The Effect of Quercetin towards Adipocytes Count in Toxoplasma gondii Profilin-exposed Adipocytes In Vitro

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Abstract. Toxoplasma gondii is one of the protozoan causes of chronic infection that allegedly causes obese (infectobesity). Some previous studies have showed that profilin Toxoplasma gondii has a role in inflammation by promoting interleukin-12 (IL – 12) which induce adipocyte dysfunction through the hyperplasia and hyperproliferation of adipocyte cells. Those processes lead to metabolic syndrome which increase adipocytes count through reducing insulin receptor’s sensitivity. On the other hand, Toxoplasma gondii, as an obligate intracellular parasite, can also damage the pancreatic beta cells. In response to inflammation, adipocytes produce Reactive Oxygen Species (ROS). To scavenge ROS antioxidants are required. Quercetin, an exogenous antioxidant, can be widely found in natural products that might be a promising candidate for development of antioxidant treatment interventions to prevent adipocytopathy. This research aims to explore the effects of quercetin towards Adipocytes Count stimulated from T. gondii profilin-exposed adipocytes. This research using visceral adipocyte rat that was cultured in Dulbecco's Modified Eagle Medium (DMEM). After 70% confluency, adipocytes were exposed to 20 µM T. gondii profilin and treated with four doses of quercetin; 31.25, 62.5, 125, and 250 µM that incubated 48 hours. After incubation period, adipocytes were observed using inverted microscope and were captured in high power field magnification using camera. Adipocytes were counted from each captured photo and all groups were analyzed using Analysis of variance (ANOVA) test. The results showed that quercetin significantly reduced adipocyte cell count T. gondii profilin-exposed adipocytes compared to untreated cells (ANOVA p = 0.00). The effective dose to lower adipocyte cell count was 31.25 µM. This study implies that quercetin has a potent antioxidant that can prevent toxoplasmosis-mediated adipocytopathy.

Key word: quercetin, adipocyte count, Toxoplasma gondii profilin

1. INTRODUCTION

Due to its high prevalence, obesity is a major global health issue, including in developing countries and children (Ng M, et al. 2014). The increasing number of obese patients demands more research about pathogenesis of obesity to identify more effective novel intervention targets. Lifestyle interventions and body weight reduction alone have shown unsatisfying results, especially because of the problems of discontinuity (Reever GM, et al. 2013). Several studies described infection through inflammatory pathways as a potential cause of obesity, further defined as infectobesity (Vasilakopoulou A and Roux C W 2007; Hedge V and Dhurandar NV 2013). The revelation of infectious agents playing roles in obesity leads to the idea of specific treatment modalities for obese people due to infection (Hedge V and Dhurandar NV 2013).
One of the potential pathogens involved in obesity pathogenesis is *Toxoplasma gondii* (*T. gondii*). This apicomplexan protozoa infected 30% of population around the globe (Reever GM, et al. 2013). As an intracellular parasite, *T. gondii* can infect all nucleated cells, including adipocytes (Toulah, Fawzia H, et al. 2011). As other members of the apicomplexan phylum, the actin cytoskeleton change during gliding movement is essential for *T. gondii* during host cell invasion. Profilin is an important component for actin polymerisation during actin-dependent gliding movement of *T. Gondii* (Plattner, Fabienne et al. 2008). Profilin-like protein of *T. gondii* is also an immunogenic element that stimulates inflammatory pathway through its recognition by the endosomal pattern recognition receptor (PRR), Toll-like receptor 11 (TLR 11) (Andrade WA. et al. 2013; Susanto, et al. 2014; Yarovinsky F 2014; Iskandar A et al. 2016).

As a response to inflammation, cells will generate of reactive oxygen species (ROS). ROS production leads to random and unregulated intracellular oxidation, which in turn triggers oxidation of iron, intracellular lipids, proteins, and DNA, resulting in vast intracellular molecular damage. Many diseases potentially arise as the consequence, such as neurodegenerative diseases, atherosclerosis, aging process, and metabolic syndrome (Holmström, Kira M and Toren F. 2014). To scavenge ROS and intracellular damage, antioxidants are needed. Body cells are equipped with endogenous enzimatic antioxidant. Glutathione (GSH), a cystein protein contain tripeptide, has an important role in cellular redox (Holmström, Kira M and Toren F. 2014).

Synthetic antioxidants are commercially available, but the safety and toxicity risks of synthetic antioxidants are higher than natural antioxidant (Ebrahimzadeh, et. al. 2008). Flavonoid is one of the most well recognized exogenous natural antioxidants, which is produced outside the body. Quercetin, a type of flavonoid, can be widely found in natural products, such as onion, cherry, tomato, broccoli, apple, green tea, black tea, grape, or blueberry (Fazel S et al. 2015 ; Ratnawati R and Hernowati TE. 2015). Previous studies showed that quercetin inhibits proliferation and differentiation of pre-adipocyte culture by decreasing the expression of adipokytokines, such as CCAAT/enhancer binding protein alpha (C/EBPα) and sterol regulatory element binding protein 1c (SBREP-1c) (Ratnawati R and Hernowati TE. 2015).

This study aims to explore the potency of quercetin to scavenge ROS free radicals and to stimulate GSH endogenous antioxidant by exposure of *T. gondii* profilin-exposed adipocytes. *T. gondii* profilin can be recognised by TLR 11 and stimulate inflammatory pathway that has causal relationship with adipocytopathy. The results of this study could be implied in development of potential antioxidant treatment interventions to prevent toxoplasmosis-mediated adipocytopathy.

### 2. MATERIAL AND METHOD

#### 2.1. Experimental design

This research used true experimental study using adipocyte culture that exposed to profilin *T. gondii* and treated by quercetin. Samples were divided into 6 groups. Each group contains four replication samples each, namely : Negative control (maturated only); Positive control (maturated and 20 µM *T. gondii* profilin exposed); Q 31.25 (maturated, 20 µM *T. gondii* profilin and Quercetin 31.25 µM exposed); Q 62.5 (maturated, 20 µM *T. gondii* profilin and Quercetin 62.5 µM exposed); Q 125 (maturated, 20 µM *T. gondii* profilin and Quercetin 125 µM exposed), and Q 250 (maturated, 20 µM *T. gondii* profilin and Quercetin 250 µM exposed).

**Adipocyte culture**

Adipocyte culture was developed from adipose tissue of 1 month old wistar rats. Adipose tissue was collected from peritoneal and retroperitoneal regions. The tissue was munched mechanically by scalpel or scissors and digested enzymatically by type 1 collagenase (Worthington).

The obtained cells were maintained in a culture flask and nourished using Dulbecco’s Modified Eagle Medium (DMEM) (Gibco©) containing sodium bicarbonate, L-glutamine, antibiotics (100U/ml
Pennicillin and 100 mg/ml Streptomycin (MP Biomedicals, LCC)), and supplemented with 10% of heat-inactivated fetal bovine serum (Gibco©).

Adipocyte culture were kept at 37° C, 5% CO₂ environment. Culture media were changed every 48 hours until confluency was achieved (Ratnawati R and Hernowati TE. 2015; Zhu S et al 2010). After the cells in the culture flask were confluent, the researchers then subcultured the cells into 12-well culture plates. The cells in the culture plates achieve the same treatment as in the culture flask.

2.2. Profilin and quercetin exposure
All of the confluent groups of pre-adipocyte culture were maturated by 0,1 µM dexamethasone, 0,5 mM isobutylmethyloxanthine, and 0,1 µM insulin (Ratnawati R and Hernowati TE. 2015). Simultaneously with maturation process, some of the cell cultures were exposed to 20 µM *T. gondii* profilin (MyBioSource) (positive control and four quercetin treatment groups) and quercetin (Sigma) (four quercetin treatment groups) dissolved in DMEM then incubated for 48 hours (Ratnawati R and Hernowati TE. 2015; Mochamad R, et al. 2013).

2.3. Adipocyte count measurement
After 48 hours of incubation, adipocytes were observed using inverted microscope and were captured in high power field magnification using camera.

2.4. Statistical analysis
The number of adipocytes in each groups were presented as mean and standard error of the mean (SEM) of four independent replications. Homogen and normal data (p>0,05) were analysed by ANOVA test.

2.5. Ethical statement
All procedures involving animals were in accordance with the ethical standards of Faculty of Medicine, Brawijaya University (No 99/EC/KEPK-PSPD/03/2017).

3. Result
The results showed that the mean of adipocyte number in positive control group (95) was higher compared to negative control (81.33). The decline in ROS levels were observed in all quercetin treatment groups (Q 31.25= 84.67, Q 62.5 = 71.67, Q 125 = 73.67 and Q 250 = 57.33) compared to negative or positive control groups (Figure 1).
From statistical analysis using ANOVA, the results showed that quercetin significantly reduced adipocyte cell count T. gondii profilin-exposed adipocytes compared to untreated cells (ANOVA p = 0.00).

4. DISCUSSION

During T. gondii infection, profilin acts as a pathogen associated molecular pattern (PAMP) recognised by the endosomal Toll-like receptor, TLR-11 (Yarovinsky F 2014). This recognition further stimulates the inflammatory pathway, eventually leading to the production of ROS (Furukawa S, et al. 2004).

The results showed there was significant difference between negative and positive control, which corresponds with the theoretical basis that profilin of T. gondii generates intracellular oxidative stress. The excessive ROS production stimulated by T. gondii profilin was unable to be neutralized by endogenous antioxidants alone (Marī M, et al. 2009), thus it stimulates intracellular molecular damage of lipids, proteins, and DNA. These damages in turn becomes the basic mechanisms of diseases, including hiperplasia of adipocyte that can lead to obesity and metabolic syndrome (Furukawa S, et al. 2004); Holmström, Kira M and Toren F. 2014).

The results here showed significant differences between control and all doses of quercetin groups. These results confirmed the theoretical basis that quercetin acts as an antioxidant in T. gondii profilin-exposed by significantly decreasing ROS levels (Fazel S, et al. 2015; Mochamad R, et al. 2013). Consistent with this results, a previous study by Lee et al. in 2013 observed quercetin effectivity as a ROS scavenger using H2DCFDA staining in fibrosarcoma culture in vitro. The study revealed that quercetin treatment was able to scavenge ROS level at doses of 5, 10, or 50 50 µg/ml (Lee DE, et al. 2013). In this study, the researchers found no significant difference among three different doses (31,25, 62,5 and 125 µM) of quercetin groups. This indicated that all doses employed in this study have similar effectivity as antioxidant, where the minimum dose of 31.25µM showed similar effectivity to those of higher doses (62.5 and 125 µM).
5. Conclusion
This study implies that quercetin has a potent antioxidant that can prevent toxoplasmosis-mediated adipocytopathy.

Acknowledgements
This research was supported by BPPM of Faculty of Medicine, Universitas Brawijaya, and Indonesia’s Ministry of Research and Technology and High Education.

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