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RETINOIC ACID EFFECTS AND FOLLOWING EFFECTS THROUGH CHITOSAN TOWARDS PREGNANCY MICE

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ABSTRACT

Vitamin A is an essential nutrient for reproduction and growth. Retinoic acid is one among lipid soluble form of vitamin A. The function of retinoic acid is regulator and transcription of gene. It takes time to excrete from the body, so that an excessive dosage will cause teratogenic effects especially in the skeletal growth. In first trimester of pregnant mothers, in particularly, it may result abnormalities in mild and severe congenital and even fetal death. However, chitosan (β -1,4-2 amine-dioxy-D-glucosamine) could be reduce its effect through repairing chondrocyte tissue and bone damaged by stimulate the performing of extra cellular matrix protein of osteoblast and chondrocyte. The purpose of the research was to observed oral route effect of retinoic acid and reducing its effect through chitosan on the pregnant mice. This study was divide into two consecutive researches consist of 16 pregnant mice were induced by oral administered retinoic acid in a dose 60 mg/kg body weight and 28 pregnant mice induced chitosan of several dose i.e. 15, 30, and 45 mg/kg body weight, respectively, oral administered following retinoic acid, both for congenital skeletal observation. Result conduct of the research was excessive retinoic acid which it induced on pregnancy mice will decreased number of delivery life fetus, on the contrary death and resorption fetus was increased. Meanwhile, the delay ossification of mice fetus with it chitosan treatment has no significantly different for congenital fetal malformation

Key words: retinoic acid, chitosan, pregnant mice, congenital fetal malformation

INTRODUCTION

The critical time on the organogenetic phase is an important period for normal development of the organ and organ system. Furthermore, the developing embryo was exposed into toxic agents caused impairing the development. Retinoic acid as lipid soluble form of vitamin A, usually was used as the treatment on dermatologic disease. Unfortunately, retinoic acid was one among teratogenic agents. Instead of wider used of vitamin A as essential nutrient for reproduction and growth, retinoic acid has slowly metabolic characteristic. Remain metabolite was accumulated and collected in the tissue. Accumulated effect of remain metabolite may caused body intoxication. On the other hand, increasing vitamin A consumption to the excessive dose in more than ten times Recommended Dietary Allowance (RDA) as a trigger of intoxication of the body will caused morphology malformation, shortening of cartilage, decreasing total amount of the limb and ossification disorder. Retinoic acid as irreversible lipophilic compound tends to enter fetal circulation through diffuse via placenta barrier to produce negative effect into the fetal development such as pregnancy failure, abnormalities organ from mild to severe congenital abnormalities and the final defect was death.

Vitamin A is an essential nutrient for reproduction and growth. Retinoic acid is one among lipid soluble form of vitamin A. The function of retinoic acid is regulator and transcription of gene. It takes time to

excrete from the body, so that an excessive dosage will cause teratogenic effects especially in the skeletal growth. In first trimester of pregnant mothers, in particularly, it may result abnormalities in mild and severe congenital and even fetal death. However, chitosan (β -1,4-2 amine-dioxy-D-glucosamine) could be reduce its effect through repairing chondrocyte tissue and bone damaged by stimulate the performing of extra cellular matrix protein of osteoblast and chondrocyte. The purpose of the research was to observed oral route effect of retinoic acid and reducing its effect through chitosan on the pregnant mice.

METHODS

This study was divide into two consecutive researches consist of 16 pregnant mice were induced by oral administered retinoic acid in a dose 60 mg/Kg body weight and 28 pregnant mice induced chitosan of several dose i.e. 15, 30, and 45 mg/Kg body weight, respectively, oral administered following retinoic acid, both for congenital skeletal observation.

This was a laboratory experimental study using randomized posttest only control group design. Samples were female BALB/C strain mice (*Mus musculus*) divide into two groups each consist of 16 pregnant day 10th animals. Treatment group were induced retinoic acid dosage 60 mg/Kg body weight orally administered, and control group were induced by 1 cc sesame oil each sample. Both groups were induced single dose in the pregnant day 10th

of mice. All experimental animals were sacrificed on the pregnant day 18th. The analysis unit was placenta implantation, resorption, living and dead delivery fetus, fetal body weight, and fetal external abnormalities.

RESULT AND DISCUSSION

On the first research, result conduct of the research was excessive retinoic acid which it induced on pregnancy mice will decreased number of delivery life fetus, on the contrary death and resorption fetus was increased. Meanwhile, the delay ossification of mice fetus with it chitosan treatment has no significantly different for congenital fetal malformation

The result of descriptive analysis of the both treatment and control groups were revealed that mean fetal body weight in control group has (mean $\pm$ standard deviation) 1.00 ± 0.24 and placenta implantation 8.00 ± 1.56 , whether in the treatment group has mean fetal body weight 0.96 ± 0.23 and placenta implantation 7.23 ± 2.24 , means no significantly difference ($P > 0.05$). The result of normality test indicated that all samples in the field of reproductive outcome mainly the number of placenta implantation and fetal body weight had normal distribution ($P > 0.05$), whether living delivery, dead delivery and resorpted embryo showed skewed distribution ($P < 0.05$). The analysis of homogeneity test indicated that number of placenta implantation and fetal body weight had homogenous variance ($P > 0.05$).

The result of independent two sample t-test showed that the mean of placenta implantation had significantly difference on 0.366 ($P > 0.05$), and the mean of fetal bodyweight had significantly difference on 0.711 ($P > 0.05$). Both placenta implantation and fetal bodyweight of the test indicated that induced retinoic acid oral administration single dose of 60 mg/Kg body weight provided no significantly

difference. Furthermore, induced retinoic acid oral administration single dose of 60 mg/Kg body weight to the both living and dead delivery fetus has significantly difference on value 0.008 ($P < 0.05$) through Mann-Whitney test.

On the treatment group, external congenital abnormalities of the fetus mice could happen on the 68 living fetuses (58.2% population of living delivery fetus). On the contrary, 100% of whole population of living delivery fetus (80) was normally development on the control group. According to the living delivery fetus mice, comparison between treatment group and control group had significantly difference. External congenital abnormalities was observed mainly palatoschysis, pocomelia, kinky tail, ectrodactily, micromelia, agenesis tail, anotia, asymmetrical auricle, simpodia, sindactyli, talipes, teratoma, short tail and many more, respectively (Table 1).

Observation on the living delivery fetus induced retinoic acid oral administration single dose of 60 mg/Kg body weight, showed that teratogenous chemical exposure may impede fetal growth and differentiation of the external skeletal causing fetal abnormalities. The severity of abnormalities was depending on the teratogenic characteristics and embryonic growth stage during exposure. When exposure was occurred at early stage of organogenesis, the path may traces back into the growth organ or extremities.

The main purpose of the second research that treated by chitosan following induced retinoic acid oral administration single dose of 60 mg/Kg body weight will decrease fetal malformation of the skeleton and stimulate delay ossification. Skeleton observation through Alizarin Red S bone staining into delivered fetal mice for both, life or dead. Morphology malformation of mice fetuses was analyzed by Wilcoxon Signed Rank Test, and the delay ossification was analyzed by one way analysis of variance follow by Least Significantly Difference to looking for different group.

Table 1. Total abnormality morphology of mice fetuses

Groups	Total Pregnant Mice	Abnormality Morphology (%)								
		Palato Schysis	Poco-melia	Kinky Tail	Ectro Dactily	Micro Melia	Extra Budding	Syndac-tily	Agenesis Tail	Others
Control	10	0	0	0	0	0	0	0	0	0
Treatment	13	79,4	60,1	45,6	32,4	22,1	22,1	20,6	17,6	47

Source: Puspitadewi, T.R., Darmanto W., and Poernomo B. 2008.

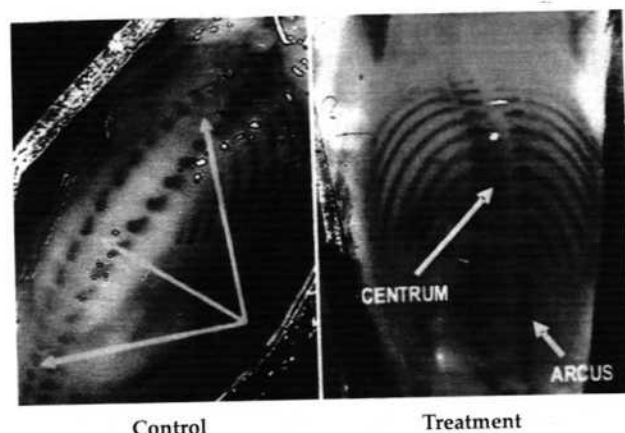


Figure 1. Vertebrae column on delivery fetal mice

Centrum will developed on the treatment group, instead none on the control Source: Prijatni, I., Darmanto W., and Poernomo B. 2008.

The result of the second research shows at the three different chitosan groups are not significantly

difference on the development malformation morphology. Therefore, observation for the delay ossification founded significantly difference ($P < 0.05$), especially on the bones like sacrum, bone part caudal, phalanges part distal of both, forelimb and hind limb.

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