experimental Design

The animals as well as their food pellets used in the study were obtained from Lampung Veterinary Center, Indonesia. During the study all mice were kept in a room at the temperature of 25°C and 12:12-hour light-dark cycle with free access to water and pellets. All animal care and treatment procedures were approved by the Ethics Committee, Faculty of Medicine, University of Lampung, Indonesia. By using a completely randomized design, pregnant females (n=24) of Swiss albino mice (*Mus musculus* L.) divided into four groups consisted of six mice each. Group 1 treated orally with distilled water (as control), whereas group 2, 3 and 4 were consecutively given extract at the dose of 45 mg/40g BW, 90 mg/40g BW and 135 mg/40g BW. The treatments were given once daily for 12 days, from the day 6 up to day 17 of pregnancy, and on the day 18 the fetuses were taken by caecarean section.

Histological Preparation

The fetal tibia of right hind leg were taken by amputation and fixed in 10% formaldehyde for 24 hours, embeded in paraffin, cut at a thickness of 6 µm and stained with Hematoxylin-Eosin.[12] The histological slides of longitudinal section of the proximal tibial epiphysis were observed under light microscope at 400x magnification. The thickness of each cell zone of the epiphyseal cartilage namely zone of reserve, proliferation, maturation and calcification were measured using micrometer.

Data Analysis

The data are presented as Mean ± SD and analyzed statistically using a one-way ANOVA. Least Significance Difference (LSD) test was used as the post hoc test. Both ANOVA and LSD tests were set at the level of significance of $\alpha = 0.05$.

RESULTS AND DISCUSSIONS

Total number of fetuses, fetal weight and fetal length of each mice group are presented in Table 1. These three parameters significantly decreased with increasing treatment dose ($\alpha = 0.05$). Effects of methanol extract of rumput teki on the development of tibial epiphyseal cartilage of mice were summarized in Table 2. The results of ANOVA and LSD test showed that all of four parameters of tibial epiphyseal cartilage growth namely the thikness of reserve, proliferation, maturation and calcification zones were significantly different between treatments ($\alpha = 0.05$).

Less number of fetuses and lower fetal weight and length by the increasing doses of extract as shown in Table 1 clearly indicates that plant extracts of nut grass inhibit fetal growth of mice. These data confirm what Molyneux et al.[13] has stated that many phytochemicals have benefits on the one hand but also the ugliness on the other. On the one hand there are many studies that show that *C. rotundus* extract provides healing benefits in various diseases, but on the other hand some chemical contents of these plants are shown to have toxic properties, quercetin is among the examples.[14] Quercetin was revealed to possess toxicity and physiological effects on several invertebrates such as insects and earthworms.[15]

Table 1 The number of fetus, fetal weight and length of pregnant mice given nut grass extract of different doses

Extract doses (mg/g BW)	N	Total of fetuses	Fetal weight (g)	Fetal length (mm)
0	6	65	2.01[a]	20.12[a]
45	6	54	1.46[b]	13.47[b]
90	6	36	1.11[c]	8.13[c]
135	6	16	0.79[d]	5.78[d]

Values in the same column followed by the same superscript are not different at α=5%.

Table 2 The thickness of reserve, proliferation, maturation, and calcification zones of fetal tibial epiphysis of mice treated with methanol extract of nut grass

Extract doses (mg/g BW)	reserve zone (µm)	proliferation zone (µm)	maturation zone (µm)	calcification zone(µm)
0	309.63±1.30[a]	215.70±0.29[a]	94.99±0.64[a]	98.69±0.57[a]
45	228.93±1.78[b]	144.24±0.53[b]	90.33±1.18[a]	153.33±1.17[b]
90	223.93±1.70[c]	139.60±1.23[c]	71.88±0.29[c]	228.90±0.35[c]
135	145.42±0.83[d]	130.84±0.31[d]	61.67±0.32[d]	309.56±1.78[d]

Data are presented as mean ± SD. Values in the same column followed by the same superscript are not different at α=5%.

Coumarin is another chemical that can be extracted from nut grass[16], this phytochemical was known to cause hepatotoxicity in both human and animals.[17] Bamboo is one of monocot plants containing some phytochemicals similar to that of *C. rotundus* such as p-hydroxybenzoic acid, β-sitosterol-β-D-glucopyranoside. Bamboo shoots extract, as indicated, exhibited abortifacient activity in female rats and was reported to cause teratogenic effect.[18]

The data in Table 2 seems to confirm the detrimental effects of nut grass extract on fetal development of mice. All parameters associated to tibial epiphyseal cartilage growth and development showed a pattern of change that fits the principles of bone formation and differentiation. The smaller the thickness of the reserve, proliferation, and maturation zones and followed by increasing thickness of calcification zone as the extract concentration increases clearly depicted that the ethanol extract of rumput teki negatively affects cartilage cells.

Proliferation of chondrocytes is highly dependent on the sex steroid hormone, mainly estrogen, either in pre- or postnatal developmental period.[19] As has revealed, chondrocytes endogenously produce estrogen that stimulates chondrocyte proliferation and protects from spontaneous apoptosis and promotes longitudinal growth of epiphyseal plate.[20] *Cyperus rotundus* is rich of flavonoid derivatives. These phytochemicals act as both agonists and antagonists of the human estrogen receptors.[21] Thus, it is logical if the fetuses exposed to the anti estrogenic extract of nut grass suffer from bone retardment.

Due to limited scientific studies on biological acivities of nut grass phytochemicals, especially in relating to teratogenic and congenital effects, it is hard to give indepth explanation regarding causal-effect relationship between variables of this study. However, based on the data obtained it can be concluded that methanolic plant extract of *Cyperus rotundus* L. causing fetal skeleton retardation in mice.

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