The effects goat milk yoghurt casein on malondialdehyde (MDA) level of rats (Rattus norvegicus) exposed by 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD)

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Abstract. Dioxins are a group of compounds that are poisonous (toxic) and it was a factor to trigger cancer (carcinogenic substance). The most toxic dioxin was 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Dioxins can cause health problems due to the nature of persistent and can bioaccumulate in the fat body. Casein goat milk yogurt potential as an antioxidant. This study aimed was to determine the effect of goat milk yogurt to dioxin exposure were observed in the levels of Malondialdehyde (MDA) of heart rats (Rattus norvegicus). It was an experimental study by used a completely randomized design (CRD). The rats (Rattus norvegicus) were divided into 6 groups, the each namely were: the negative control group (K-), control casein group (KK), the positive control group (K+), treatment 1 group (K1), treatment 2 group (K2), and treatment 3 group (K3). Heart MDA levels measured by used thiobarbituric acid (TBA) with UV-Vis spectrophotometry method at a wavelength of 530 nm. MDA heart analysed by one-way Analysis of Variance (ANOVA) confidence level α = 5%. The results showed that the best yogurt casein dose used to inhibit MDA in TCDD-rats is 900 mg/kg. Casein goat milk yogurt can prevent the increase of MDA significantly (p<0.01) as a result of exposure to TCDD. Goat's milk yogurt casein dose of 900 mg/kg is the best dose. The conclusion is the provision casein goat milk yogurt can prevent the increase of MDA caused by exposure to TCDD.

Keywords: Dioxins, Casein goat milk yogurt, Heart, and MDA

1. Introduction
Dioxins are by-products of a compound of various processes performed by humans, namely in the manufacture of certain classes of pesticides and the burning of materials containing chlorine hydrocarbons [1]. The burning of materials containing chlorinated hydrocarbons, such as Polyvinylchloride (PVC), Polychlorinated biphenyl (PCB), hospital waste, household waste, and burning paper bleached with chlorophenol would generate dioxins [2]. The most toxic dioxin is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) [3].

Dioxins can lead to various health problems due to the nature of persistent and can bioaccumulate in the fat body [4]. Some diseases that can be caused by exposure to dioxin has been studied, namely cardiovascular disorders [5]. Dioxins are chemicals that produce oxygen radicals in the cell nucleus. Free radicals as reactive compounds that can damage cells in the body [6].

Improvement of Reactive Oxygen Species (ROS) cause lipid peroxidation, as a result of the reaction of the fatty acid chain was cut into a compound toxic to cells, such example is Malondialdehyde (MDA),
which can damage cells in the body. Malondialdehyde (MDA) is one indicator of free radicals. The higher levels of free radicals in an organ of the higher levels of MDA [7].

Yoghurt is a product produced from milk fermented with a starter of Lactic Acid Bacteria (LAB) [8]. Components of the main protein in milk is casein which is a source of peptides and have health effects as antioxidants. Fermented milk (yogurt) is shown to have a higher antioxidant activity than pure milk [9]. The use of casein goat's milk yoghurt is expected to be an alternative to prevent increase or reduce the amount of free radicals caused by exposure to dioxin.

2. Method

2.1. Chemicals and Reagents
Preventive be given in the form of casein yogurt goat's milk, which is made from goat's milk Peranakan Etawa (PE) fresh from Surabaya Valenta Goat Milk with starter yoghurt (YOGOURMET Yoghurt starter, LYO-SAN, INC 500 Aeroparc, CP 598, Lachute, QC, Canada, J8H 4G4), containing Lactobacillus bulgaricus, Streptococcus thermophilus and Lactobacillus acidophilus. 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 by oral force feeding.

2.2. Casein manufacture of goat's milk yoghurt
Goat's milk yoghurt was centrifuged at a speed of 12000 rpm for 10 minutes at a temperature of 50°C and filtered using a filter paper to separate the casein with water soluble extract (WSE). The filtrate produced in freeze dried and stored at a temperature of -20°C, the process is to maintain a stable pH.

Measurement of MDA by TBA using the method describe by [10].A heart pieces were crushed and homogenized by centrifuge at a speed of 1000 rpm for 10 minutes. The supernatant was measured with a spectrophotometer absorbance at λ of 532 nm.

2.3. Animals
24 white rats (Rattus norvegicus) Wistar strain were used in the experiment with age 8-12 weeks and weigh 150-250 grams. Animals are 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 of negative groups (normal), a group of casein (casein yogurt goat milk a dose of 600 mg/kg), positive group (TCDD dose of 100 ng/kg), the treatment group 1 (casein yogurt goat milk 300 mg/kg and TCDD 100 ng/kg), group 2 (casein yogurt goat milk 600 mg/kg and TCDD 100 ng/kg) and the treatment group 3 (casein yogurt goat milk 900 mg/kg and 100 ng TCDD/kg). Casein volume goat milk yogurt and TCDD that goes into each rat (Rattus norvegicus) 1 ml. Rats (Rattus norvegicus) euthanized by means of cervical dislocation, then surgery by making an incision in the abdomen. The heart is obtained by removing the abdominal contents beforehand and washed with 0.9% physiological saline. The heart is cut and wrapped with aluminium foil, dipped in liquid nitrogen for 10 seconds, then stored in a refrigerator at a temperature of 20C as a material for examination MDA.

2.5. Data analysis
Data obtained from the results of treatment were analysed by using Microsoft Office Excel and SPSS version 22 for Windows with one-way analysis of variance Analysis of Variance (ANOVA) and the advanced test Honestly Significant Difference (HSD) α = 5%.
3. Results and Discussion
With the effects of goat milk yogurt casein on levels Malondialdehyde (MDA), rats (*Rattus norvegicus*) were exposed by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) show significant differences from each treatment group. Results MDA statistical analysis using one-way ANOVA test followed by or Tukey HSD test with a confidence level of 95%. The average value of MDA white rat heart (*Rattus norvegicus*), each treatment group shown in Table 1.

| Group   | The average levels of MDA (ng/ml solvent) |
|---------|-----------------------------------------|
| K (-)   | 1486.37 ± 39.37a                        |
| KK      | 1436.50 ± 56.38a                        |
| K (+)   | 1739.00 ± 97.02c                        |
| P (1)   | 1693.37 ± 87.85c                        |
| P (2)   | 1607.12 ± 67.62bc                       |
| P (3)   | 1515.87 ± 83.07ab                       |

Description: the same notation indicated not statistically significant different (p < 0.01).

Results of statistical analysis one-way ANOVA test showed that administration of casein goat milk yogurt can prevent the increase in MDA levels (p < 0.01), it is seen with the 3 types of notations on advanced test Tukey (Table 1).

K (-) indicates a significant difference (P < 0.01) in group K (+), P (1), and P (2), but not significantly different at P (3) and have same notation with KK. 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. In group K (-) and KK finds that there are levels of MDA, but the treatment was not given a second dose of TCDD exposure as toxic substances that enter the body. The MDA levels showed that in normal or healthy conditions there is still a free radical. Free radicals in the body which are derived from oxygen is inhaled. Oxygen is able to generate a lot of energy but a by-product of the reaction of the formation of the energy will generate ROS.

Group K (+) indicates the average level of the highest MDA (1739.00 ± 97.02), due to the K (+) is given at a dose of TCDD exposure 100ng/kg as toxic substances that enter the body. Increased levels of MDA, as a result of exposure to TCDD administration which triggers increased production of ROS in the heart, causing oxidative stress and lipid peroxidation. TCDD exposed to a white rat (*Rattus norvegicus*) will get into the cells and the binding of AHR in the cytoplasm. The bond to form a new complex compounds with AHR Nuclear Translocator (ARNT) and interact with the Dioxin-Responsive Enhancer Elements (DRE) located on the surface of the target genes and activate various transcription factors in the cell nucleus. AHR-ARNT heterodimer induce cytochrome P450 genes and lead to increased production of cytochrome. Cytochrome P450 enzymes produce ROS which is specifically the result of biochemical activity of toxic agents.

Group P (1) differed significantly with K (-), KK, and P (3), but did not differ significantly with P (2), and note equal to K (+). Group P (1) shall be treated casein goat's milk yoghurt 300mg dose/kg and exposure to TCDD dose of 100ng/kg is known to prevent the increase in MDA, but in small quantities. This happens due to the antioxidant content of casein in goat milk yogurt that can inhibit free radicals in the body, so the increase in MDA can be prevented. However, dosing in P (1) is far from K (-) as indicated by differences in notation.

Group P (2) differ significantly from the K (-) and KK, but did not differ significantly with K (+), P (1), and P (3). Group P (2) is given goat's milk yoghurt casein treatment dose 600mg/kg and exposure to TCDD dose of 100ng/kg, showed a decline in the average levels of MDA compared with K (+), but
it has not seemed significant. Dosing in P (2) they show differences with the notation K (-), so the dose is less effective than expected.

Group P (3) differ significantly from the K (+) and P (1), but did not differ significantly with K (-), KK, and P (2). Group P (3) shall be treated casein yogurt goat milk dose of 900mg/kg and exposure to TCDD dose of 100ng/kg decreased the average levels of MDA significantly compared with K (+) and the average yield of MDA approached the group K (-). This shows that the larger the dose of goat's milk yoghurt casein is given, the greater the decrease in MDA. Dosage in group P (3) is able to provide the expected effects, the MDA produced in normal amounts, so as to inhibit tissue damage.

In this study, the best dose casein to inhibit MDA in rats (*Rattus norvegicus*) is 900 mg/kg. Decreased levels of MDA in treatment group goat milk yogurt preventive casein (P1, P2, and P3), casein because goat's milk yogurt contains bioactive peptides which works as an antioxidant scavenger (catchers) free radicals that inhibited lipid peroxidation reaction. Bioactive peptides fermented goat milk will stabilize superoxide radicals by donating a hydrogen atom (H). Superoxide radicals captured by casein bioactive peptides and goat's milk yogurt will prevent the occurrence of initiation formation of lipid radicals that are unstable because of loss of one hydrogen atom (H) of lipid molecules. Other than that Kullisaar *et al.* [11].

### 4. Conclusion

The provision of preventive therapy casein yogurt goat milk at a dose of 300mg/kg and 600mg/kg have the same effect in preventing an increase in the levels of Malondialdehyde (MDA) heart rat (*Rattus norvegicus*) exposed to TCDD, whereas preventive therapy casein yogurt goat milk with a dose 900mg/kg is the best dosage because it can prevent the increase in MDA levels with decreased levels of MDA approach normal group (K).

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