The Effect of Goat Milk Yoghurt Casein Antioxidant Activity on Kidney Tumor Necrosis Factor Alpha (TNF-α) and Interleukin 1 Beta (IL-1β) Levels in Rattus norvegicus Exposed by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)

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Abstract: Dioxins are highly toxic and carcinogenic compounds. The most dangerous dioxin is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). TCDD can cause health problems that accumulate over time. Goat milk yoghurt casein can reduce free radicals caused by TCDD. Goat milk yoghurt casein contains bioactive peptide that acts as an antioxidant. The aim of the current research was to determine the effect of goat milk yoghurt towards TNF-α and IL-1β levels after TCDD exposure. This research was an experimental study using a completely randomized design (CRD). Rattus norvegicus were divided into 6 groups: K- (negative control), K+ (positive control induced by TCDD 100 mg/kgBW), KP (placebo control given goat milk yoghurt casein 300 mg/kgBW), K1 (given TCDD 100 mg/kgBW and goat milk yoghurt casein 300 mg/kgBW), K2 (given TCDD 100 mg/kgBW and goat milk yoghurt casein 600 mg/kgBW), and K3 (given TCDD 100 mg/kgBW and goat milk yoghurt casein 900 mg/kgBW). Quantitative analysis with one-way ANOVA and post hoc Tukey test showed that goat milk yoghurt could prevent TNF-α increase significantly (p<0.05) from control groups, and also prevent IL1-β increase although not significantly different from control groups (p>0.05). It can be concluded that goat milk yoghurt casein 900 mg/kgBW can prevent the increase of TNF-α and IL1-β. Thus, goat milk yoghurt casein could be used as an antioxidant source against TCDD exposure.

1 INTRODUCTION

Earth ecosystem is continuously contaminated by various pollutants. Several pollutants have better durability against environmental degradation because they are chemically, biologically, or even to photolytic reaction and may persist for a long time in the environment (Ritter et al., 2007). Gupta and Ali., 2012). Pollutants able to persist for a long time in the environment is called persistent organic pollutants (POPs). There are several persistent environmental pollutants, one of them is dioxin (Alharbi et al., 2018).

Dioxins are produced out of combustion of household and industrial waste, especially chlorine compounds such as chemical industry, pesticide, plastic, pulp, paper, and so on (Winarti and Munarso, 2005). The most toxic dioxin is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). According to International Agency for Research on Cancer (IARC), TCDD is included in group I, which means it is carcinogenic to human (World Health Organization, 1997). Toxicity effects caused by TCDD are cancer, reproductive and growth disorder, immune system disorder, and affecting immune system (Patrizi and de Cumis, 2018).

TCDD toxicity is mediated by Arylhydrocarbon Receptor (AhR). Upon TCDD exposure, activated AhR would move towards nucleus and form dimer compound with Ah Receptor Nuclear Translocator (ARNT) (Harvey et al., 2015). TCDD-AhR-ARNT compound complex will then form Reactive Oxygen Species (ROS) and is toxic to various organs, including kidney 1 Oxidative stress by TCDD will induce NF-κB which is a proinflammation transcription factor. Active NF-κB will increase COX-2 expression (Shen et al., 2005; Wan et al., 2014).

Goat milk is known as natural ingredient with the most complete component and can neutralize acid and...
toxin in the body (Getaneh et al., 2016). Protein in goat milk consists of casein as much as 74% of total protein, whey protein as much as 17%, and non-protein nitrogen as much as 9% (Al-Saadi et al., 2014). Casein is a source of peptide and has antioxidant properties (Khan et al., 2019). Yoghurt is produced from fermentation process of milk lactose by lactic acid bacteria (LAB).

1. Yoghurt is proven to have antioxidant activity higher than fresh milk.
2. Administration of casein from goat milk yoghurt is hoped to be an alternative antioxidant source to lower free radicals out of TCDD exposure as observed based on the level of TNF-α and IL-1β.

2 MATERIALS AND METHOD

2.1 Materials

Yoghurt was made by yoghurt starter containing 3 LAB strains: L. bulgaricus, S. thermophilus, and L. acidophilus (Yogourmet®, Lyo-SAN INC: 500 Aeroparc, C.P. 589, and Lachute, QC. Canada, J8H, 464). Chemical used was TCDD with more than 99% purity (Supelco Analytical Bellefonte, PA, Cat No:48599). Stock solution of 10 µg/mL TCDD was diluted into 100 ml of corn syrup as solution dosage. Animal model used were 24 male Rattus norvegicus of Wistar strain, aged 8-12 weeks, weighing 150-200 grams obtained from animal house D’wistar Bandung. Rattus norvegicus were kept in Bioscience Institute, Brawijaya University. Laboratory condition was kept in below normal temperature of 24±2°C and given 12 hours light/dark lighting durations. Acclimatization was conducted for 14 days before the experiment with animal general condition observation occurred every day. Feed and drink were given ad libitum.

3 METHOD

3.1 Making of Goat Milk Yoghurt and Goat Milk Yoghurt Casein Preparation

Initially mother working culture was made by adding 0,35 grams of starter powder (Yogourmet® containingL. bulgaricus, S. thermophilus, and L. acidophilus) into 70 ml of pasteurized goat milk and incubated in 45°C for 4 hours until it reached pH 4,4-4,5. Yoghurt making followed with the addition of working mother culture in 3% concentration into 480 ml into pasteurized goat milk and incubated in 45°C for 4 hours until it reached pH 4,5-5. Goat milk yoghurt was centrifuged in 5°C with 1200 rpm for 5 minutes and then meshed to separate casein and whey. Afterwards, casein was freeze dried to stabilize casein pH. Goat milk yoghurt casein was stored in -20°C until used.

3.2 Goat Milk Yoghurt Casein Antioxidant Activity Test:

Goat milk yoghurt casein with 50 µg/mL, 75 µg/mL, 100 µg/mL, 125 µg/mL, and 150 µg/mL concentration were added into 2 mL DPPH 0,1 mM respectively. The solutions were mixed and incubated in room temperature for 30 minutes in dark room. These solutions were then measured for absorbance with spectrophotometer in λmax 516 nm. The same treatment was done to blank solution containing 2 mL DPPH 0,1 mM and 1 mL methanol p.a.

3.3 Research Design

This research has been ethically approved with certificate from Research Ethical Committee of Brawijaya University (Komisi Ethik Penelitian Universitas Brawijaya—KEP). Rattus norvegicus were randomly divided into 6 groups. Each group contained 4 Rattus norvegicus as repetition for every treatment. Negative control group (K-) was given standard feed and drink without treatment. Place of control group (KP) was given goat milk yoghurt casein 600 mg/kgBW/day. Positive control group (K+) was exposed to TCDD 100 ng/kgBW/day. Treatment group 1 (P1) was given goat milk yoghurt casein 300 mg/kgBW/day and exposed to TCDD 100 ng/kgBW/day. Treatment group 2 (P2) was given goat milk yoghurt casein 600 mg/kgBW/day and exposed to TCDD 100 ng/kgBW/day. Treatment group 3 (P3) was given goat milk yoghurt casein 900 mg/kgBW/day and exposed to TCDD 100 ng/kgBW/day. Goat milk yoghurt casein was given orally by being diluted in reverse osmosis water, while TCDD exposure was given orally diluted with corn syrup. The volume of casein solution and TCDD given was 1 ml for each. Treatment was conducted for 21 days and ended with Rattus norvegicus euthanized with cervical dislocation.
3.4 Measurement of TNF-α and IL-1β Level in Kidney

*Rattus norvegicus* was dissected and their kidneys taken and put into 10% formalin. Kidney samples were made into histopathological preparates with immunohistochemistry stain (IHC) of TNF-α and IL1-β antibody, and followed by preparates reading under light microscope with 400x magnification. The measurement of TNF-α and IL-1β was calculated by Immunoratio software.

3.5 Data Analysis

Obtained TNF-α and IL1-β data were analyzed by one-way Analysis of Variants (ANOVA) statistical analysis followed by Tukey test with the help of SPSS for Windows software.

4 RESULTS

4.1 Kidney TNF-α Level

The result of P1 treatment group (20,10007,554), P2 (17,8750 ± 9,141), and P3 (12,9550 ± 4,826) did not show significantly different results with positive control (K⁺), but were significantly different with negative control (K⁻). This provides information that goat milk yoghurt casein in 300, 600, and 900 mg/kgBW dosage managed to protect kidney from TCDD exposure injury, indicated by the inhibition of TNF-α increase, even if the inhibition is not significantly different when compared to TNF-α level from positive control (K⁺). The average level of TNF-α for every treatment can be seen in Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>TNF-α Level (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K⁻</td>
<td>23,50 ± 0,000a</td>
</tr>
<tr>
<td>K⁺</td>
<td>23,7467 ± 2,555a</td>
</tr>
<tr>
<td>KP</td>
<td>21,0300 ± 8,327ac</td>
</tr>
<tr>
<td>P1</td>
<td>20,1000 ± 7,554ad</td>
</tr>
<tr>
<td>P2</td>
<td>17,8750 ± 9,141ad</td>
</tr>
<tr>
<td>P3</td>
<td>12,9550 ± 4,826ad</td>
</tr>
</tbody>
</table>

Annotation: Different notation showed significant difference between treatment groups (p<0,05)

Table 1. The Influence of Goat Milk Casein on Kidney TNF-α Level on Average

4.2 Kidney IL-1β Level

The value from treatment group P2 (31,495 ± 2,7554) and P3 (21,615 ± 25,502) showed IL-1β increase inhibition compared to positive control (K⁺), however, there were no statistically significant difference. This provides information that goat milk yoghurt casein was able to protect against TCDD exposure in 600 and 900 mg/kgBW dosage, indicated by inhibition of IL-1β level increase. The average value of IL-1β level for every treatment group can be seen on Table 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>IL-1β Level (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K⁻</td>
<td>31,070 ± 33,267a</td>
</tr>
<tr>
<td>K⁺</td>
<td>35,700 ± 0,000a</td>
</tr>
<tr>
<td>KP</td>
<td>22,310 ± 20,79a</td>
</tr>
<tr>
<td>P1</td>
<td>43,140 ± 7,318a</td>
</tr>
<tr>
<td>P2</td>
<td>31,495 ± 2,7554a</td>
</tr>
<tr>
<td>P3</td>
<td>21,615 ±25,502a</td>
</tr>
</tbody>
</table>

Annotation: Different notation showed significant difference between treatment groups (p<0,05)

5 DISCUSSIONS

Administration of TCDD to positive control caused increase of TNF-α level (23,7467 ± 2,555) which is higher compared to negative control (23,50 ± 0,000) and increase of IL 1-β level (35,700(World Health Organization. 1997)0,000) which is higher compared to negative control (31,070 ± 33,267). The increase of TNF-α and IL 1-β was caused by TCDD exposure being accumulated in the body. TCDD will bind with AhR in cytosol and disturbed lysosomal enzymatic activity. TCDD-AhR complex would trigger CYP1A1 and CYP1B1 protein expression and induce intracellular ROS increase. This caused lipid peroxidation and lipid acid conversion through lipoxygenase pathway which will then disturbed glomerulus function (Jigyasi and Kundu, 2013). ROS is able to bind H atom from lipid, protein, and nucleotide existing within cell organelles or DNA and thus caused disfunction and DNA structure injury as well as cell organelles damage. AhR activity by TCDD also induce inflammatory cell response within kidney tubules, signified by the increase of TNF-α and IL 1-β (Manabe, 2011).
Goat milk yoghurt casein exhibited strong antioxidant activity (4.52 µg/mL), and thus able to prevent inflammation in the kidney. Administration of goat milk yoghurt casein has the most effective effect in 900 mg/kgBW/day dosage which could lower oxidative stress level, indicated by the inhibition of TNF-α level (12.9550 ± 4.826) increase and IL-1β (21.615 ± 25.502) level increase. This treatment showed highest difference compared to positive control (K+), which showed TNF-α level being 23.7467 ± 2.5555 and IL-1β level being 35.700 ± 0.000. The kidney protective effect exhibited by goat milk yoghurt casein against TCDD was possible for casein’s function as antioxidant. Milk fermentation process by LAB produces bioactive peptides (Wan et al., 2014). Goat milk yoghurt bioactive peptides would stabilize superoxide radicals by donating hydrogen atom (H). Superoxide radicals were captured by bioactive peptides from goat milk yoghurt casein and this prevented unstable radical from forming out of lipid molecules losing one hydrogen atom (H). Moreover, bioactive peptides also prevent oxygen molecule from transferring electron to peroxyl radicals and thus prevents propagation process, stopping free radicals from reacting with oxygen (Kullisaar et al., 2003).

6 CONCLUSION

Goat milk yoghurt casein may prevent the rise of proinflammatory cytokine TNF-α and IL-1β level in animal model Rattus norvegicus exposed to TCDD and thus can be used as alternative nutrition with antioxidant that can prevent cell damage caused by toxic environmental pollutants.

REFERENCES


Jugyasi, J., Kundu, R. 2013. Dose and duration dependent toxicity of Dioxin (2,3,7,8’TCDD) to few lysosomal enzymes in mice kidney. IOS Journal of Environmental Science, Toxicology and Food Technology 7(3):64-68.


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