The Effect of Monosodium Glutamate Administration on Estradiol Levels on Pregnant Wistar Rats

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ABSTRACT
The purpose of this study was to see the influence of the MSG administratsion to the estrogens levels, progesterone levels, and the quantity of infant on pregnant Wistar rats. The design of this study was experimental research with post test only control group design on 24 samples pregnant albino Wistar rats. The sample was divided into four groups, one control group and three treatment group. The control group was not given the MSG while the treatment group 1, 2 and 3 were given MSG with a dose of 43.2 mg, 86.4 mg, and 172.8 mg for 16 days pregnancy. The levels of estradiol rats were measured using Enzyme Linked Immunosorbent Assay (ELISA) in the Biomedicine Laboratsory Medical Faculty of Andalas University. Data analysis was conducted by using the Kruskal Wallis Test to know the levels of estradiol and then continued with Mann Whitney Test. The results of this study were the MSG have an effect on estradiol levels (p-value < 0.05). In conclusion, the median estradiol levels were higher in the treatment group compared to the control group.

Keywords: *Monosodium Glutamate, Estradiol Levels, Pregnant Wistar Rats.*

1. INTRODUCTION

Monosodium Glutamate (MSG) is a chemical that is often added to food ingredients and is better known as vetsin, micin, or MSG [1]. MSG was discovered in 1908 by Kikunae Ikeda [2]. Monosodium glutamate (MSG) contains 78% glutamate acid, 12% sodium and 10% water has a solubility of 74gr / 100ml water (very soluble in water) [3].

Monosodium Glutamate can enhance the taste of food but some results of MSG study showed that even though MSG is safe for consumption, it also can cause toxicity against certain organs function as it affects the appetite and promote weight loss [4], affect the activities of the motion [3], can damage the arcuate nucleus in the hypothalamus [5], MSG can also cause uterine fibroids in animals, and decreased quantity of animal infant [6]. Organ damage caused by MSG may also occur due to the oxidative stress and apoptosis as well as hypoxia [7], whereas the placenta is one of glutamate receptor which is the manufacturer of the steroid hormone in pregnancy [8].

Estradiol is pregnancy Hormone, and produced by the placenta in large quantities from steroid precursors in the blood, both from the adrenal glands of the fetus and the mother [9]. When pregnancy approaches term, normal pregnancy in humans is in a hyperestrogenic condition, this increase occurs with gestational age [10]. Estrogen hormones function to maintain pregnancy and maintain fetal development, increased hormone estradiol can significantly acceleratse labor or preterm[11].

Glutamate can cause uterine fibroids in the rats by increasing estrogen levels, this is associated with the use of MSG which is Excitotoxicity into cells [12]. Excitotoxins are amino acids such as glutamate, aspartate and cysteine, if given to neurons they will be stimulated and die. Glutamate is absorbed very quickly in the

digestive tract such as proteins containing glutamate acid in food, absorption of glutamate can cause a surge in glutamate levels in blood plasma [13]. Study about effect MSG on pregnant rats still very rare [14]. This research aims to determine the influence of Monosodium Glutamate administratsion on estradiol levels on pregnant Wistar rats.

2. MATERIAL AND METHODS

The type of research is experimental research which aims to determine the effect of MSG on the levels of estradiol Wistar rats [15]. The research design used was post test only control group design, the design used to measure the effect of treatment in the experimental group [16]. The way to do this is to compare the treatment results with the control group after treatment in experimental animals [17,18].

This research was conducted at Animal House Biomedical Laboratsory of the Medical Faculty of Andalas University for experimental animal care and treatment. Examination of estradiol hormone levels was carried out in the Biomedical Laboratsory of the Medical Faculty of Andalas University.

The time of the study was carried out for one year. The population in this study was Wistar rats, the samples used were pregnant rats that had inclusion criteria, rats with female sex mated first with male rats, weighed 200-250 gr. The number of samples is 24 pregnant rats and divided into 4 groups. One groups control and the number of treatment groups was the same, namely 6 individuals per cage.

The Wistar Female were mated by examining the estrus phase where during the estrus phase estrogen levels increase and the blood supply of the vagina and blood supply to the vagina increases, so that the vaginal epithelium has cornification (horned epithelium) with mucus secretions, and the female rats's vagina looks swollen and red [19]. Female Rats are mated with male rats which are placed in the same cage, the next day a vaginal examination is done to determine whether there is a pregnancy or not.

Determination of pregnancy can be detected by the formation of a vaginal plug that closes the vagina of the mouse from the cervix to the vulva, this is due to coagulation of semen that forms a vaginal plug so that the vaginal and vaginal plugs are closed until then the cement clots release and fall [20,21]. Pregnant rats were separatsed from male rats, then randomized and divided into four groups. Rats were placed in cages, each cage contained 6 rats.

The experimental room is controlled at room temperatsure. Food and drinks are given in ad libitum. Giving a dose of Monosodium Glutamate in this study using MSG was weighed and then dissolved in 2 ml of distilled water and administered orally to treated rats using the gavage needle sonde. Giving MSG for rats using a comparison table of body surface area of experimental animals for conversion of human doses with 70 kg to 200 grams of rats body weight is 0.018 [21]. The dosage is given to the control group and the treatment group, each given MSG in the control group, was only given plain water, while the treatment group was treated with a dose of 43.2 mg, 86.4 mg and 172 mg respectively, the treatment was given for 16 days of pregnancy, on the 17th day blood was collected in rats.

Rats blood is taken through retroorbital by inserting a 2 cm capillary tube into the branch of the ophthalmic vein located in the orbital median sac. Then centrifugation at 3000 rpm for 15 minutes. The next step is to examine the level of estradiol hormone measured using the ELISA (Enzyme-Linked Immunosorbent Assay) method kit that is done with Duplo.

Examination of estradiol hormone, rats serum using the ELISA method, was carried out in the Unom FK Biomedical laboratsory. This study has received ethical approval from the Research Ethics Committee at the Medical Faculty of Andalas University. Data on estradiol obtained were analyzed by Kruskal Waliss and Mann Whitney test.

3. RESULT

In this study a normality test for the levels of estradiol was carried out using the Shapiro Wilk test. Statistical analysis was continued with the Kruskal-Wallis test. The following will illustratse the median value of estradiol in the control group and treatment after MSG administratsion

Table 1. Test results for Kruskal-Wallis Estradiol Hormone (pg) Wistar rats pregnant in the Control Group and Treatment

Group	N	Estradiol (pg/ml) Median	(Min-Max)	*p**
Control	6	57,257	(49,140-67,016)	0,003
Groups 1	6	63,605	(52,445-79,172)	
Groups 2	6	154,748	(55,697-190,834)	
Groups 3	6	140,653	(98,745-187,873)	

**p-value* 0,003<0,05

Table 1 shows that estradiol levels in the control and treatment groups had significant differences ($p < 0.05$) where the value of $p < 0.003$. The median level of estradiol hormone in the control group was 57.257 pg/ml, treatment group 1 was 63.605 pg/ml, treatment group 2 was 154.748 pg/ml and group 3 was 140.653 pg/ml. To

see the differences in each group, the Mann-Whitney test was performed as shown in table 2

Table 2. Results of the Mann Whitney test on estradiol levels in pregnant Wistar rats in the Control Group and Treatment Group

Group	Groups	*p**
Control	Groups 1	0,310
	Groups 2	0,015*
	Groups 3	0,002*
Groups 1	Groups 2	0,041*
	Groups 3	0,002*
Groups 2	Groups 3	0,818

Value-p*with of the Mann Whitney test

Based on table 2, the results of the Mann-Whitney test showed that there were significant differences between the control group and treatment group 2 (p = 0.015) and treatment group 3 (*p* = 0.002), there were also significant differences between group 1 and treatment group 2 (*p* = 0.041) and treatment group 3 (p = 0.002.

4. DISCUSSION

Effect of Monosodium Glutamate (MSG) on Estradiol Level in Wistar Rats pregnant This study showed a significant difference in estrogen hormone levels between the control group and the treatment group (p <0.05) where the p-value was 0.003. The median level of estrogen hormone in the control group was 57.257 pg/ml, treatment group 1 was 63.605 pg/ml, treatment group 2 was 154.748 pg/ml and group 3 was 140.653 pg/ml. The Mann-Whitney test results showed there were significant differences between the control group and treatment group 2 (*p* = 0.015<0,05) and treatment group 3 (*p* = 0.002<0,05), there were also significant differences between group 1 and treatment group 2 (*p* = 0.041<0,05) and treatment group 3 (*p* = 0.002<0,05). MSG can increase serum estradiol levels.

An increase in estrogen levels was also found by Obochi et al., 2009 where there was an increase in the estrogen hormone in females after being given MSG [12]. Monosodium Glutamate causes the activation of the aromatase enzyme which catalyzes the conversion of testosterone (DHEA) to estradiol, resulting in an increase in the synthesis of the hormone estradiol. As stated by Kanova and Bicikova 2010, that pregnant women with high aromatase ratses can cause the placenta to secrete more estradiol [9].

Glutamate in the brain is a major excitatory neurotransmitter in the central nervous system which has been shown to play an important role in complex communication networks, which are contained among all cells in the brain16. Glutamate receptors are found in the cortex cerebri, cerebellum, hypothalamus, and hippocampus. A number of researchers have shown that glutamate receptors are also present in various organs referred to in the brain [22].

This study showed that Estradiol hormone in experimental animals given Monosodium Glutamate showed higher estradiol levels in the treatment group compared to the control group. Animal studies conducted by Husarova and Ostanitkova report that, oral MSG administratsion in experimental animals can result in loss of neuronal responses in the hypothalamus [18]. Decreasing hypothalamic responses in response to hormonal feed back can affect the process of induction and inhibition of homon one with other hormones, such as antagonistic hormone work or in conditions that require not to be induced a hormone to balance the physiological process of the body such as in the pregnancy process. During pregnancy, serum estradiol levels are maintained at a level that is not too high to prevent preterem labor or abortion due to contraction of myometrium [9].

The same thing was stated by Zhang and Bhavnani (2006) that the induction of glutamate in experimental animals stimulated an increase in calpain and caspase 3 which could result in an increase in the apoptosis ratsio in cells. The same study also found that brain cells and mitochondrial autosoma cells did not affect estrogen so that estrogen levels increased in serum in experimental animals [20].

5. CONCLUSION

Administratsion of MSG results in an increase in serum levels of estradiol in rats .

RECOMMENDATIONS

This study is limited to looking only at estradiol levels in pregnant Wistar rats. Further research is needed on the impact of giving MSG to reproductive organs, hormones during other pregnancies in Wistar pregnant rats. Further research is needed on the impact of MSG on toxicity caused by MSG in Wistar mouse fetuses.

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