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Catechins of GMB-4 Clone Inhibits Adipogenesis Through PPARγ and Adiponectin in Primary Culture of Visceral Preadipocyte of *Rattus Norvegicus* Wistar

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KEYWORDS

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Abstract Catechins of green tea (*Camelia sinensis*) GMB4 clone may serve as a potential therapeutic anti-obesity agent, probably through its effects on preadipocytes. Thus, to evaluate such anti-obesity effects, we performed series of in vitro experiments using primary cultures of visceral preadipocytes from *Rattus norvegicus* strain Wistar. Quick Cell Proliferation assay, Oil Red-O staining, ELISA and immunocytochemistry were used to determine the effects of 25 µM, 50 µM, 75 µM, and 100 µM catechins on primary culture of preadipocytes, particularly on cell viability and differentiation as well as on expression of relevant obesity genes i.e. PPARγ and adiponectin levels. The results showed that there were no significant differences on preadipocytes viability among control and catechins treatments except in cells treated with 50 µM catechins (means ± SD = 128 ± 2.47) which resulted 28% higher viability than control ($p = 0.037$). Catechins inhibited preadipocytes differentiation into adipocytes, at 100 µM up to 78% lower than control. The level of PPAR-γ apparently was reduced by catechins, but statistically significant only at 75 µM ($p = 0.029$). In contrast, the adiponectin level on preadipocytes increased by catechins at 75 µM and 100 µM (0.786 ± 0.126 and 0.673 ± 0.319 ng/ml; control: 0.077 ± 0.017, $p \leq 0.01$). In conclusions, our data revealed that desired anti-obesity effects of catechins of green tea GMB4 clone on visceral preadipocytes were concentration-dependent; at dosage 50 µM catechins enhanced cell viability; at more than 75 µM inhibited differentiation of preadipocytes and was associated with lower PPAR-γ and higher adiponectin levels.

Introduction

Obesity occurs as a result of imbalance between energy intake and energy expenditure. This excess energy is stored in adipose tissue in the form of triglyceride. Over consumption of energy, sugar and saturated fat along with lack of physical exercise has triggered an increase in obesity (Choe, Huh, Hwang, Kim, & Kim, 2016; Pellegrinelli, Carobbio, & Vidal-Puig, 2016). The increase of triglyceride in adipose tissue inside the body may occur due to hyperplasia and hypertrophy through adipogenesis. Hyperplasia is the increase of adipocytes whereas hypertrophy is the increase of the size of adipocytes. The development of adipocytes begins with the proliferation and differentiation of preadipocytes cells into mature adipocytes. PPARγ playing a major role in adipogenesis induces the differentiation of adipocytes. PPARγ is a potential inductor in adipogenesis and also capable to trigger transdifferentiation in cultures of adipocyte myoblast, mainly through co-expression with C/EBPα (CCAAT/enhancer binding protein) (Rosen, Walkey, Puigserver, & Spiegelman, 2000; Tontonoz & Spiegelman, 2008; Siersbaek, Nielsen, & Mandrup, 2010). The

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activation of PPARγ induces the differentiation of adipocyte and the accumulation of lipids by modulating a large amount of regulator genes of adipogenesis. The same pattern is also applies to the metabolism and the absorption of lipids (Lefterova, Haakonsson, Lazar, & Mandrup, 2014; Moseti, Regassa, & Kim, 2016). Adiponectins are adipocytokines acting as anti atherogenesis, anti-inflammation and anti-diabetic (Hsu & Yen, 2007). Adiponectins play a direct role in the regulation of metabolic pathways in adipose tissue, the liver and skeletal muscles (Ajuwon & Spurlock, 2005) The genetic expression of adiponectin in mature adipocytes are regulated by several transcription factors, including PPARγ, C/EBP-α, liver receptor homolog-1, dan SREBP1 (Cho et al., 2007).

PPARγ is a regulator of adipogenesis and has been a subject of extensive study regarding to obesity. PPARγ is mostly expressed in adipose tissue and is required for the differentiation of adipocytes, both in vivo and in vitro (Stienstra, Duval, Müller, & Kersten, 2007). Cells which do not contain PPARγ are incapable to differentiate into adipocytes and experiences an ablation on specific adipocytes which causes hypocellularity of adipocytes and adiposity decrease (Berger, 2005).

In an in vivo study with rodents, specific activation of PPARγ induces the differentiation of preadipocytes into adipocytes. The work of (Ajuwon & Spurlock, 2005) showed that catechins, quercetins and kaempferols suppress the differentiation of preadipocytes 3T3L1 by suppressing the expression of SREBP-1 and the transcription factors PPARγ and C/EBP-α. Once the adipocytes were fully differentiated, there was no significant increase in the expression of PPARγ (Cho et al., 2007). Several studies showed the effects of EGCG in decreasing the expression of iNOS through competitive inhibition of arginine and tetrahydrobiopterin bonds, and this shows that the gallate structure of the catechins plays an important role (Chien, Chen, Lu, & Sheu, 2005). Isolated EGCG was capable to decrease the mRNA of PPARγ, SREBP-1, C/EBPα andTNFα genes of human visceral adipocytes; however, adiponectin was largely unaffected by EGCG (Santangelo et al., 2007). Flavonoids is also known to affect lipolysis and adipogenesis in adipocytes. Several flavonoids exhibit lipolytic activity synergistic with epinephrine in preadipocytes in rats (Kuppusamy & Das, 1994).

Green tea, which contains polyphenolic flavonoid compounds called catechins as its main component, is widely consumed throughout Asia (Kao, Hiipakka, & Liao, 2000). Catechins are dominant componds of polyphenol, consisting of epicatechins, epigallocatechins, epicatechins-3-gallate and epigallocatechins-3-gallate. Green tea extract is known for its antidiabetic and anti-obesity effects (Broadhurst, Polansky, & Anderson, 2000). Green tea polyphenols can reduce the fat deposits of induced Wistar rats with high-fat diets (Tian et al., 2013). Ratnawati et al. (2009) have performed isolation and purification of catechins and EGCG from green tea GMB4 clone obtained from the Gambung Institute for Tea and Quinine. One hundred grams of green tea contained 12 to 14% isolated catechins. The result of HPLC analysis showed that EGCG and ECG were main components from catechins isolation of green tea GMB-4 clone (Susanti et al, 2015). Catechins are a class of catechin compound group consisting of epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), epigallocatechin gallate (EGCG), catechin, gallocatechin, catechin gallate and gallocatechin gallate. EGCG is a predominant catechin which has content from 48% up to 55% in total polyphenols of green tea leaves (Velayutham, Babu, & Liu, 2008).

Green tea catechins administered to the preadipocyte culture during adipocyte differentiation will decrease lipid accumulation and decrease the expressions of markers of adipocyte differentiation, including PPARγ, FAS, SCD-1 and C/EBP-α. Catechins stimulate the

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expression and secretion of adiponectin in adipocytes (Cho et al., 2007).

Neither in-vivo nor in-vitro research on catechins isolated from green tea GMB4 clone in relation to metabolic syndromes have ever been performed. As such, this study was needed to understand the effects of catechins isolated from GMB4 clone on rat preadipocyte culture toward the transcription factor protein PPARγ and adiponectin.

Material and methods

Cell Isolation and Cultivation

Fibrous tissue and blood vessels were discarded, then the fibroblast like adipose tissue was washed and minced. Tissue suspension was incubated with 5 mg/7ml type I collagenase (Sigma, USA) for 60 minutes at 37°C in a shaking water bath. Incubation was terminated by adding serum-free culture medium (DMEM/F12 (1:1)). After filtration with a microfilter (250 µm), the cell suspension was rotated at 2,500 rpm (700xg) for 7 minutes, and the fat layer (mature adipocytesand fat droplets) in the supernatant was discarded. Pellets which contained fibroblast-like preadipocytes were resuspended with culture media then rotated at 2,500 rpm (700x g) for 7 minutes. Pellets were then resuspended with culture media. The cell suspension was grown on acover glass over a culture plate and incubated at 37°C, 5% CO_2 for 24 hours. The cells were washed once every 3 days (Ratnawati, Indra, & Satuman, 2008; Indra & Widodo, 2005).

Stimulating Differentiation of Adipocyte

Upon preconfluence, the rat preadipocytes were grown in adipogenic media (DMEM/F12), enhanced with 66 nM insulin, 100 nM dexamethasone, 0, 2 mM IBMX and 10 µg/ml TZD) for cell differentiation. The cell suspension was grown on a cover glass over a culture plate and incubated at 37°C, 5% CO_2 for 24 hours. The cells were washed once every 3 days (Santangelo et al., 2007).

Treatment of Catechins Isolated from Green Tea GMB4 Clone

Catechins were isolated from green tea GMB4 clone from the Gambung Institute for Tea and Quinine Research, Bandung, which was performed at the Chemistry division at the Faculty of Sciences at Institute of Technology Bandung (Ratnawati et al., 2009). (Ajuwon & Spurlock, 2005) The catechins which were dissolved in serum-free medium was exposed to several different concentrations: 25, 50, 75 and 100 µM for 3 replications each (Ajuwon & Spurlock, 2005). The cells were incubated for 24 hours then measured for levels of PPARγ and adiponectin, whereas proliferation, differentiation and expression of the two proteins were observed on the cell (Ajuwon & Spurlock, 2005). Protein levels on the culture media were measured using ELISA, whereas proliferation, differentiation and protein expression were observed usingquick cell proliferation assay, Oil Red-O staining, and immunocytochemistry, respectively.

Measurement of Preadipocyte Viability using Quick Cell Proliferation Assay

Cell viability was measured using a quick cell proliferation assay kit from Biovison, USA. Live cells were counted previously using a hemocytometer. Cells were stained using trypan blue; live cells would not absorb the trypan blue, while the dead cells would absorb its color (Hemmrich, Von Heimburg, Cierpka, Haydarlioglu, & Pallua, 2005). Preadipocytes in 100 µl culture medium on a 96-well culture plate containing 5 x 10^3 cells per well were incubated for 24 hours at 37˚C. A 10µL solution of quick cell proliferation assay [WST-1 reagent (lyophilized) and electro coupling solution (ECS)] was added to each well. The cells were then incubated for 30 minutes to 4 hours at 37 °C and 5% CO_2. After incubation, cells were then shaking for 1 minute (formazan produced by the cells would appear as a yellowish residue on the bottom of the well). Each sample was then

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measured for its absorbance at 650 nm with an ELISA reader. This works by measuring metabolic activity which shows cell viability. Metabolic activity was measured on the cell population by incubating Tetrazolium salt on the cells (Sambrook, Fritsch, & Maniatis, 1989). Viable cells would break down the tetrazolium into yellowish formazan. The ELISA reader then analyzes the color of the formazan. The intensity of the yellowish color of the formazan reflects the number of viable cells.

Measurement of the Differentiation of Preadipocytes using Oil Red-O Staining

Oil Red-O is a coloring agent that specifically binds fatty compounds (Négrel and Dani, 2001) thus the measurement of lipid droplets for this study was performed using Oil Red-O coloring. Oil Red-O staining was used to measure cell differentiation. The colored cells would be the differentiated cells, which would exhibit lipid droplet formations (Rode & Eisel, 2008). Adipocytes were washed using PBS then stained with 0.5% Oil Red-O sourced from Sigma, USA (Ajuwon & Spurlock, 2005).

Measurement of Adiponectin Levels using ELISA (Enzyme Linked Immunoabsorbent Assay)

Procedures for the measurement of adiponectin levels followed the ELISA kit manual from Otsuka Pharmaceutical. Ltd., Japan. A sample of adipocyte media (1:100 µL) containing adiponectin was mobilized on an ELISA antimouse adiponectin plated micro plate. The sample was then incubated for 1 hour at 29°C and rinsed with 200 µL wash buffer for 3 times. 100 µL secondary antibody (biotinylated rabbit antimouse) which was incubated for 60 minutes at 27°C then washed again with 200 µL wash buffer for 3 times. Substrate solution was then added, followed by incubation for 15 minutes at 27°C then ended by adding 100 µL stop solution and reading it at λ 450 nm on the ELISA reader.

Measurement of PPARγ Levels using ELISA (Enzyme Linked Immunoabsorbent Assay)

Procedures for the measurement of PPARγ levels followed the ELISA kit manual from Cusabio Biotech Co., Ltd., China. There was 100 µLof the suspension sample solution from the total isolated protein of the adipocytes and blanks were placed into the well then incubated for 2 hours at 37°C. The solution was then discarded and then added with 100 µL biotin antibody working solution, then incubated for 1 hour at 37°C. After that it washed with 200 µL wash buffer for 3 times, then added with 100 µL HRP avidinand incubated for 1 hour at 37°C. Then, it washed again with 200 µL wash-buffer for 3 times, then it was added with 90 µL TMB substrate then it was incubated for 10 to 30 minutes at 37°C. It was ended by adding 50µL to stop solution, finally it was read at λ 450 nm on the ELISA reader.

Observation of the Expression of PPAR-γ and Adiponectin using Immunocytochemistry

The procedures used were based on (Harmon, Patel, & Harp, 2002) which for this research was used to identify the presence of PPARγ and adiponectin in the preadipocytes. For each treatment, the cells were fixated with absolute methanol for 20 minutes. The cells were washed with PBS pH 7.4 for 3 times at 5 minutes each. The fixated cells were storable at 4°C in PBS before being treated for immunocytochemistry. Cells were then dropped with H_2O_2 solution inPBS for 20 minutes then washed again with PBS pH 7.4 three times for 5 minutes. Afterwards, the blocking serum Fetal Bovine Serum (FBS) 10% (w/v) was dropped onto the cells for 30 minutes, then washed with PBS pH 7.4 three times for 5 minutes each (primary antibody incubation in FBS serum 1:250 at 4°C for 24 hours, then cells were placed in room temperature for 15 minutes. Cells were then washed with PBS twice for 5 minutes each. Cells were then incubated with the secondary antibody IgG Biotin *anti rabbit* in PBS 1:500 for 1 hour at room

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temperature. Then, cells were washed with PBS pH 7.4 three times for 5 minutes each. Cells were dropped with SA-HRP in PBS (1:400) for 60 minutes at room temperature, then it was washed with PBS twice for 5 minutes each. Finally, the cells were dropped with Diamino Benzidine (DAB) in DAB buffer. Cells were washed with tap water then aquadest for 10 minutes. Then, the cells were placed on an object glass and dropped with entellant and placed in room temperature. The cell preparate was then observed under a microscope then the number of brown-colored cells was counted (indicating the expression of PPARγ and adiponectin. Expression of PPARγ can be seen in the brown-stained cell nuclei. The expression of adiponectin is visible in the brown-stained cytoplasms.

Data Analysis

After all the data from observation and measurement were gathered, analysis was performed using parametric statistics. The resulting data are presented in mean±SD. The data is in the form of quantitative and qualitative data. All data were analysed using SPSS version 15. For quantitative data, one-way ANOVA analysis was used.

Results and discussion

This research was conducted in-vitro using preadipocytes isolated from *Rattus norvegicus* Wistar grown in adipogenic medium then exposed for 24 hours to catechins isolated from green tea GMB4 clone with a concentration of 25 µM, 50 µM, 75 µM and 100 µM. The results were the development of fibroblast-like preadipocytes into mature adipocytes as marked by fibroblasts formations which transform into lipid droplet formations as seen in Figure 1.

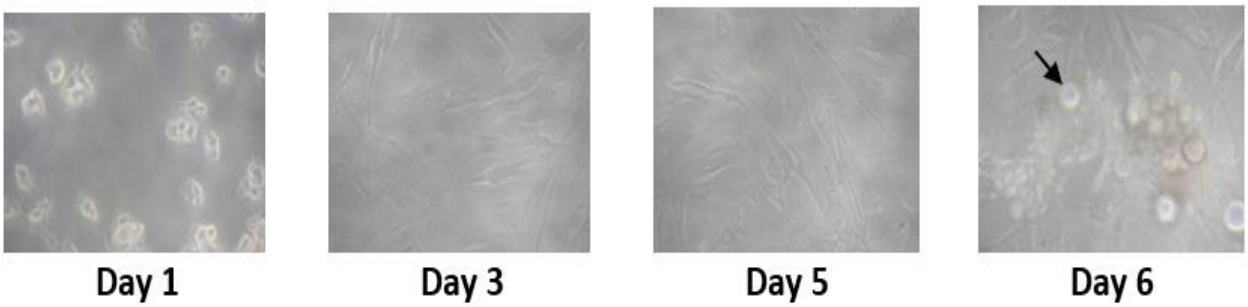

Figure 1. The development of fibroblast-like preadipocytes into mature adipocytes were marked by fibroblast formations on day 1 until day 3, where the fibroblast-like preadipocytes have yet developed into preadipocytes that look like fibroblasts (elliptical); on day 5, the preadipocytes where incubated in differentiating medium, so that on day 6 the lipid droplets are visible (400X magnification using the Olympus CX31 photomicroscope).

The Viability of Preadipocytes

The effect of isolated catechins on the viability of *Rattus norvegicus* Wistar preadipocytes was performed using *Quick Cell Proliferation Assay.* As can be seen in Table 1, after 24 hours catechins exposure, the catechins concentrations of 25 µM, 50 µM, 75 µM caused higher viability of preadipocytes at (109±16.36), (109±16.36), and (117±14.92) consecutively compared to control. Interesting that 100 µM catechins concentration affected a lower viability of adipocytes compare to control at (95.92±1.16).

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Table 1. Results of the Quantification of the Viability of Preadipocytes, Preadipocyte Differentiation, PPARγ Levels (pg/ml), and Adiponectin Levels (ng/ml) analysed using One-way ANOVA

Treatment Catechins	Viability of Preadipocytes	Preadipocyte Differentiation	PPARγ Levels (pg/ml)	Adiponectin Levels (ng/ml)
Control	100±2.21 (a*	8.67±3.83 (c*	13926.67±4751 (bc*	0.077±0.017 (a*
25 µM	109±16.36 (ab*	5.07±2.69 (b*	21926.67±3442 (c*	0.215±0.089 (a*
50 µM	128±2.47 (b*	5.53±3.04 (b*	13326.67±6694 (bc*	0.520±0.126 (ab*
75 µM	117±14.92 (ab*	7.80±2.60 (bc*	1660±1000 (a*	0.786±0.126 (b*
100 µM	95.92±1.16 (a*	1.87±1.36 (a*	5660±2253 (ab*	0.673±0.319 (b*

Note: *Different notations denote significant differences
*Same notations denote negligible differences

It seems that a different catechins concentration had a different effect, as 50 µM of catechins might trigger 28% higher cell viability but 100 µM of catechins might trigger lower cell viability. Moreover, the others catechins concentration presented lower viability than 50 µM concentration, however, there were still higher than control.

The inhibition of the differentiation of Preadipocytes

In order to discover the inhibition of preadipocytes differentiation, Oil Red O was used to stain lipid droplets. After being induced with differentiation stimulators, lipid droplets were identified. The density of the lipid droplets in the control group tend to be higher than the 25µM (5.07±2.69) and 50 µM (5.53±3.04) treatment groups. The density of lipid droplets at a concentration of 75 µM (7.80±2.60) tend to be higher than at 25 µM (5.07±2.69) and 50 µM (5.53±3.04), but is not unlike the control group. At a concentration of 100 µM (1.87±1.36), the density of the lipid droplets was found to be far lower than the control group, as well as the 25 µM (5.07±2.69), 50 µM (5.53±3.04), and 75 µM (7.80±2.60) treatment groups.

Thus, from this research, we can conclude that exposure of catechins with a concentration of 100 µM on *Rattus norvegicus* Wistar preadipocytes is capable of inhibiting the differentiation of preadipocytes into adipocytes, with a differentiation inhibition percentage of 78% compared to control.

The PPARγ Levels of Preadipocytes

To understand the influence of catechins on PPARγ, measurement of the levels and expressions of PPARγ was performed by using ELISA. on *Rattus norvegicus* Wistar preadipocytes exposed to catechins isolated from green tea GMB4 clone with concentrations of 25 µM; 50 µM; 75 µM and 100 µM with 3 replications. Exposure to catechins isolated from green tea GMB4 clone on preadipocytes resulted in a significantly lower measured level of PPARγ than the control group (Table 1). At a concentration of 25 µM (21,926.67±3,442), a far higher amount of PPARγ than the control group was observed (13,926.67±4,751), at a concentration of 50 µM (13,326.67±6,694) levels PPARγ did not differ significantly from control. At a concentration of 75 µM (1,660±1,000), PPARγ levels were far lower than the control group. At a concentration of 100µM (5,660±2,253), PPARγ levels were lower than control. The highest level of PPARγ was observed at a concentration of 25 µM (21,926.67±3,442) and the lowest at 75 µM (1660±1000) as presented in Table 1.

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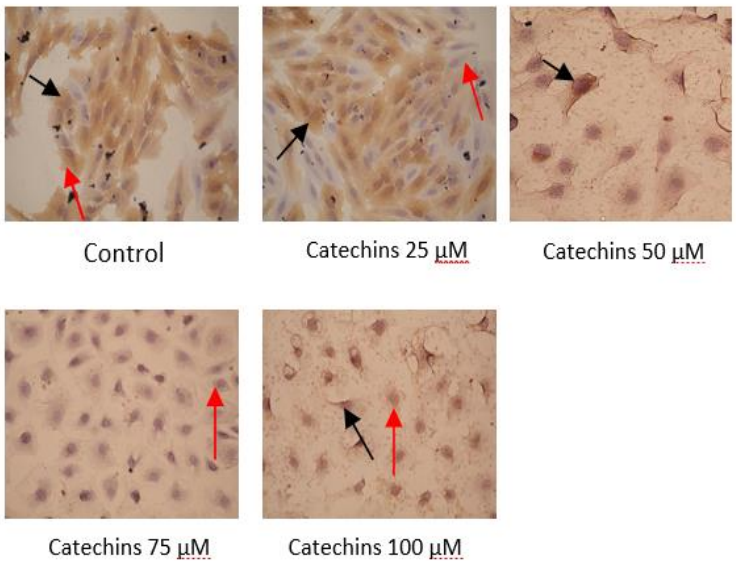

Figure 2. Expression of PPARγ in preadipocytes (marked by arrows) upon exposure to catechins for 24 hours. Expression of PPARγ appears to be higher in the control group; however, in the treatment group exposed to catechins, the expressions are lower. Black arrows denote positive PPARγ expression, and red arrows denote negative or no PPARγ expression (400X magnification with an Olympus CX31 photomicroscope).

After induction in differentiation medium and exposed with catechins for 6 days, cells were found to have formed a monolayer and confluent, thus PPARγ were observed using immunocytochemical staining using DAB (Diamino Benzidine). Figure 2 showed higher PPARγ expression levels in the control group than the treatment groups which were exposed to catechins. Similar results were found in the 25 µM treatment groups which had slightly lower expression levels than the 50 µM treatment group. A significant difference was found in the 75 µM treatment group where no PPARγ expression was observed. This is shown by the preparation of cell smears which did not absorb the DAB stains and resulted in blue-colored cells, however, PPAR expression levels were higher at a concentration of 100 µM. Thus, it can be concluded that the lowest expression levels of PPARγ were found at an exposure concentration of 75 µM and 100 µM catechins on preadipocytes of *Rattus norvegicus* Wistar. This seems to be the best concentration level of PPARγ which is the lowest, but not for 25 µM that was higher than control and 50 µM that was equal to control.

The Adiponectin Levels of Preadipocytes

Adiponectin levels were measured using ELISA on preadipocytes of *Rattus norvegicus* Wistar exposed to catechins isolated from green tea GMB4 clone at concentrations of 25 µM; 50 µM; 75 µM and 100 µM with 3 repetitions show significantly different levels from control. The highest levels of adiponectin were found at a concentration of 75 µM (0.078±0.012) and the lowest at 25 µM (0.215±0.089). Exposure to catechins with a concentration of 25 µM on preadipocytes increased adiponectin level (0.215±0.089) compared to control (0.77±0.017), so did concentrations of 50 µM (0.052±0.126), 75 µM (0.78±0.0126) and 100 µM (0.673±0.319). It referred to Table 1.

Immunocytochemical staining using DAB (Diamino Benzidine) showed lower expression of adiponectin in the control group than the treatment groups which were exposed to catechins. In Figure 3, expressions of adiponectin in the control group and the 25 µM

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treatment group were low, whereas at 50 µM expressions were higher albeit insignificantly. Significant differences from control were found at concentrations of 75 µM and 100 µM where expressions were found to be higher. This is identified by the prepared cell smears which did not absorb the DAB staining, the cells being bluer. From these results, it can be understood that higher adiponectin levels were found at concentrations of 75 µM and 100 µM, the lowest levels at concentrations of 25 µM, whereas 50 µM was not too different from control.

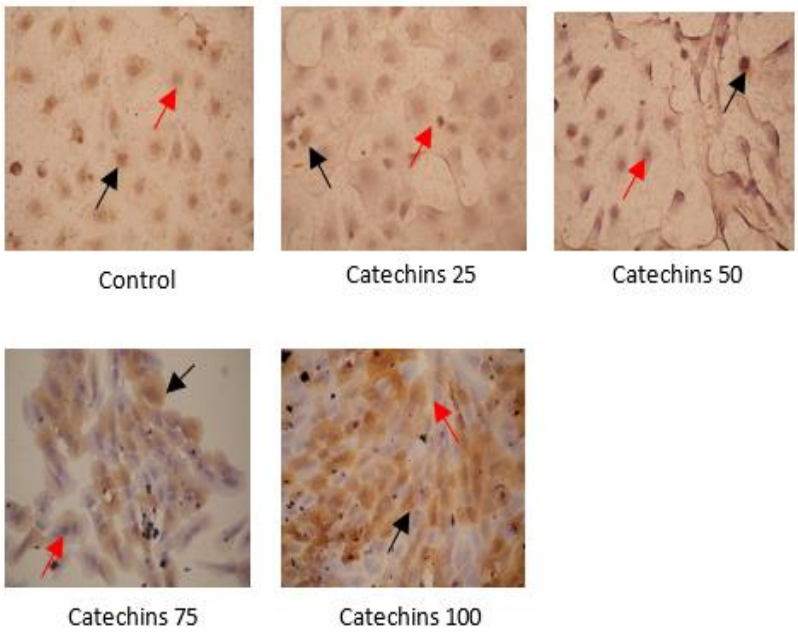

Figure 3. Expressions of adiponectin (arrows) on preadipocytes upon exposure to catechins for 24 hours. In the control group, expressions were found to be low, however, in treatment groups, expressions were lower at concentrations of 75 µM and 100 µM. Black arrows denote adiponectin in expression or positive, whereas red arrows denote no expression or negative. (400X magnification using an Olympus CX31 photomicroscope).

This study found that at a concentration of 100 µM of catechins were able to inhibit the differentiation of preadipocytes into adipocytes. At 75 µM the number of cells that differentiated increased, albeit insignificantly compared to control and the 25 µM and 50 µM treatment groups. The study showed that exposure of catechins at a concentration of 100 µM can significantly decrease cell differentiation compared to 75 µM. It is suspected that at this concentration, catechins are capable of inhibiting the maturation of adipocytes and accelerate apoptosis. (Passamonti et al., 2009) found that catechins, EGCG at a concentration of 100 µM, significantly increases the number of cells that go through apoptosis. Catechins are capable of suppressing the differentiation of adipocytes (Ajuwon & Spurlock, 2005), this is in line with (Bost, Aouadi, Caron, & Binétruy, 2005) who found that tea catechins are capable of suppressing the expression of PPARγ and C/EBP-α on 3T3-L1 preadipocytes. Adipogenic inhibition by the tea catechins seems to occur during the differentiation phase. Once the 3T3-L1 preadipocytes were fully differentiated, the catechins did not affect lipid metabolism nor the accumulation of triacylglycerol (Ardévol, Bladé, Salvadó, & Arola, 2000; Furuyashiki et al., 2004).

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Furthermore, according to (Passamonti et al., 2009), it is suspected that EGCG plays a role in the inhibition of the signal paths of ERKs in adipocytes which eventually inhibits lipogenesis. ERKs are necessary for the differentiation of cell 3T3-L1 preadipocytes into adipocytes. Specific transcription factors, PPARγ, is a substrate to ERK and their phosphorylation lowers PPARγ activity and inhibits the differentiation of adipocytes (Lin, Della-Fera, & Baile, 2005). The data on cell viability showed that the catechins did not affect cell viability, except at a concentration of 50 µM, where cell viability was higher than control. This data is supported by (Ajuwon & Spurlock, 2005) who showed that catechins did not affect the viability and cytotoxicity of 3T3-L1 preadipocytes.

This study found that flavonoids, in this case catechins, intervene adipogenesis by inhibiting the differentiation of preadipocytes of Rattus norvegicus Wistar through decreasing the expression of the adipogenic transcription factor PPARγ. This study found that the expression of PPARγ decreases along with an increase in the concentration of the exposure of catechins, whereas the expression of adiponectin increased. Confirmed with the immuno-cytochemical results, exposure to the catechins did decrease expressions of the intracellular protein PPARγ. Based on results of one-way ANOVA, the lowest levels of PPARγ were found at concentrations of 75 µM and 100 µM. This shows that the catechins at said concentrations are indeed capable of lowering levels of PPARγ upon to exposure for 24 hours. These results are in line with the studies of (Ajuwon & Spurlock, 2005) where catechins (3, 3' 4', 5, 7-flavanpentol) at a concentration of 100 µM were capable of inhibiting the mRNA expression of PPARγ. These results are also in line with (Santangelo et al., 2007) where EGCG 100 µM were capable of decreasing mRNA levels of PPAR, SREBP-1, C/EBP and TNF gene in human visceral adipose tissue. Adipose tissue produces and secretes PPARγ and C/EBPα which play a role in the initial stages of the differentiation of adipocytes (Rosen et al., 2000). Adiponectin is adipocytokine which plays a role as antiatherogenic, antiinflammation and antidiabetic (Hsu & Yen, 2007). The expression of adiponectin on mature adipocyte is regulated by several transcription factors, including PPARγ, C/EBP-α and SREBP1 (Cho et al., 2007). These results are in line with our results of highest adiponectin levels at catechins concentrations of 75 µM and 100 µM. The decrease in adipogenesis is suspected to be through the inhibition of PPARγ as reported by (Hsu & Yen, 2007) which showed that the exposure of flavonoids on 3T3-L1 preadipocytes are capable of inhibiting adipogenesis by suppressing the expressions of PPARγ, C/EBPα, and leptin as well as increasing the expression of adiponectin. When green tea catechins are administered during the differentiation of adipocytes on preadipocyte culture, lipid accumulation and expression of markers of adipocyte differentiation, including PPARγ, FAS (fatty acid synthase), SCD-1, and C/EBP-α will decrease. Catechins, in this case (-) catechins stimulate the expression of the protein and the secretion of adiponectin in adipocytes (Cho et al., 2007).

This study needs to be supported by further research on the addition of the duration of exposure to catechins so the concentration can run optimally. Aside from that, further research is also in order regarding the inhibition of the other adipocytokines leptin, resistin and TNF-α through the inhibition of the transcription factors SREBP-1 and C/EBP-α. A further in-vivo study is also needed, to support the potential of

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catechins isolated from green tea GMB4 clone as a therapeutic agent for obesity.

Conclusion

Exposure to catechins isolated from green tea GMB4 clone improved the cell viability of preadipocytes by up to 28% at a concentration of 50 µM, compared to control. The differentiation of preadipocytes into adipocytes tends to decrease along with an increase in the concentration of catechins. The catechins show lower expressions of PPARγ and adiponectin at a concentration of 75 µM compared to control.

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