



Research Article

Inhibitory Effects of Tuber Extract of Nut Grass (*Cyperus Rotundus L*) on the Growth of Rat Fetuses

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Article History

Received: 24.09.2019

Accepted: 03.10.2019

Published: 23.10.2019

Abstract: In this paper we studied the effect of tuber extract of nutgrass (*Cyperus rotundus L*) on fetal weight and length of white rat (*Rattus norvegicus*) Sprague Dawley. Pregnant female rats (n=24) are grouped into four consisted of six rats each. Group-1 (control) only received distilled water. Group-2, 3 and 4 consecutively received tuber extract of nut grass at the dose of 22.5, 45, and 90 mg/kg body weight. Extract was given orally using gavage needle on day 6th for 13 days until day 18th of pregnancy. On day 18th of pregnancy, female rats were laparotomized under deep anaesthesia. All fetuses were taken to measure their body weight and length. The results showed that both fetal weight and fetal length of the rats were significantly decreasing with increasing doses of the extract. In conclusion, tuber extract of nut grass (*Cyperus rotundus L*) has inhibitory effects against fetal growth of rats during pregnancy.

Keywords: fetal growth, nutgrass, *Cyperus rotundus*, teratogenic.

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INTRODUCTION

Pregnancy defined as the process of embryo formation and development within the female body. The process includes the influx and migration of spermatozoa, conception, growth of embryo, implantation, formation and growth of the placenta, and growth and development of the fetus (Manuaba *et al.*, 2010).

During pregnancy, fetal growth and development as well as the health status of the mother are greatly affected by maternal dietary intake (Marangoni *et al.*, 2016). The use of drugs during pregnancy, for instance, can seriously affect fetal growth and development. In many cases, drugs exposure during the critical period of skeletal development causes growth retardation (a teratogenic disorder) on limb (Katzung, 2014).

Dwarfism, characterized by the lower body weight and length compared to normal, is a common teratogenic disorder occurs in foetuses exposed to teratogenic agents during organogenesis (Pace, 2012). Teratogenic agents have many sources in nature, including those from plants that are commonly seen as safe and commonly used as traditional medicine (Costa *et al.*, 2012).

One of the plants that is widely used as traditional medicine by people in many Asian countries but is thought to contain teratogenic phytochemicals is nutgrass (*Cyperus rotundus L.*) (Parson & Cuthbertson, 2009).

The nutgrass tuber is reported to be efficacious as antifungal, anti-inflammatory, antidiabetic, antidiarrheal, cytoprotective, antimutagenic, antimicrobial, antibacterial, antioxidant, cytotoxic, analgesic and antipyretic (Lawal & Oyedeji, 2009). Phytochemical studies showed that the nutgrass tuber contains alkaloids, flavonoids, tannins, starch, glycosides, furochrome, saponins and sesquiterpenoids (Lawal & Oyedeji, 2009; Busman *et al.*, 2018).

Despite its medicinal efficacy, nut grass herbs are also reported to cause side effects. Long term use of this grass herb cause fertilization impairment in mice (Nurcahyani, 2016; Rabbani *et al.*, 2014).

This grass tuber is rich in flavonoids. This phytochemical is known to act like antiestrogenic receptors, if fetuses exposed to this compound bone retardation may occur, resulted in imperfect growth of the fetus (Collins-Burrow *et al.*, 2012; Shiezhadeh, 2013).

Coumarin is another phytochemical contained in the nutgrass tuber. This compound is known to have a hepatotoxic effect on human and animals when given continuously. Consumption of coumarin is known to increase abnormalities of the liver functions (Abraham *et al.*, 2010; Iwata *et al.*, 2016; Morimoto *et al.*, 1999).

Quercetin is the next other chemical known to have anti-inflammatory and antiviral activities. This compound also cause toxic effects and interfere with the physiological process of the body and has a biphasic effect on the development of cancer

cells (Li *et al.*, 2016; Selin-Rani *et al.*, 2016; Van der Woude, 2006).

Tuber extract of nut grass also known to contain p-hydroxybenzoic acid, β -sitosterol, β -D-glucopyranoside are also found in the extracts of the nut grass. These compounds are reported to be teratogenic and cause abortion in female mice (Saraswathy & Vidhya, 2013).

Given the nutgrass (*Cyperus rotundus* L.) extract contains substances that are suspected of teratogenic, we have investigated its inhibitory effects on the growth of fetuses in laboratory rats (*Rattus norvegicus*).

MATERIALS AND METHODS

Plant Samples And Extraction

Plant samples of nutgrass (*Cyperus rotundus* L.) collected from the District of Tanggamus, Lampung. Fresh tubers of the grass were washed and sun dried. Then the dried tubers were ground to be a powder form. By using Soxhlet apparatus the powder was extracted with alcohol 96% (Reg No. GBL 8109032909A1, Batch No. C910 202) as the solvent. Extraction carried out at the temperature of 35°C and the rotation of 60 rpm for 60 minutes.

EXPERIMENTAL ANIMALS

Female rats (*Rattus norvegicus*) Sprague Dawley aged 12-16 weeks and weight of 200-250 g, were obtained from Lampung Veterinary Center, Indonesia. All animals were housed at the temperature of 25°C and 12:12-hour light-dark cycle with free access to water and pellets *ad libitum*. All animal care and treatment procedures were approved by the Ethics Committee, Faculty of Medicine, University of Lampung, Indonesia.

EXPERIMENTAL DESIGN AND TREATMENT

One the test rats (n=24) got pregnant, the animals grouped into four consisted of six rats each. Group-1 (control) only received distilled water. Group-2, 3 and 4 consecutively received tuber extract of nut grass at the dose of 22.5, 45, and 90 mg/kg body weight. Extract was given orally using gavage needle on day 6th for 13 days until day 18th of pregnancy.

FETAL SIZE MEASUREMENT

On day 18th of pregnancy, female rats were laparotomized under deep anaesthesia. All fetuses were removed and washed using distilled water. After being dried with using tissue paper, the fetal length and weight were measured using calliper and Mettler Toledo Analytic Balance, respectively.

STATISTICAL ANALYSIS

The data are presented as Mean \pm SD and analyzed statistically using a one-way ANOVA. Least Significance Difference (LSD) test was used as the post hoc test.

RESULTS AND DISCUSSION

The effects of tuber extract of nut grass (*Cyperus rotundus* L.) on body weight of rat fetuses are presented in Figure 1. Analysis of variance of the data in Figure 1 resulted in F value of 9.7747 (lower 3.0725 – upper 4.8740).

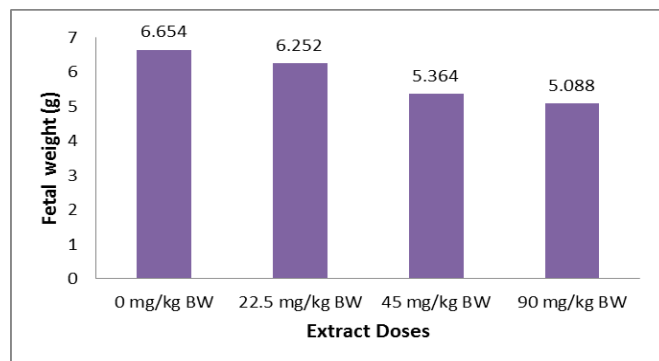


Fig.1 Effects of tuber extract of nut grass (*Cyperus rotundus* L.) on the fetal weight of rat

Results of post hoc test using LSD (Least Significant Difference) against the mean weight values of the fetuses are shown in Table 1 below.

Table 1 LSD test results against mean difference of fetal weight of rats given tuber extract of nut grass

Extract doses	Mean	Difference			Critical difference (lower upper)
0 mg/kg	6.654				0.6919 -0.9568
22.5mg/kg	6.252	0.402			
45 mg/kg	5.364	1.29**	0.888*		
90 mg/kg	5.088	1.566**	1.164**	0.276	

*) significantly differ at $\alpha = .05$; **) significantly differ at $\alpha = .01$

Figure 2 shows the fetal length of rat given tuber extract of nut grass (*Cyperus rotundus* L.). F value of the ANOVA against the data resulted in F=9.3929 (lower 3.0725 – upper 4.8740).



Fig.2 Effects of tuber extract of nut grass (*Cyperus rotundus* L.) on the fetal length of rat

LSD test against the difference of mean values of the fetal length shown in Figure 2 are presented in Table 2 below.

Table 2 LSD test results against mean difference of fetal length of rats given tuber extract of nut grass

Extract doses	Mean	Difference			Critical difference (lower - upper)
0 mg/kg	42.67				2.5303 - 3.4992
22.5 mg/kg	41.746	0.924			
45 mg/kg	38.778	3.892**	2.968*		
90 mg/kg	37.27	5.4**	4.476**	1.508	

*) significantly differ at $\alpha = .05$; **) significantly differ at $\alpha = .01$

The research findings indicate clearly that tuber extract of nut grass (*Cyperus rotundus* L.) has inhibitory effects on the growth of rat foetuses. Phytochemical study revealed that nut grass contains quercetin and flavonoids in their tubers. Quercetin is known to have a toxicity effect in cells. This compound is known to react in inhibiting tyrosine kinases. Receptor tyrosine kinases are known to be receptors of growth factor (Nidugala *et al.*, 2016; Maruyama, 2014; Muha & Muller, 2013).

Flavonoids in the roots of the nutgrass are also known to have cytotoxic effects. Because flavonoids are also compounds that can be used as anticancer, flavonoids also have very strong side effects. This is because flavonoids work by apoptosis and suppress cells with high proliferation, such as the bone marrow (Borek, 2001; Lin *et al.*, 2009; Susianti, 2015).

Anticancer compounds such as flavonoids and saponins are thought to inhibit osteoprogenitor cell division so that the formation of osteoblasts is disrupted and consequently the absorption of calcium by these cells becomes inhibited (Astika, 2000).

In a previous study, the effect of the puzzle grass tuber extract (*Cyperus rotundus* L.) in pregnant mice resulted in malformations in the fetal body parts of the mouse (*Mus musculus*) accompanied by a decrease in the thickness of the chondrocyte reserve zone, proliferation zone and bone maturation zone fetus mice (*Mus Musculus*). Meanwhile, the thickness of the zone is an important parameter of bone formation or body frame development (Nurchayani *et al.*, 2016; Kini & Nandeesh, 2012).

During pregnancy, there are several factors that can affect the baby's weight, namely maternal factors, placental factors, fetal factors and environmental factors. One of the maternal factors that influence baby's weight is the presence of teratogens during pregnancy (Roland *et al.*, 2014; WHO, 2006)

Decreasing fetal weight is the mildest form of exposure to a compound that is teratogenic. Weight loss is an indicator of the occurrence of growth barriers due to interference with the processes that underlie growth (cell division, metabolism and synthesis) (Capra *et al.*, 2013; Emily *et al.*, 2015; Wilson *et al.*, 1990).

In humans, estrogen has an important role in fat metabolism. Estrogen is known to inhibit lipolysis in fat metabolism. Whereas, *Cyperus rotundus* is a plant that rich in flavonoids. This phytochemical is an agonist and antagonist in the estrogen receptor (antiestrogenic). Therefore, if the fetus is exposed to antiestrogenic compounds from the grass extract, there will be bone retardation and weight loss (Collins-Burrow, 2012; Luglio, 2014; Shiezadeh *et al.*, 2013; Busman *et al.*, 2016).

CONCLUSION

Because of both body weight and body length of rat foetuses are significantly lower by the higher doses of extract, it can be concluded that tuber extract of nut grass (*Cyperus rotundus* L.) has inhibitory effects on the growth of rat foetuses during pregnancy.

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