The Effects of Isolate of Goat Casein Yoghurt Milk to MDA Level and Gastric Histopathology of Rats Exposed to 2,3,7,8-Tetrachlorinedibenzo-P-Dioxin (TCDD)

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Abstract. Dioxins are toxic compounds belonging to organochlorine compounds are highly reactive and produce free radicals that can damage stomach cells. 2,3,7,8-Tetrachlorinedibenzo-p-dioxin (TCDD) is the type of dioxin that has the highest toxicity. Casein goat milk yogurt has the potential as an antioxidant. This study aims to determine the preventive effect of goat milk yoghurt on exposure to dioxin observed levels of malondialdehyde (MDA) and gastric histopathology. Experimental research using Completely Randomized Design (RAL). The white rats (Rattusnorvegicus) were divided into 6 groups, the normal group, casein control (goat milk yoghurt dose 600 mg / kg BW), positive control (TCDD dose 100 ng / kg BW), treatment 1 (goat milk yoghurt 300 mg / kg BW and TCDD 100 ng / kg BW), treatment 2 (goat milk yoghurt 600 mg / kg BW and TCDD 100 ng / kg BW), and treatment 3 (goat milk yoghurt 900 mg / kg BW and TCDD 100 ng / kg BW). Gastric MDA levels were measured using the Thiobarbituric Acid (TBA) assay method with 530 nm wavelength UV-Vis spectrophotometric measurement and gastric histopathology using Hematoxylin-Eosin (HE) staining observed in the form of gastric mucosal cell damage. Gastric MDA levels were analyzed using one way ANOVA statistical test, α = 5% and gastric histopathology analysis were descriptively processed. The results showed that goat milk yoghurt with dose of 600 and 900 mg / kgBW was the best dose in preventing the increase of MDA level of gastric from white rat (Rattusnorvegicus) exposed TCDD significantly (p <0,01), and dose 900mg / kgBB prevented erosion of gastric mucosal cells. The conclusion of this research is giving goat milk goat yoghurt able to prevent the increase of MDA level and erosion of gastric mucosal cells caused by TCDD. 

Keywords : Dioxin, Casein goat milk yoghurt, Gastric, MDA, Gastric histopathology

1. Introduction
Dioxins are air pollutants that can arise due to various activities such as industrial by-products or chlorine-burning [1]. 2,3,7,8-tetrachlorinedibenzo-p-dioxin (TCDD) is the most toxic type of dioxin among other types of dioxins.

TCDD can cause a variety of health problems because of its persistent nature and can accumulate in body fat, such as heart problems. TCDD is a chemical that produces radical oxygen in the cell nucleus. Free radicals as reactive compounds that can damage cells in the body. If the body is unable to neutralize existing free radicals, these free radicals can interfere with tissue or organ cells [2]. Tissue cells that can be disturbed are the stomach. Malondialdehyde (MDA) is one indicator to measure oxidative stress.
caused by the accumulation of free radicals [3]. The higher levels of free radicals in an organ, the higher the level of MDA organs [4].

Yoghurt is the product produced from milk fermented with a starter of Lactic Acid Bacteria [5]. The main protein component in milk is casein which is a source of peptides and has a health effect as an antioxidant. Fermented milk (yogurt) has been shown to have higher antioxidant activity than pure milk [6]. The benefit of goat milk casein yogurt is expected to be an alternative to prevent an increase or reduce the number of free radicals due to TCDD exposure.

2. Method

2.1. Chemical material

Preventive provided in the form of casein goat's milk yogurt, which is made from Etawagoat's milk fresh from Surabaya Valenta Goat Milk with starter yoghurt (Yogourmet Yoghurt Starter, LYO-SAN. INC 500 Aéroparc, C. P. 598, Lachute, QC. Canada, J8H 4G4), it's containing Lactobacillus bulgaricus, Lactobacillus acidophilus, and Streptococcus thermophilus. Induction of cardiac toxic TCDD as inductors used 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD Sigma 48599). Giving TCDD diluted with corn oil for every 100 ng / ml TCDD in 100 ml of corn oil.

2.2. Casein manufacture of goat's milk yoghurt

Goat's milk yoghurt was centrifuged at a speed of 12000rpm for 10 minutes at a temperature of 5°C and filtered using a filter paper to separate the casein with water-soluble extract (WSE). The filtrate product in freeze-dried and stored at a temperature of -20°C, that process is to maintain the stability of pH.

2.3. Experimental animal

Animals used in this study were white rats (Rattus norvegicus) Wistar strain, male, aged 8-12weeks, and weight 150-250 grams were 24 tails. Animals were adapted for seven days to adjust to the conditions in the laboratory.

2.4. Research design

This research was conducted an experimental study using a completely randomized design (CRD). Rats (Rattus norvegicus) were divided into 6 groups, consist of the negative control, control casein (casein yogurt goat milk a dose of 600 mg/kg), a positive control (TCDD dose of 100 ng/kg), treatment 1 (casein yogurt goat milk 300 mg/kg and TCDD 100 ng/kg), treatment 2 (casein yogurt goat milk 600 mg/kg and TCDD 100 ng/kg) and treatment 3 (casein yogurt goat milk 900 mg/kg and TCDD 100 ng/kg). Casein volume goat milk yogurt and TCDD that goes into each rat (Rattus norvegicus) 1 ml. Animals euthanation by cervical dislocation, then surgery by making an incision in the abdomen at the linea alba with open the skin and fascia. Gastric obtained by removing the abdominal contents first and washed with 0.9% physiological NaCl. The gastric cut into two parts. The first part wrapped with aluminum foil, dipped in liquid nitrogen for 10 seconds, then stored in a refrigerator at a temperature of 2°C as a material for examination MDA. The second part is inserted into an organ pot containing formaldehyde for histopathological examination of the stomach.

2.5. Data analysis

Measurement of MDA by TBA method using a gastric organ is cut into small pieces and crushed. Homogenized by centrifuge at speed of 1000rpm for 10 minutes. The supernatant result was measured with a spectrophotometer absorbance at $\lambda = 532$ nm. Data obtained from the results of treatment were analyzed by using Microsoft Office Excel and SPSS version 22 for Windows with one-way analysis of variance (ANOVA) and the advanced test Honestly Significant Difference (HSD) $\alpha = 5\%$. Histopathology of the stomach with Hematoxylin-Eosin staining method (HE) were observed the erosion of gastric mucosal cells by using a Nikon microscope H600L, DS Fi2 300 megapixel camera, 400x magnification.
3. Results and Discussion

This study aims to determine the preventive effects of goat milk yoghurt casein administration against gastric MDA levels of white rats (*Rattus norvegicus*) exposed to 2,3,7,8-tetrachlorinedibenzo-p-dioxin (TCDD) showed significant differences from each treatment group which can be seen in Table 1. The test results of MDA with statistical analysis using one way ANOVA test followed by a test or Tukey's Honestly Significant difference at 95% confidence level. The average value of MDA stomach white rats (*Rattus norvegicus*) treatment group are presented in Table 1.

**Table 1. The average levels of Gastric MDA.**

| Group | The average levels of MDA (ng/ml) |
|-------|----------------------------------|
| K (-) | 1644.50±89.09<sup>a</sup>        |
| KK    | 1588.38±76.63<sup>a</sup>        |
| K (+) | 1928.38±116.10<sup>c</sup>       |
| P (1) | 1857.75±97.01<sup>bc</sup>       |
| P (2) | 1715.13±77.45<sup>ab</sup>       |
| P (3) | 1685.25±80.35<sup>ab</sup>       |

Information: Value of MDA with different notations indicate significant differences (p <0.05) between the treatment groups.

Group K (-) were significantly different (p <0.05) with group K (+) and group P (1), But not significantly different from group P (2) and P (3) together with a group notationKK. The value of the negative control group is the default value of MDA rat (*Rattus norvegicus*) in a normal state in which the mice not given TCDD. Differences from group K (-) and KK, which is in the given preventive casein KK goat milk yogurt with a dose of 600 mg/kg, so hopefully, there is the effect of changes in levels of MDA of preventive given. Group KK significantly different from group K (+) and group P (1), but not significantly different from group P (2) and P (3) and same notation with the group K (-). MDA shown in the test results prove that the state of the body healthy mice still contained the content of free radicals which supported the statement Guyton & Hall (1996), that, consequently of the metabolic processes of biochemical systems (biological oxidation) in the body is able to produce free radicals as much as 2.5% of the total oxygen requirement or as much as 3.4 kg/24 hours.

In the group K (+) significantly different from group K (-), casein control group, the group P (2) and P (3), but not significantly different from group P (1). Indicate that exposure of TCDD will trigger a form Reactive Oxygen Species (ROS) that cause oxidative stress in the stomach that can increase levels of MDA [7].

In group P (1) significantly different from group K (-) and KK, while not significantly different from group K (+), group P (2) and P (3). Based on these data, showed that casein goat milk yoghurt acts as an antioxidant that prevents the increase in MDA levels in the stomach despite the preventive level of 300 mg/kg have not shown the average levels of MDA that approaching MDA levels in the negative control group (A).

Group P (2) not significantly different from group KK, Group C (-), and group P (1), but significantly different from group K (+), and have the same notation as groups P (3). In the group P (3) significantly different from group K (+), Not significantly different from group K (-) and group KK, and have the same notation as group P (2). Based on the results of the Tukey test average of 2 treatment groups with 3 treatment groups had the same notation as a negative control group. This shows that the group treated with casein therapy yoghurt 600 mg/kg (E) and 900 mg/kg (F) both have the same effect of decreasing levels of MDA in the gastric organ.

The treatment group therapy with casein yogurt goat milk 600 mg/kg (E) and 900 mg/kg (F) show decreased levels of MDA in gastric organ due yoghurt goat milk contains bioactive peptides that work
as antioxidants through inhibition of lipid peroxidation by scavenger (trap) free radicals. This is consistent with the statement Korhonen & Pihlanto [8], that the bioactive peptides goat milk yogurt can reduce levels of MDA through the enzymatic inhibition of lipid peroxidation and nonenzymatic. Superoxide radicals captured by casein bioactive peptides and goat's milk yogurt will prevent the occurrence of the initial formation of lipid radicals that are unstable because of the loss of one hydrogen atom (H) of lipid molecules. Furthermore, it can inhibit the electron transfer molecular oxygen on peroxyl radicals and prevent free radical process propagated so that will not react with oxygen [9].

Figure 1. Gastric histopathological profiles of rats in groups A to F.

Gastric histopathological results of each treatment group showed differences in gastric mucosal cell damage, necrosis pyknotic, neutrophil cell infiltration, and the number of chief cells. Figure of gastric negative control group (healthy mice) (A) shows at the mucosal columnar epithelium simplex seen with parietal cells and chief cells are still arranged and neatly lined with a cylindrical shape simplex. This is in accordance with the opinion from Puspitasari [10], those chief cells are basophilic because many contain mitochondria and granules for producing the enzyme pepsinogen, while the parietal cells are acidophilic because these cells produce HCL or stomach acid. The morphology of the parietal cells and large oval is normal. Also visible is the neutrophil cells in small amounts are still normal because neutrophils have a role to fight against foreign agents that are pathogenic.

Unlike the histopathologic picture in the positive control group (C) which indicates severe cell damage. Compounds TCDD can increase ROS levels found in the stomach. Through the intermediary of ROS, TCDD would cause lipid peroxidation of the cell membrane and will form lipid radicals. Lipid radicals formed binds with oxygen and forming a chain of free radicals, resulting in oxidative stress. TCDD induction of oxidative stress which causes an unbalance between free radicals and antioxidants is an important aetiological mechanism of many diseases [11,12]. Due to the oxidative stress can lead to damage to the gastric mucosa. On the histopathologic picture is seen on epithelial erosions accompanied by inflammatory cell infiltration (neutrophils) compared to the negative control. Erosion on the gastric mucosa as the effects of the free radicals that stimulate white blood cells to the gastric mucosal cells look much on the histopathological picture. White blood cells will produce H₂O₂ to kill some types of bacteria and fungi as well as for cell growth, but he did not attack specific targets so that he will also attack the fatty acids polyunsaturated cross cell membranes, organelles, or DNA, which can
cause structural damage and cell function. The visible presence of inflammatory cell infiltration involving inflammatory cells such as neutrophils, lymphocytes, monocytes, and macrophages, but the inflammatory cells that dominate are neutrophils. Erosion on the gastric mucosa as the effects of the free radicals that stimulate white blood cells to the gastric mucosal cells look much on the histopathological picture. White blood cells will produce H$_2$O$_2$ to kill some types of bacteria and fungi as well as for cell growth, but he did not attack specific targets, so that he will also attack the fatty acids polyunsaturated cross cell membranes, organelles, or DNA, which can cause structural damage and cell function. The visible presence of inflammatory cell infiltration involving inflammatory cells such as neutrophils, lymphocytes, monocytes, and macrophages, but the inflammatory cells that dominate are neutrophils. White blood cells will produce H$_2$O$_2$ to kill some types of bacteria and fungi as well as for cell growth, but he did not attack specific targets, so that he will also attack the fatty acids polyunsaturated cross cell membranes, organelles, or DNA, which can cause structural damage and cell function. The visible presence of inflammatory cell infiltration involving inflammatory cells such as neutrophils, lymphocytes, monocytes, and macrophages, but the inflammatory cells that dominate are neutrophils. White blood cells will produce H$_2$O$_2$ to kill some types of bacteria and fungi as well as for cell growth, but he did not attack specific targets, so that he will also attack the fatty acids polyunsaturated cross cell membranes, organelles, or DNA, which can cause structural damage and cell function. The visible presence of inflammatory cell infiltration involving inflammatory cells such as neutrophils, lymphocytes, monocytes, and macrophages, but the inflammatory cells that dominate are neutrophils. White blood cells will produce H$_2$O$_2$ to kill some types of bacteria and fungi as well as for cell growth, but he did not attack specific targets, so that he will also attack the fatty acids polyunsaturated cross cell membranes, organelles, or DNA, which can cause structural damage and cell function. The visible presence of inflammatory cell infiltration involving inflammatory cells such as neutrophils, lymphocytes, monocytes, and macrophages, but the inflammatory cells that dominate are neutrophils. White blood cells will produce H$_2$O$_2$ to kill some types of bacteria and fungi as well as for cell growth, but he did not attack specific targets, so that he will also attack the fatty acids polyunsaturated cross cell membranes, organelles, or DNA, which can cause structural damage and cell function. The visible presence of inflammatory cell infiltration involving inflammatory cells such as neutrophils, lymphocytes, monocytes, and macrophages, but the inflammatory cells that dominate are neutrophils. White blood cells will produce H$_2$O$_2$ to kill some types of bacteria and fungi as well as for cell growth, but he did not attack specific targets, so that he will also attack the fatty acids polyunsaturated cross cell membranes, organelles, or DNA, which can cause structural damage and cell function. The visible presence of inflammatory cell infiltration involving inflammatory cells such as neutrophils, lymphocytes, monocytes, and macrophages, but the inflammatory cells that dominate are neutrophils.

Neutrophils are leukocyte cell types most in parts of white blood cell that is 50-70% among the other leukocyte cell. There are 2 kinds of neutrophils that neutrophils rod and (Stab) and neutrophil segments (polymorphonuclear). Differences stem from both the neutrophil is a young form of neutrophil segments are often referred to as neutrophils core having a horseshoe shape of a horseshoe. Along with the process of maturation, the form will essentially be segmented and segmented neutrophils. According Riswanto [13], the cell has a broad cytoplasm pale pink and purple colored fine granules. Neutrophils serves as the body's defense against foreign substances, especially against bacteria. Neutrophils in the blood circulation of approximately 10 hours and can live for 1-4 days, when in the extravascular tissue. According to Fournier [14], neutrophils are responsible for the process of the withdrawal of other inflammatory cells to localize the damage by releasing pro-inflammatory cytokines, in addition to neutrophils also play a role in mucosal repair. One hour after tissue injury, the neutrophils will be attracted and become the predominant cells in the injured tissue during the first 2 days and the numbers will increase on the second day. Neutrophils will phagocytosis damaged cells and kill bacteria, but it will issue the neutrophil proteases to break down damaged tissue, then the neutrophils will experience apoptosis (programmed cell death) and degradation by macrophages [15].

Damage to the gastric mucosal cells by TCDD also lead to necrosis. Necrosis is cell death induced pathological state and is irreversible [16]. Necrosis is visible is the nucleus of cell necrosis pyknotic which looked dark and wrinkled as DNA correlated into a solid mass. According to Hidayat et al. [17], the exposure to substances that can cause free radicals continuously will result in necrosis and cell damage.

Histopathologic features in the treatment group 1 (D) with therapy casein yogurt 300 mg/kg body weight did not differ much with the picture of the positive control group (C) is an only visible reduction in erosion of the epithelial cells of the gastric mucosa and is still visible necrosis pyknotic although not as much as the positive control group. In the treatment group 2 (E) with casein therapy of 600 mg/kg showed an improvement of epithelial erosion, absence of necrosis pyknotic, and accompanied by a
reduction in inflammatory cells when compared to the treatment group 1. In the treatment group 3 (F) with therapeutic doses of casein yoghurt 900 mg/kg, pyknotic necrosis and epithelial erosion had decreased significantly showed better results when compared to the treatment group 3 and shows the exact same results with gastric healthy mice.

Histopathologic features in the control group casein (B) with yoghurt casein therapy of 600 mg/kg, showed equally good results with the negative control group. The purpose of the casein control test to see the effects only given casein alone. It has been proven that goat milk yoghurt cause act as anti-inflammatory and antioxidant. The content of bioactive peptides and it contains lactic acid bacteria play a role in eliminating microbial pathogens [18]. Goat milk casein which acts as antioxidants peroxyl radical change (the result of lipid peroxidation) become less reactive and break the chain reaction of free radicals [19].

4. Conclusion
The given of preventive therapy casein yoghurt goat milk at a dose of 600 mg/kg and 900 mg/kg has the same effect and the best dosage because it can prevent the increase in MDA levels of gastric rat (*Rattus norvegicus*) exposed to TCDD with decreased levels of MDA approach MDA group normal (K). And a dose of 900 mg kg is the best dose that is capable of preventing damage to the gastric fundus rat exposed to TCDD.

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References
[1] UNEP 2003 *Standardized toolkit for identification and quantification of dioxin and furan releases.* Geneva-Switzerland: Inter-Organization Programme for the Sound Management of Chemicals, Diambib 2 September 2004, dari http://www.pops.int/documents/guidance/Toolkit_2003.pdf
[2] Price S A and Wilson L M 2006 *Patofisiologi.* Ed 6 (Jakarta: EGC)
[3] Arkhaes N 2008 *Kadar Malondialdehida (MDA) Serum Sebagai Indikator Prognosis Keliuan Pada Sepsis Neonatorum* [Tesis]. Program Pascasarjana Magister Ilmu Biomedik dan Program Pendidikan Dokter Spesialis-I Ilmu Kesehatan Anak Universitas Diponegoro Semarang
[4] Luczaj W and Elzbieta S 2003 DNA Damage Caused by Lipid Peroxidation Products *Cellular and Molecular Biologi Letter* 8 391-413
[5] Shah N, Caballero B, Trugo L C and Finlas P M 2003 Yoghurt: The Product and its Manufacture. *In Encyclopedia of Food Sciences and Nutrition Academic Press:* New York, U.S.A. pp 6252-6259
[6] Liu, Je-Ruei, et al 2005 Antioxidative Activities Of Kefir *Asian-Aust. J. Anim. Sci.* 18 (4) 567-573
[7] Doi H, Baba T, Tohyama C and Nohara K 2013 Functional Activation of Arylhydrocarbonreceptor (Ahr) in primary T-cell by 2,3,7,8 tetrachlorodibenzo-p-dioxin
[8] Korhonen H and Pihlanto A 2006 Bioactive Peptides: Production And Functionality *International dairy journal* 16 945-960
[9] Kullisar T, Songisepp E, Mikelsaar M, Zilmer K, Vihelemm T and Zilmer M 2003 Antioxidative Probiotic Fermented Goats Milk Decreases Oxidative-Stress-Mediated Atherogenicity in Human Subjects *British Journal of Nutrition* 90 449-456
[10] Puspitasari D A 2008 *Gambaran Histopatologi Lambung Tikus Putih (Rattus norvegicus) Akibat Pemberian Asam Asetil Salisilat* Institut Pestanian Bogor
[11] Ciftci, Osman., Murat, OlcayDisli and Timurkan N 2012 Protective effects of protocatechuic acid
on TCDD-induced oxidative and histopathological damage in the heart tissue of rats. *Toxicology and Industrial Health* **29** (9) 806-811 Department of Pharmaceutical Toxicology, University of Inonu, Turkey

[12] Yoshida R and Ogawa Y 2000 Oxidative Stress Induced by 2,3,7,8-TCDD: An Application of Oxidative Stress Markers to Cancer Risk Assessment of Dioxins *Indust. Health Journal* **38** 5-14

[13] Riswanto 2013 *Pemeriksaan Lab Hematologi* (Yogyakarta: Alfamedia and Kanal Medika)

[14] Fournier B M 2012 *The Role of Neutrophils During Intestinal Inflammation* Epithelial Pathology Research Unit, Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, Georgia: USA 5, 354-366

[15] Martin P and Leibovich S J 2005 Inflammatory cells during wound repair: the good, the bad, and the ugly *Trends in Cells Biology* **15** (11) 599-607

[16] Tambayong J 2000 *Patofisiologi untuk Keperawatan* (Jakarta: Buku Kedokteran EGC)

[17] Hidayat A, Christijanti W and Marianti A 2013 Pengaruh Vitamin E terhadap Kadar SGPT dan SGOT Tikus Putih Galur Wistar yang Dipapar Timbal *Unnes Journal Pangandan Argoindustri* **3** (4) 1412-1422

[18] Pessione E and Simona C 2016 Bioactive molecules released in food by Lactic Acid Bacteria: Encrypted Peptides and Biogenic Amines *Journal Frontiers in Microbiology*

[19] Jetawattana S 2005 Malondialdehida (MDA), A Lipid Oxidation Product : Free Radicals In Biology and Medicine *Journal Biology Mediciene* Department of Radiation Oncology Free Radical and Radiation Biology The University of Iowa for 77:222, Spring 2005 Iowa City, IA 52242-1181