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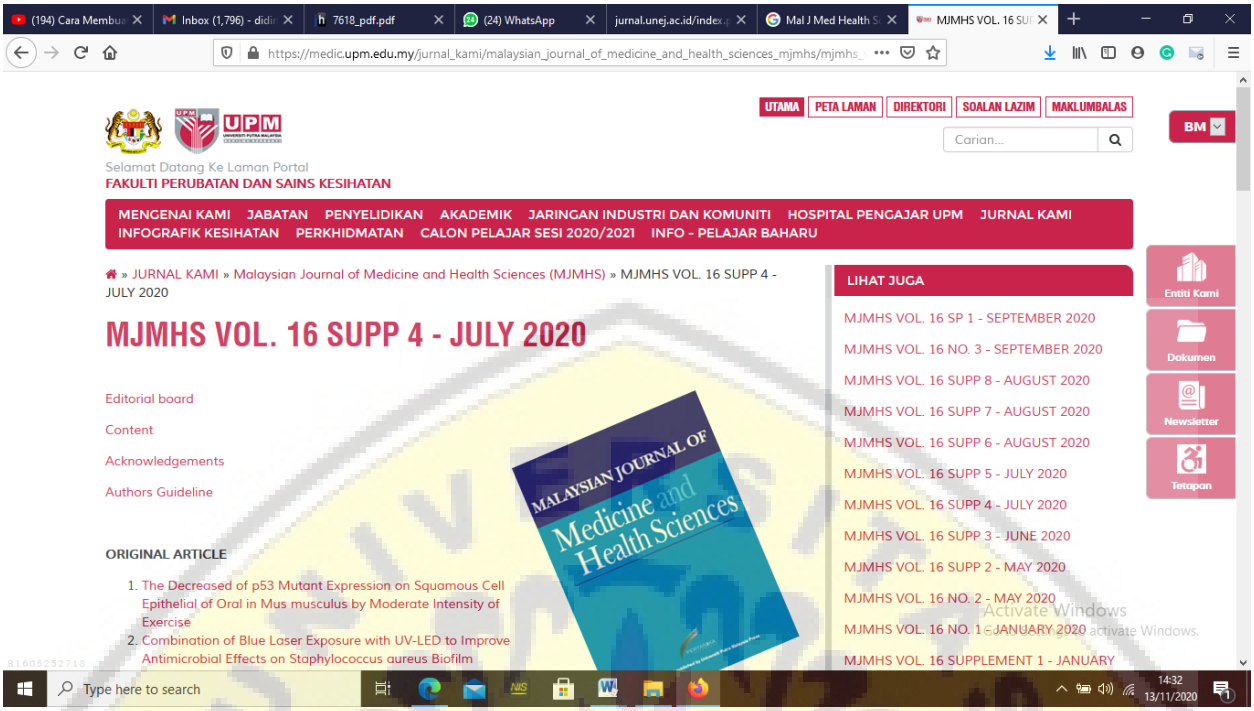

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ORIGINAL ARTICLE

Analysis of Tooth Enamel Structure and Mechanical Properties in Rats Induced Mono Sodium Glutamate (MSG)

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ABSTRACT

Introduction: Formation of enamel begins in intrauterine. The process is prone to disturbances, for example bad nutrition intake. Monosodium Glutamate (MSG) is a food additive that is added to meals to improve the taste. Unmeasured use can result in physical abnormalities, growth, and immune system disruption. This research aim of this study was to analyze MSG consumption on rats during gestation and gestation to lactation on enamel structure and mechanical properties in their first offspring. **Methods:** Three groups of male rats, aged 21 days, which were born from mice induced by MSG during gestation (group 1), during gestation to lactation (group 2) and those without MSG (group 3 as a negative control group). Monosodium Glutamate is given daily at the dose of 1.54 mg/gr (body weight/BW) orally, which starts on the fifth day of gestation until partition (23 days) in the first group and until weaning time (44 days) in the second group. Analysis of the structure and properties of enamel was performed on the lower left first molar using scanning electron microscope (SEM) and Vikers microhardness test. **Results:** The average enamel hardness in MSG induced mice during gestation, gestation and lactation periods, and without MSG was 242.7 Vickers hardness (HV); 238.3 HV and 309.1 HV respectively, while the porosity in the enamel structure is 13,1909%, 18,147% and 7,039%. **Conclusion:** MSG intake in mice during gestation and gestation to lactation results in abnormalities in the structure of the enamel and its mechanical properties in offspring.

Keywords: Monosodium Glutamate (MSG), enamel, enamel structure, physical properties of enamel

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INTRODUCTION

Enamel is the outermost layer of the dental crown that covers the dentine. The inorganic content is the highest of all kind of tissues of human body, which is composed of hydroxyapatite (HAP) ($Ca_{10}(PO_4)_6OH_2$) crystals, trace elements, and other inorganic substances such as carbon (C), magnesium (Mg), sodium (Na), and fluoride (F)) (1). These substances affect the level of enamel hardness which varies on each surface. Hardness is one of the mechanical properties of enamel that supports the function of mastication. These properties are influenced by the structural patterns of enamel rods, chemical components and locations. The maximum hardness of enamel is 3.5 gigapascals (GPa) on the enamel surface and gradually decreases on the deeper surface. In general, the stability of hardness is 2-2.5 GPa, which is in the region of 100-600 mm from the dentinoenamel junction (DEJ) (2). A good enamel structure influences its mechanical properties, for example a perpendicular enamel rod has a lower hardness and modulus of elasticity compared to the parallel ones. In addition, the distribution of chemical composition, and the degree of mineralization influence its mechanical properties. Although the deficiency is only 1% of the hydroxyapatite composition causes a decrease in modulus of elasticity ± 3 Gpa (3).

Abnormalities of enamel structures can be caused by disruption during development because ameloblast cells (enamel-forming cells) are very sensitive. Such disturbances can occur systemically or locally. For example are infections, trauma, nutritional disorders, hormones etc (4). Systemic and local disturbances impair the formation of tissue matrix or mineralization, so that the composition and the enamel thickness is decreased. The conditions cause the teeth to be more sensitive, easily damaged, and also changes in color and shape. This disorder is irreversible (5).

Usually to improve the taste of food using monosodium glutamate (MSG), is a sodium salt of glutamic acid [$C_5H_8NO_4NaH_2O$]. There is no limit set on the amount of usage. Usually monosodium glutamate (MSG) is used only in certain foods, but MSG is often added in Chinese

and Japanese foods such as noodles, meatballs, and sauces. Some research shows that the use of MSG in the diet induces an increase in oxidative stress, leading to a tendency for CHD and arteriosclerosis incidences (6). MSG also known as glutamic acid or L glutamic acid (GLU) is an amino acid that can be synthesized in the body and is found in food sources of protein. Glutamic acid functions as an intermediary metabolism. In addition, the brain acts as an excitatory neurotransmitter. The GLU most consumed by humans comes from a protein diet. Some foods also contain a little in the form of free glutamate and MSG (7). Administration of MSG in Wistar rats during the neonatal period causes destructive lesions of the hypothalamus nucleus which causes obesity syndrome, stunting, and hypogonadism (8). Seo, et.al.(9) reported that chronic administration of MSG induces malfunction of the Hypothalamus-Pituitary-Adrenal (HPA) axis and decreases behavioral activity in adult mice. The hypothalamus is the part of the brain responsible for reproduction of several important hormones and chemicals that control cells and organs. Transcription factors and signaling molecules are the result of hypothalamic secretion that functions for the process of differentiation and proliferation in the early stages of gestation. Identification of some changes and inhibition in its development causes a pattern of dysfunction of specific hormones (10).

During morphogenesis of the teeth, signaling molecules provide interaction between and within the mesenchyme and epithelial tissue layers. These molecules are used repeatedly in the process of development. Enamel knots are the center of signaling which regulate the pattern of tooth development and are associated with folding epithelial sheets. Gene mutations and disorders and network signaling cause abnormalities in tooth formation in both humans and mice (11). This study was to analyze structure and mechanical properties of enamel in rat whose their mothers were induced with MSG during gestation and gestation to lactation.

MATERIALS AND METHODS

Material and equipment

Monosodium Glutamate (Ajinomoto®), feeding tubes 3 ml, scalpel, Microhardness Vickers Tester (Wilson, China), Diamond Disc, Scanning Electron Microscope (SEM) (Inspect F50, USA). Wistar rats (*Rattus norvegicus*).

Animal preparation

Rats were obtained from the Integrated Research and Testing Laboratory of Gadjah Mada University, Yogyakarta. The procedure of this study was in accordance with the guidelines for the ethics committee on the use of experimental animals and was approved by the Health Research Ethics Committee of the University of Jember (674/H.25.1.11/ KE/2015). Female wistar rat, aged 2-3 months, average body weight 200-250 grams, which have been adapted, mated overnight and searched for vaginal plugs via smear. If a plug is found, it is considered as the first day of gestation (12).

Experiment design

Pregnant rats were separated into other cages and divided into 3 groups. The first group, rats were induced with MSG during gestation, the second group was induced MSG during both gestation and lactation, and the third group was not given any MSG. MSG dose was set to 1.54mg/gr BW (body weight) orally. Administration of MSG was done orally using a feeding tube which starts on the fifth day of gestation until partition (23 days) in the first group and until weaning time (44 days) in the second group (13).

Five of 21-days-old rats (offspring) (F1) from each group were sacrificed. The Left mandibular first molars removed, cleaned using saline, and dried to was analyzed the surface of the enamel structure and its mechanical properties. The level of porosity of the enamel surface was analyzed using scanning electron microscope (SEM), while the hardness of the enamel was used to observe the mechanical properties which analyzed using Vickers microhardness test. SEM and microhardness tests were performed on the buccal surface and cups slope of the teeth. SEM assessment was carried out by 5000x magnification, then analyzed with using Image J 1.49V software.

Preparation of the enamel surface structure test using SEM and microhardness test

The mandibula was cleaned of soft tissue then it was cut and separated from others teeth. Molar teeth are cleaned using a toothbrush and clean water then dried (Figure 1).

SEM Test

Prepared samples were coated in gold. Samples were cleaned using a hand blower and then placed in a holder inserted into the specimen chamber. Shooting using SEM with a magnification of 5000x. Shooting of each sample was carried out on the buccal surface and the slope of the tooth cusp (Figure 1).

Microhardness test

Specimens were invested with buccal surfaces facing upwards, on pipes with a diameter of 2 cm and a height of 1 cm that had been filled with resin. The samples were placed under the focus of the adjustable Vickers Microhardness. Compressive loads are chosen to put pressure on the teeth. In this study the compressive load is 50 grams (Figure 1) (14).

Statistic test

The data obtained were analyzed statistically with One Way ANOVA using IBM SPSS 24 statistical software with a confidence level of 95%. One-way ANOVA analysis was carried out after the Levene test was carried out as data for its homogeneity. The Kolmogorov-Smirnov test was performed to determine the normality of the data and the homogeneity of the data was tested using the Levene test. . Post hoc test uses the Tukey test.

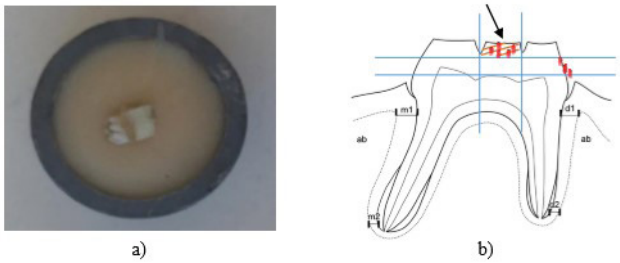

Figure 1: Samples were prepared for SEM and microhardness testing. (a) Samples planted in resin. (b) the location of the area to be tested on SEM and microhardness.

RESULTS

Enamel surface structure analysis

The abnormality of the enamel structure is observed at the level of porosity on its surface. The results of SEM observations were assessed using Image J 1.49V software, which shows the porosity on the surface of the enamel structure in percentage. The porosity was marked by a red color. The average percentage of enamel surface porosity in each group is presented in the diagram (figure 2).

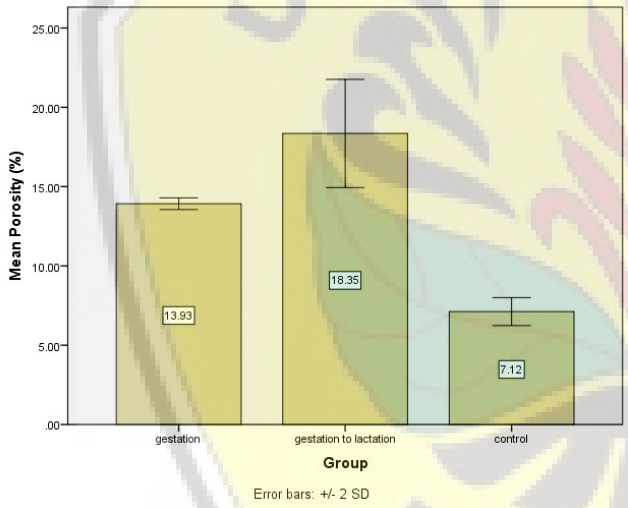

Figure 2: Histogram of the average percentage of enamel porosity

Figure 2 illustrates that the group of mice induced with MSG during gestation to lactation had the highest percentage of porosity than other groups (13.90%). The group without MSG (control group) had the smallest porosity percentage (0.661). All three groups had significant differences. the porosity levels of the three groups were significantly different. These results indicate that MSG induced during gestation and lactation causes changes in the quality of the enamel structure indicated by the occurrence of porosity. An illustration of enamel porosity can be seen in Figure 3.

Mechanical Properties

Enamel hardness test is used to analyze the mechanical properties using Vickers microhardness tester. The mechanical hardness value of rat enamel in the group

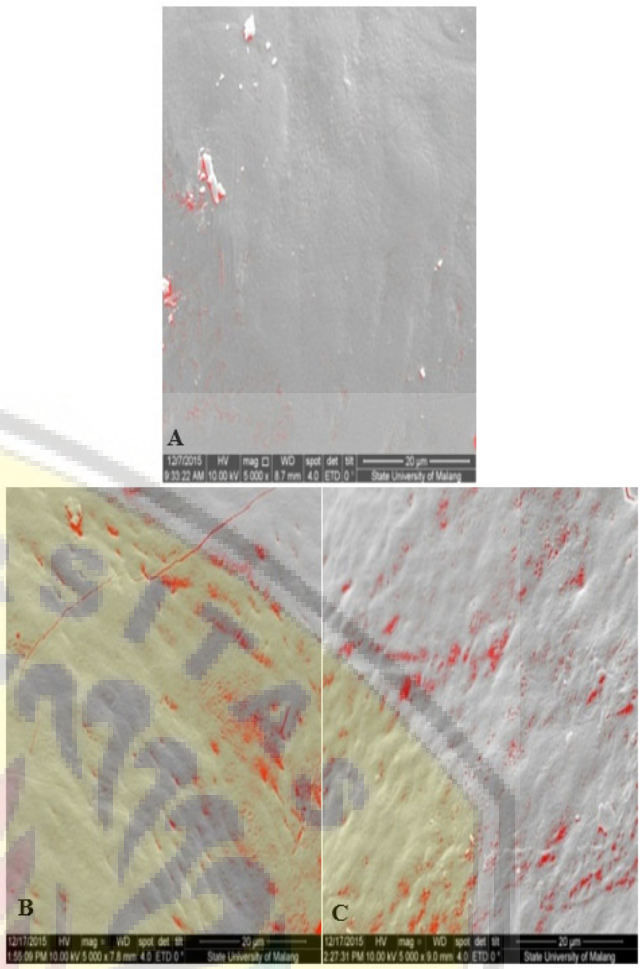

Figure 3: ImageJ processing results on the enamel structure in the buccal region (magnification 5000x). The red color indicates a porosity of the enamel structure. A: group without MSG shows no red color (without porosity); B. MSG-induced group during the gestation period has a low percentage of porosity; C. The MSG-induced group during the gestation period until the lactation period has a greater percentage of porosity. Figure C shows more red colors, compared to B.

whose mother was induced by MSG was lower than the group without MSG. In addition, MSG intake during pregnancy to breastfeeding results in the lowest value of enamel hardness in offspring. The data can be seen in Figure 4.

The average of enamel hardness of offspring from maternal without MSG intake was 309.1HV. The group of maternal induced by MSG during gestation and during gestation to lactation had a value of 242.7HV and 238.3HV respectively. MSG intake in maternal during gestation and lactation periode resulted in the lowest enamel hardness on their offspring. The average of enamel hardness was not significantly different compared to the group of mice whose mothers only received MSG intake during gestation. The control group had the highest level of hardness and was significantly different when compared to the group of rats whose mothers received MSG intake during pregnancy and during pregnancy until lactation. Images of Vickers microhardness test are shown in figure 5.

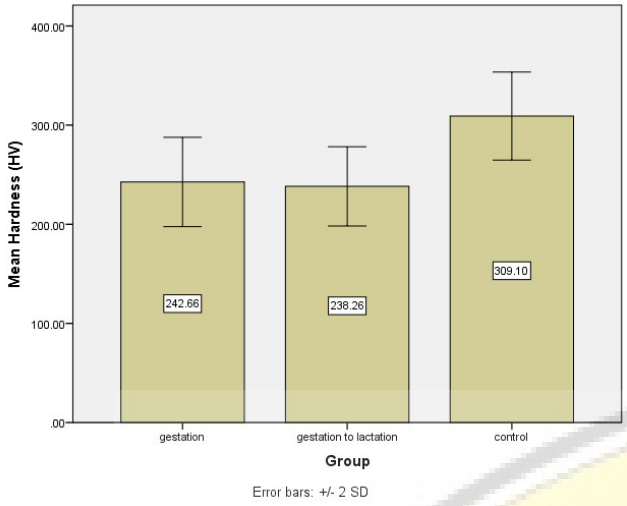

Figure 4: Bar chart of enamel hardness rate

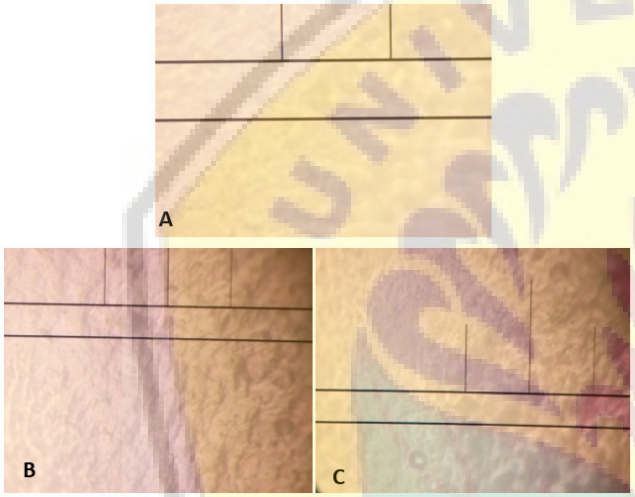

Figure 5: Images of the measurement of enamel hardness with Vickers Microhardness Tester (magnification 100x). The area between the lines is the site of the measurement.

One way ANOVA test showed a significant difference in porosity of enamel structure and mechanical properties (hardness of enamel) (Table I).

Post Hoc Tukey tests were conducted to determine differences between groups. The Post Hoc Tukey results revealed a significant difference between groups control (3) and group induced with dan kelompok yang diinduksi dengan MSG in enamel hardness. In enamel porosity groups 1, 2, 3 show significant differences (Table II).

DISCUSSION

The proper structure and physical properties of enamel depend on factors that have influenced its growth since the embryonal period. Teratogenic substances and nutritional deficiencies during pregnancy become one of the factors that have a significant effect on teeth development (15). In humans, teeth are first formed, approximately 6 weeks in utero, whereas in mice, their molar teeth are formed in the age of 12.5 days

Table I: One Way ANOVA test

		Sum of Squares	df	Mean Square	F	Sig.
Hard-ness	Between Groups	15753.232	2	7876.616	16.878	.000
	Within Groups	5600.164	12	466.680		
	Total	21353.396	14			
Poros-ity	Between Groups	320.009	2	160.004	153.222	.000
	Within Groups	12.531	12	1.044		
	Total	332.540	14			

Table II : Result of Post Hoc Tukey[a] test

		Porosity			Hardness	
Group1	N	Subset for alpha = 0.05				
		1	2	3	1	2
control	5	7.1194				309.1000
gestation	5		13.9252		242.6600	
gestation to lactation	5			18.3494	238.2600	

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 5.000.

in utero (16). Teeth bud development is prone to both systemic and local disruption. Any damages formed on the enamel are irreparable. In general, enamel damage occurs systemically, so it is usually used to detect the presence of calcium metabolic disorders during development (17).

The results of this study indicate that MSG induction during gestation causes abnormalities in tooth structure, with indicate enamel porosity. Gestational period is a critical condition in fetal development. The occurrence of disruption during the intrauterine period will cause changes in the composition of tissues that affect the organ system. Besides being influenced by the condition of the placenta, nutrient intake plays an important role in the growth of the fetus (18). Glutamic acid is a stimulatory neurotransmitter that is abundant in the central nervous system (19). MSG is L-glutamate which originanated from a diet, will be an exotoxic protein if consumed exceeds normal limits. Exotoxins which are digested by the mother during pregnancy will be streamed into the fetus through the placenta and passed into the baby through breast milk. This will contribute to the occurrence of neurodegenerative diseases, obesity disorders, hyperactivity, pain and diabetes in the offspring (20). The research was also supported by von Diemen and Trindade's research, stated that MSG consumed during pregnancy and breastfeeding (21 days of life) at a dose of 20mg / day caused changes in growth patterns as indicated by the Lee index, body weight and nasal-anal length (NAL) lower than normal controls. The study shows a phenomenon that is consistent with the results of this study, the abnormalities in the enamel

structure in the maternal who consume MSG during pregnancy and breastfeeding.

Monosodium glutamat able affect neurotransmission function in mammalian. In the mechanism of neurotransmission involve metabotropic glutamate receptor (mGluR) and ionotropic receptor (21). This process occurs through interactions with receptors which are centered in the area of the central nervous system and responsible for regulation of energy expenditure and storage. MSG promotes neuroendocrine dysfunction when given in large amounts during the neonatal period in mammals (22). Excessive glutamate causes the GLU receptor to be exposed to glutamate with high doses locally, which causes the arcuate fibers of neurons in the hypothalamus to experience fatigue due to excessive excitatory (GLU is an excitatory neurotransmitter). This triggers damage to arcuate neurons. In addition, excessive doses cause GLU penetration in areas in neurons, which results in cell death, so that the growth of the hypothalamus and its secretions is disrupted (23). In accordance with research of von Diemen, et al.(7) that oral administration of MSG during pregnancy caused a decrease in secretion of growth hormone by the hypothalamus, in offspring and the occurrence of degenerative changes in cerebrum neurons (24). Glutamate which is induced when mice are immature subcutaneously or orally will induce hypothalamic damage (20). Intake in the neonatal stage exacerbates damage to the hypothalamic nucleus which includes the arcuate nucleus and ventromedial nucleus which causes weight gain, fat deposition, decreased motor activity and growth hormone secretion (25). This hormonal imbalance causes disruption of amelogenesis process. Growth hormone receptors are detectable at the time of preameloblast cell division, preodontoblast differentiation, and ameloblast secretion. Sasaki, et al.(26), shows that, hypothyroidism during prenatal and post natal periods results in abnormalities in enamel and interferes with enamel mineralization. In addition, GH deficiency decreases preameloblast and preodontoblast rRNA expression and synthesis of two proteoglycans (decorin and biglican). All of these will cause defects in the formation of hydroxyapatite crystals and teeth mineralization.

Damage to the hypothalamus nucleus due to MSG exposure plays a role in the mechanism of weight gain (obesity), fat deposition, decreased motor activity and growth hormone secretion. Obesity is one of the causes of diabetes mellitus (25). In this study (data not shown), there was an increase in body weight that received MSG intake during pregnancy and lactation compared to controls. In the study did not observe an increase in blood glucose in the parent mouse or offspring. The negative effect of MSG on experimental animals was shown in the Tala'a and Hussin studies, namely the administration of MSG in Wistar rats dose 15mg for 30 to 75 days resulting in weight gain, liver weight, hepatocytes 24-26%, hyperplastic, apoptosis, buffer cells and increased AST and ALT serum (27). In the pancreas, the intake of MSG during the neonatal period for 1 month and 9 months, causes a decrease in the number of pancreatic beta cells, which allows diabetics in the future (28). Maternal who is pregnant with diabetes has an adverse effect on the development of the fetus due to deviations from DNA methylation. Maternal diabetic shows the suppression of Apex1 gene expression in the offspring. Apex1 plays a role in the proliferation of dental ephitelial stem cells (DESCs) through promoter methylation (29). This supports the results of this study which showed an abnormality in the surface structure of rat tooth enamel due to MSG intake from the mother during gestation and lactation.

Formation of tooth enamel occurs at certain times during the growth stage. Enamel comes from ameloblast cells. During its growth, it is very easily influenced by environmental changes because ameloblasts are very sensitive cells. Abnormal ameloblast, can result in changes in enamel profile in permanent or deciduous teeth. This can be seen in the Developmental Defects of Enamel (DDE) abnormality, which shows a wide variety of abnormalities ranging in the color of the teeth to enamels that are not fully formed. Causative substances that play a role in these disorders occur genetically and systemically (30).

Any damage to the secretory ameloblast during amelogenesis resulted in thin enamel, whereas disruption during maturation results in an abnormality, marked by the presence of a white bands or blurry areas due to porosity in the corresponding enamel region. That is because ameloblasts are very sensitive secretory cells, so that even with a minuscule changes or disturbances caused ameloblasts unable to degrade and lose matriks organic. It will become a residue that lies between the apatite crystal in the enamel. This will disrupt and inhibit the growth of hydroxyapatite crystals (30). Furthermore, the disruption in the secretory stage impairs the crystal extension, resulting in enamel hypoplasia or if the abnormality occurs during maturation causes the thick but soft enamel to be formed, making it prone to decay (31). These conditions, affect the stage of odontogenesis in mice. Amelogenesis is one of the stages of odontogenesis with several phases, for molar teeth it begins at 12.5 days in utero (16). Receptor interaction which are centered in central nervous system regulate the process. Glutamat acid elicits neuroendocrine dysfunction when given in large amounts during the neonatal period in mammals (22).

This research shows that MSG administration causes low teeth hardness. Such weakness of teeth on the test results are due to the high porosity value (figure 1). Akin to some studies which state that good hydroxyapatite crystal organization has a profound effect on enamel resistance to wear and fracture. This resistance reflects

the balance between various functional pressure vectors and good transfer of occlusal load to dentin. From the mechanical point of view of fracture, the direction of the crystal and the thin layer of protein between the crystals are the two main contributors to natural constitution of the teeth (32). In addition, other mechanical strengths such as shear bond strength depend on the size, volume of the surface area of the porosity of the structure (33). Normally, the enamel structure is composed of hydroxyapatite crystals. The crystals are dense, porous, thin and long and are surrounded by organic matrices and water. Hydroxyapatite crystals that are arranged regularly give rise to 2 porous categories arising from the space between the crystals in the nucleus of the prism (small gap) and between the prisms itself (large gap). The large percentage of porosity in enamel indicates an abnormality, which is common in hypomineralized enamel or enamel hypoplasia cases (34). In the enamel with hypomineralization, the size of the crystal width is smaller, approximately 5 nm compared to normal enamel, which is 50nm. Small enamel crystals cause large pores on the enamel surface (35).

CONCLUSION

It was concluded that the induction of MSG during gestation and lactation causes disruption of teeth development that affects the mechanical and structure of enamel of the offspring. The enamel structure that appears porous contribute in the level of hardness.

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