The activity of casein derived from goat milk yogurt as an antioxidant on histopathology of rat’s liver exposure by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)

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Abstract. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a toxic contaminant which persistent in the environment. Accumulation of this compound on the body through food chain can generate adverse effect in human health. The aim of this research was to observe the activity of casein derived from goat milk. A total of twenty-four male Wistar rats of twelve weeks old were used in this research. The rats were divided into six groups: (1) control group, (2) placebo group which given casein 600 mg/kgBW, (3) TCDD group given with TCDD 100 ng/kgBW, group P1 given casein 300 mg/kgBW and TCDD, (5) group P2 given casein 600 mg/kgBW and TCDD, (6) group P3 given casein 900 mg/kgBW and TCDD. Provision of casein and TCDD were given orally for 21 days. Liver sample collected for histopathological examination with hematoxylin Eosin (HE) stained. Experimental method was based on completely randomized design. The histopathological were analyzed by Kruskal-Wallis test followed by Mann-Whitney test to know the differences of the treatments. Result of the research showed that casein could protect the rat liver from damage in TCDD intoxicated rats. Significant increase (P<0.05) shows from group given with casein dose 900 mg/kgBW.

1. Introduction
Dioxin is the common name for a group of prominent pollutant chemicals which belongs to persistent organic pollutants (POPs). Dioxin consists of 210 types of chemicals, which are 75 types of polychlorinated dibenzo-p-dioxin (PCDD) and 135 types of polychlorinated dibenzofuran (PCDF) [1].

The highest toxic in the dioxin is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The effects of TCDD’s toxicity for the human are some disorders toward nervous system, reproductive system, endocrine system, and degrade the immune system [2]. In addition, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) can cause chloracne (a skin problem symptomized by hyperkeratosis and black spots or blackheads), kidneys problems, liver problems, and anemia. 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) is also influential to oxidative stress [3,4].

The liver is the most important organ in the process of metabolism and detoxification. The exposures of the toxic chemicals make the liver problems get worse. The liver is potential to have some defects because it is the first organ after digest system that will be exposure by those toxic chemicals [5].

The TCDD which is given at a dose of 10 up to 100 ng/kg body weight/ day during thirteen weeks to the female Sprague Dawley mice can cause the mutation of the liver such as hepatocellular degeneration, inflammation, necrosis, vacuolization on the cytoplasm, the alteration of the lobular
form, increased production of superoxide anion, peroxidation lipid, the stand breaks of DNA, and glutathione decrease at the brain tissue and liver [6]. The given of TCDD per-oral at dose 0.1 ng/kg of body weight during twenty-seven days in the liver histopathology description is the hepatocyte vacuolization, inflammatory, and the increasing of Kupffer, necrosis, and the increasing amount of Malondialdehyde (MDA) blood plasma [7].

When having stress, the free radicals trigger some biochemical reactions which cause the changes on cellular components. The free radical is an atom or molecules which have one or more unpaired electrons in an orbital [8]. The free radical can damage some vital cellular components; those are fat, protein, and DNA [9]. Oxidative stress due to free radicals can be defined by measuring one of the parameters as MDA [10].

Basically, the body already has antioxidant system to prevent free radicals. Yet, in the high oxidative stress condition, the supply of body antioxidant is not enough, so that the cell components damage can probably happen. Therefore, some extra antioxidant outside the body is needed, obtained from foods that contain of antioxidant or by consuming antioxidant goat milk yogurt [10,11]. goat milk yogurt as the free radical preventer has been researched currently, mainly the casein derived from goat milk yogurt. Casein is a precursor protein for bioactive peptides. The casein goat milk yogurt stands as the first defense against polyunsaturated fatty acids (PUFA), which can be found in phospholipid and glycolipid of the cell membrane. Casein goat milk yogurt works as the antioxidant chain breaking [10,12].

2. Materials and Methods

2.1 Chemical
Yogurt starter contain 3 strains of lactic acid bacteria: L. bulgaricus, S. thermophilus and L. acidophilus (Yógourmet, Lyo-SAN INC: 500 Aeroparc, C.P. 589, and Lachute, QC. Canada, J8H, 464), Dioxin chemical was used 2,3,7,8-Tetrachlorodibenzo-p-dioxin. TCDD with > 99% purity (Supelco Analytical Bellefonte, PA, Cat No: 48599). The stock solutions of 10 µg/mL were diluted in 100 mL of corn oil to prepare the solution dose.

2.2 Preparation of goat milk yogurt and casein
Fresh and unprocessed goat milk were purchased from local farm (Malang, Indonesia). Goat milk yogurt was prepared using modified method. Casein was prepared by cold centrifugation at 12,000 rpm at 50 0C for 10 minutes. The casein obtained by filtration under cold condition using Whatman paper and then stored in freezer at -20 0C until used.

2.3 Animals
Wistar male albino rats age 8-12 weeks (weighing about 120-150 mg) were purchased from animal house D’wistar Bandung. Animals were housed at Biosains Institute of Brawijaya University. Laboratory conditions were maintained under normal temperature: 24 ± 2°C and a 12 h day/night cycles. Acclimatization take place for 14 days before the experiment started, observation for general health were taken. Standard diet and drinking water were given ad libitum.

2.4 Experimental design
The study approved by the local ethics committee. The animals were randomly divided into four equal groups, 24 rats divided into 6 groups. The control group without treatment, given normal food and water. The placebo group, given goat milk yogurt casein diluted in distilled water with oral dose 600 mg/kgBW/day. The TCDD group, given with oral dose of TCDD 100 ng/kgBW/day diluted in corn oil. The treatment group 1 (T1), given casein 300 mg/kgBW and TCDD 100 ng/kgBW daily. The treatment group 2 (T2), given casein 600 mg/kgBW and TCDD 100 ng/kgBW daily. The treatment group 3 (T3), given casein 900 mg/kgBW and TCDD 100 ng/kgBW daily. The volume for casein solution and TCDD solution was 1 mL for each rat. The treatment was done for twenty-two days. In the end of the treatment, the mice were sacrificed by doing cervicalisdislocasio. After it dead, it is suddenly surged and taking out the liver. The liver of the mice is used for histopathology examination is inserted into a plastic pot that had been fulfilled by Formalin 10% and labeled.
3. Result and Discussion

Energy In the necrosis, the control group (-) (0.35) has the significant result from the other groups. The control group (+) (2.8) is also significant from the other groups. The treatment group 1 (0.65) is also significant fact from the control group (-) (0.35), the control group (+) (2.8), the treatment group 3 (0.25) but does not have significant fact from the treatment group 1 (0.65) and the treatment group 2 (0.6).

![Necrosis](image1)

![Inflammation](image2)

![Degeneration](image3)

Figure 1. The TCCD effect of animal models control and treatment group

The treatment group 3 (0.25) has significant fact from the control group (-) (0.35), the control group (+) (2.8), the treatment group 1 (0.65) but does not have significant fact from the treatment group 2 (0.6). In the degeneration, there is no some real significance among the treatment groups. In the inflammatory, the control group (-) (0.5) has a significant fact from the other treatment groups entirely. However, there is no significant result among the control group (+) (1.35), the treatment group 1 (0.95), the treatment group 2 (0.85) and the treatment group 3 (0.3).

The observation upon the alteration of mice’s liver histopathology description (hepatocyte cells) with the lesion form expresses, the highest mean is captured in the control group (+) (2.8). The control group (+) is the experiment group that was given TCDD 100 ng/kg weight/day and added corn oil solvent. The control group (+) generates toxic effect in the liver (hepatocyte cells). This is can be known by comparing it to the control group (-) (0.35). Liver mostly becomes the target organ of the toxic chemicals because the most of the chemicals entering the body through the gastrointestinal system and after it is absorbed; then it is transported by vena porta to the liver. Necrosis is an irreversible alteration [5]. Cell that experienced necrosis cannot return normal. In the end of necrosis, cells will be died [13].

The Mean of the treatment group 1 (0.65), which was given TCDD at a dose of 100 ng/kg body weight/day/head and given casein goat milk yogurt at a dose of 300 mg/kg body weight/day/head, decreased if it is compared to the control group (+) (2.8) which were given TCDD at a dose of 100 ng/kg body weight/day/head and given corn oil solvent. The similar case is also occurred on the treatment group 2 (0.6) which was given TCDD at a dose of 100 ng/kg body weight/day/head and given casein goat milk yogurt at a dose of 600 mg/kg body weight/day/head. And on the treatment group 3 (0.25), that was given TCDD of 100 ng/kg body weight/day/head and given casein goat milk yogurt at a dose of 900 mg/kg body weight/day/head. This shows that there is a protection effect by casein goat milk yogurt to the exposure of TCDD. The provisions of casein goat milk yogurt can protect the liver (hepatocyte cells) from damage because the function of casein goat milk yogurt is as the antioxidant of free radicals, which is created by the toxic chemicals, and finally caused cellular
component damage. Casein goat milk yogurt is the first defender toward the lipid peroxide effect (at PUFA) in the cell membrane, phospholipid on mitochondria, endoplasm reticulum, and membrane plasma which has affinity toward casein goat milk yogurt because the casein goat milk yogurt is mostly concentrated on that parts.

Figure 2. The profile of Rat’s Liver by Hematoxylin Eosin staining. Note: A= C(-), B= P, C= C(+), D= T1 (TCDD 100ng/kgBW and casein goat milk yoghurt 300mg/kgBW), E= T2 (TCDD 100ng/kgBW and casein goat milk yoghurt 600mg/kgBW), F= T3 (TCDD 100ng/kgBW dan casein goat milk yoghurt 900mg/kgBW)

The observation to the alteration of mice’s liver histopathology description (hepatocyte cells) with lesion form degeneration expresses, the entirely group treatment, the mean is relatively the same (control group (-) (0,1), treatment group 1 (1,35), treatment group 2 (0,6) dan treatment group 3 (0,5), although the treatment group 2 (0,6) has the highest mean. The degeneration which seen microscopically is the hydropic degeneration and fatten degeneration. Degeneration is a reversible alteration. It means if the exposure that causes damage has been stopped; the cell can be reversed as like as before it is exposure [13]. In the control group (-) there is also degeneration, however, it is not possibly due to pathological factor of TCDD. It is because the physiological factor of each individual experimental mouse or because of the liver itself (hepatocyte cells) which is too delicate as its function is metabolizing any chemicals or strange objects which come into the body (Simanjuntak, 2007).
Theoretically, the process of liver damage is initiated from the degeneration process indicated by cellular swelling. The treatment with TCDD exposure seems to generate extracellular liquid coming into cytosol in a large amount. One of the alterations that caused by the toxic chemicals and can cause the formation of free radicals, is the change of the membrane cell’s characteristics and cytoplasm membrane at the cellular component, such as mitochondria and lysosomes which is impacted by lipid peroxidation [14]. After damaging cell membrane, the toxic chemicals also can reach and broke the cell nucleus, which cause the cell’s structure abnormal and going on to necrosis. Necrosis is a degradation or cell’s disorganization which is irreversible or the cell death as the influence of lesion, signed by the real morphological transformation on the nucleus as pyknosis (cell’s chromatin solidification), karioreksis (the rupture of the nucleus) and kariolisys (the nucleus disappearing) [13].

Inflammatory is a body physiological response against a damage and disorder by some external factors. Inflammation is divided into two basic patterns. Acute inflammation is a short lasting inflammatory, from several minutes up to several days and marked by vascular alteration, liquid exudation, plasma protein, and neutrophil obstruction accumulation. The acute inflammation can grow chronic if the damage agent still exists. The chronic inflammation is a proliferative response, fibroblast proliferation occurring, endothelium vascular, and mononuclear cell infiltration (lymphocyte, plasma cell, and macrophages). The inflammatory respond includes a complex device that influences vascular and cellular changing [13].

The mean changing of the mice’s liver histopathology description (hepatocyte cells) with the lowest inflammation form is at the control group (-) (0,5). It expresses that there is no damage cause agent which is potential to make a worse inflammation. Whereas, in the treatment group 1 (0,95), the treatment group 2 (0,85) and the treatment group 3 (0,3). Inflammatory cells infiltration in sinusoid, porta area, and vena centralist are dominated by lymphocyte cells.

Hence, casein goat milk yogurt is able to protect liver (hepatocyte cells) from damage. Pihlanto said that casein has an antioxidant activity as a radical scavenger and cation chelator which able to inhibit lipoxigenase-catalyzed lipid autoxidation [15] and by watching at every transformation modus histopathology description on each group treatments, it is known that the ability of casein goat milk yogurt is more outstanding in protecting liver from necrosis change instead of degeneration and inflammation change. By increased dose (11,20, and 37 mg/kg weight/ day), the protecting of casein goat milk yogurt is getting higher indicated by the decreasing of necrosis change.

4. Conclusion
The provision of casein goat milk yogurt can protect the hepatocyte cell of the mice from the damage due to the exposure of TCDD.

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