ISBN : 978-602-0860-10-7

CONFERENCE PROCEEDINGS 2016

August 23-24, 2016
Bandar Lampung, Lampung, Indonesia

UISFS
THE USR INTERNATIONAL SEMINAR ON FOOD SECURITY

"Improving Food Security : The Challenges for Enhancing Resilience to Climate Change"

Volume II

The University of Lampung

Indonesian SEARCA Fellow Association

Southeast Asian Regional Center for Graduate Study and Research in Agriculture

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USR INTERNATIONAL SEMINAR ON FOOD SECURITY

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Emersia Hotel and Resort, Bandar Lampung, Lampung, Indonesia

23 – 24 August 2016
Volume II

Organized by

Research and Community Service Institution
The University of Lampung – Republic of Indonesia,
Indonesian SEARCA Fellow Association,
SEARCA

2016

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FETAL SKELETON DEVELOPMENT OF MICE (*Mus musculus* L) THREATED WITH NUTGRASS (*Cyperus rotundus*) EXTRACT

NUNING NURCAHYANI, YAN WIRASTI, JAMSARI, DJONG HON TJONG AND HENDRI BUSMAN

ABSTRACT

Research on medical herbs have been done since the information related with their safetinesscan not be guaranted. Results of this research are used to reduce side effect of medical herbs without clinical analysis of its benefits for human. The nuttgrass*(Cyperus rotundus* L.) grows wildly in many places, can be used to treat high blood pressure, breast tumors, candida, colds, flu, and helps treat convulsions, moodiness and depression, premenstrual syndrome (PMS) and the pain and cramps associated with PMS, menopause, and antiestrogenic effects. This herb contains a volatile oil with b-pinene, cyperene, a-cyperone b-cyperone and a-cyperol as its main ingredients. It also contains alkaloids, flavonoids, triterpenes, etc. The aims of this research were to determine the effects of nuttgrass (*Cyperus rotundus* L.) given orally to pregnant mice (*Mus musculus* L) during organogenesis fase to skeleton development anatomically and histologically, by using structure of epifisialis cartilago as indicator. Research has been conducted using Complete Randomized Design. Twenty pregnant mices were devide into 4 groups: A (control with 0,4 ml aquabides), B (4,5 mg/40grBB nuttgrass extract in 0,4 ml aquabides), C(45 mg/40grBB nuttgrass extract in 0,4 ml aquabides), D (135 mg/40grBB nuttgrass extract in 0,4 ml aquabides). Data were analized with Analysis of Variance (ANOVA) to find the differences of each treatment, if there were significant differences, Least Significant Differences 5% will be done. The result showed, that nuttgrass extract given orally to pregnant mices did not cause malformations to fetal mice. It reduces fetal body weight and length. In addition, it gave effect on changing the histological structure of fetal epifisialis tibia, by reducing the thickness of proliferation zone, maturation zone, cartilage zone during mineralization processes.

Key Words: nuttgrass (*Cyperus rotundus* L.), mice (*Mus musculus* L.), epifisialis cartilage, contraception, fetus

INTRODUCTION

Nutgrass (*Cyperus rotundus* L) has been known as one of medicinal herbs and used widely by people in the world. However, until now the research has been conducted on nutgrass related to its function as medicinal herbs or traditional medicine is very few.

Recently, the nutgrass is used to treat poor appetite, diarrhea, dysentery, fevers, parasites, gastritis, indigestion, and sluggish liver (Sa'roni and Wahjoedi, 2002). Beside that, nutgrass can also be used to treat high blood pressure, vomiting blood, breast tumors, candida, colds, flu, and colic. It helps treat convulsions, moodiness and depression, premenstrual syndrome (PMS) and the pain and cramps associated with PMS, menopause. This herb contains a volatile oil with b-pinene, cyperene, a-cyperone b-cyperone and a-cyperol as its main ingredients. It also contains alkaloids, flavonoids, triterpenes. Its volatile oil has a mild estrogen-like action. The water solutions of its total alkaloid, glycosides, flavonoids and phenolic compounds have cardiotonic and hypotensive effects. Its extract can inhibit some fungi, potential antifertility plants, used in the treatment of cervical cancer (Anonim b, 2007). Based on the effect of nuttgrass to reproduction system, it may disturb the fertilization and inhibit the implantation process, inhibit intrauterine mortality, and fetuses growth retardation (Winarno and Sundari, 1997; Okfiyanti, 2008; Pasaribu, 2008).

The aims of this research were to determinethe effects of nutgrass (*Cyperus rotundus* L.) given orally to pregnant mice (*Mus musculus* L) during organogenesis period on growth and development of embryos and skeletal fetuses, anatomically and histologically,using structure of cartilage epifisialis as indicator.

METHODS

Research was conducted at Zoology Laboratory University of Lampung on April-November 2015. Nutgrass extract was made from *Cyperus rotundus* L of Lampung. Extraction of nutgrass was used for this research and it was gained by drying the nutgrass

, made to powder, diluted in methanol, and put into rotary evaporator in 35^{o} C with 60 rpm for about 1 hour. Its extract was ready to used.

Method used in this research was Complete Randomized Design. Twenty pregnant mices were devided into 4 groups and treated by gavage with A (control with 0,4 ml aquabides), B (4,5 mg/40gBW nuttgrass extract in 0,4 ml aquabides), C (45 mg/40gBW nuttgrass extract in 0,4 ml aquabides), D (135 mg/40gBW nuttgrass extract in 0,4 ml aquabides). The treatment

were given from gestation day 6 to 17. On day 18 of pregnancy , fetuses were removed by caecarean section.

Parameters observed were skeleton development on fetal mices anatomically and histologically, by using structure of epifisialis cartilago as indicator. To observe development of fetal skeleton, fetuses were processed using Allizarin red S method. Observation of histological structure of the tibial group plate preparation by paraffin method (Hematoxylin-Eosin staining)(Mc Manus dan Mowry, 1960).

The thickness of tibial epifisialis cartilage were measured, such as chondrocyt zone, proliferation zone, maturation zone, calcification of cartilage zone mineralized.

Data were analized with Analysis of Variance (ANOVA) to find the differences of each treatment, followed by Least Significant Differences at 5%.

RESULTS AND DISCUSSIONS

A. Effects of nutgrass (*Cyperus rotundus* L.)extract on Morphology of fetal mice (*Mus musculus L*)

Based on result of the effect of nutgrass (*Cyperus rotundus* L.)extract to fetal mice (*Mus musculus* L), there were no morphological abnormalities. It was found that the fetuses body weight and length decreased in the treatment group compare to those of control.

Body weight and length of fetal mice

In this research, fetal mice body weight and length were measured and analized since congenital abnormalities may cause body weight and length decreasing. It indicated that the growth and development process has been corrupt because of external and internal factors, both on animal and human. Decreasing of fetal body weight and length were one of some parameters to examine the effect of teratogenic agents. It was sensitif parameters (Wilson, 1973).

Results of observation on the body weight and length of fetuses was shown at Table 1.

Table 1. The number, body weight, and length average of fetal mice from the pregnant mice treated with nutgrass extract (*Cyperus rotundus* L.) during organogenesis

Group	Dosage(mg/40g body weight/day)	number of mice	number of fetuses	body weight(g)	length (mm)
A	0	5	54	1,65a	16,22a
B	4,5	5	46	1,31a	13,47b
C	45	5	38	1,25b	9,13b
D	135	5	22	0,86c	5,71c

Difference alphabetics in the same column showed significant among treatment groups

Statistical analysis with Anova indicated that there were significance differences (P<0,05)between body weight and length of fetuses from control and those of treatment groups. Table 1 showed that the more increase dosage extract of nuttgrass (*Cyperus rotundus* L.) given to pregnant mice, the fetuses body decrease. In these results indicated that the growth and development process was inhibited. According to Wilson (1973); Sa'roni and Wahjoedi (2002), some chemical agents not only cause to death but also caused malformation and growth retardation on fetuses, depend on dosage and time given to the sample animals.

Results showed that fetuses got malformation from different dosage of nutgrass were smaller in body length compare to those of the normal one. Growth retardation indicates the abnormalities of development processes. Some teratogenic agents caused visceral and skeletal abnormalities can be also detected from the form and function, without showing the abnormalities morphologically.

Decreasing in fetal body weight may be relate to the teratogenics effects of nutgrass(*Cyperus rotundus* L.)extract given orally to the pregnant mice (*Mus musculus* L) during organogenesis. In this research, decreasing fetal size has been appeared in the lowest dosage of the nutgrass extract given. The negative effects of nutgrass (*Cyperus rotundus* L.) extract to fetal mice happened because of disturbance in fetal circulation processes. In addition to it, the fetuses did not have specific enzyme for conducting detoksification to break the nutgrass extract . It indicated that nutgrass extract may have embryotoxic effects.

B. Nutgrass (*Cyperus rotundus* L.)extract effect on tibial epifisial cartilage of fetal mice (*Mus musculus L)*

This research in which nutgrass (*Cyperus rotundus* L.) extract was given to pregnant mice caused some abnormalities in fetal development. In this research, number of fetuses, body weight and length of fetuses, as well as tibial epifisialis cartilage has been used to determine the fetuses development.

Chondrocyt zone

Results of examination on chondrocyt layer of control and treatment group. There were hyalin cartilage which contained roundshape chondrocyt called ovoid. This chondrocyt was in inactive condition. The results on the effect of nutgrass extract on chondrocyt cartilage can be seen in Table 2.

Table2. The thickness average of cartilage chondrocyt zone of tibial epifisialisof fetal mice from the pregnant mice treated with nuttgrass extract (*Cyperus rotundus* L.) during organogenesis

Group	Dosage(mg/40g body weight/day)	thickness of chondrocyt zone (μm)	total tibial length (μm)
A	0	285,22 a	5448 a
B	4,5	266,23 a	5332 a
C	45	246,18 a	5088 b
D	135	252,43 a	5004 c

Difference superscript alphabet in the same column showed statistical difference (p<0,05)

Statistical analysis using ANOVA showed there were no significance differences (p > 0,05) between the thickness of chondrocyt zone of control with the treatment groups.

Proliferation Zone

Observation on proliferation zone showed the chondrocyt cells were doing mitotic division and become a lot of cells closed each other, and formed cells population along the fetal skeletal body axis. Data was available at Table 3.

Table. 3. The thickness average of cartilage proliferation zone of tibial epifisialisof fetal mice from the pregnant mice treated with nuttgrass extract (*Cyperus rotundus* L.) during organogenesis

Group	Dosage(mg/40g body weight/day)	thickness of proliferation zone (μm)	total tibial length (μm)
A	0	588,42 a	5880 a
B	4,5	524,40a	5680 a
C	45	446,22 b	5133 b
D	135	422,12 b	5006 c

Difference superscript alphabet in the same column showed statistical difference (p<0,05)

Statistical analysis with ANOVA showed significance differencess among the treatment group. The next analysis showed there were significance differencess (p < 0,05) between treatment group 4 with 2 and 1, between treatment group 3 with treatment group 2 and 1. However, between treatment group 4 and 3, alsotreatment group 2 and 1, there were no significance differencess.

Maturation zone

Observation on maturation zone indicated that there were hyperthrope chondrocyt and some vacuolas in the cells. Result can be seen in Table 4.

Table 4. The thickness average of maturation zone intibial epifisialiscartilage of fetal mice From the pregnant mice treated with nutgrass extract (*Cyperus rotundus* L.) during organogenesis

Group	Dosage (mg/kg body weight/day)	thickness of maturation zone (μm)	tibial length (μm)
A	0	180,22 a	5880 a
B	4,5	167,90 a	5240 a
C	45	124,12 b	5124 b
D	135	91,88 c	5004 c

Difference superscript alphabet in the same column showed statistical difference (p<0,05)

Statistical analysis with ANOVA showed significance thickness differences (p < 0,05) between the maturation zone in control with the treatment group.

Calcification of the cartilage zone

Microscopis observation of the cartilage zone calcification in control and reatment group showed the appearance of some hyperthrope and dead chondrocyt layer.This zone was thin and close to diafisis. Cartilage matrix in this zone began to get calcification by forming hydroxiapatit so there was thiny layer surrounding the hyperthrope and dead chondrocyt

The results can be seen at Table 5.

Statistical analysis with ANOVA showed significance thickness differences (p < 0,05) between the calcification cartilage zone in control with the treatment group.

Table 5. The thickness average of cartilage zone calcification intibial epifisialisof fetal mice from the pregnant mice treated with nutgrass extract (*Cyperus rotundus* L.) during organogenesis

Group	Dosage (mg/kgbody weight/day)	thickness of calcification zone(μm)	total of tibial length(μm)
A	0	281,15 a	7880 a
B	4,5	273,55 a	7442 a
C	45	231,42 b	7124 b
D	135	214,11 c	7004 c

Different superscript alphabeth in the same column showed statistical difference (p<0,05)

Based on results, it seems that nutgrass extract may inhibit calcification endochondralis of tibial epifisialis cartilage on fetal mice. It was shown from the proliferation zone, maturation zone, cartilage zone mineralized become more thick. Data showed that the thickness of chondrocyt zone was the same between control and treated

group after treatment with nutgrass extracts. It seems that nutgrass extracts affected directly to target cells which active doing mitotic division. Overall, nutgrass extract may give intervention on skeleton before DNA replication finish completely at cynthesis phase. Beside that, it also inhibited phosphodiesterase enzyme activity.

From microscopic observation on the thickness of proliferation zone, there were decreasing of proliferation thickness related to increasing of nutgrass extract dosage. At the highest dosage 135 mg/kg body weight, the structure of chondrocyt layer at proliferation zone was decrease in its thickness . In this layer the cells was unorganized and inconsistent line. The cells was not closed each other and did not formed straight line. Caused the nutgrass extract seems interact with mitotic processes by inhibiting the cells mitotic and finally inhibiting cells proliferation of the chondrocyt layer. According to Ham and Cormack (1979); Aulia, Sugianto, and Aida (2002), this zone undergo mitotic actively and playing a role as location to form new cells to substitute the damage hyperthrophy cells. At diafisis part, cells also started degenerating processes. Since nutgrass extract can inhibit cells proliferation at proliferation zone, it may affect to the next zone at tibial epifisialis cartilage. Finally, it may inhibit tibia calcification processes, so the total length of tibia decrease compare to the control. Sagi (1996) said that embryonic cells is very sensitive of the environmental effects so the mitotic division happened continuously. At mitotic phase, no membrane at the nucleus,while chromosomes spread, so nutgrass extracts will easily interact in it causing cells and tissue damage. Finally it causes malformation of growth and development. Observation on histological structure on chondrocyt layer at maturation zoneindicated decrease of the thickness of maturation zone related to increase on nutgrass extract dosage. The histological change may happen because nutgrass extract inhibit mitotic processes of chondrocyt at proliferation zone. It cause the zone below them become more thicker. The hyperthrope chondrocyt tissuewill substitute by the new chondrocyt from proliferation zone.

The result of this research showed that nutgrass extract given orally to pregnant mices did not cause malformations to fetal mice. However, it reduces fetal body weight and length. In addition, it gave effect on changing the histological structure of fetal epifisialis tibia, by reducing the thickness of proliferation zone, maturation zone, cartilage zone during mineralization processes

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