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Mangosteen Peel Extract (*Garcinia mangostana* L) and Its Compounds Lower Lipid Content on Adipogenesis Cells Model (3T3-L1)

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ABSTRACT

Obesity is one of the risk factor for dyslipidemia incident, cancer, diabetes mellitus, and cardiovascular disease. Treatment of obesity using common commercial drugs shows low rate of success, hence natural phytochemicals may provide better or more efficient therapeutic approach. Mangosteen (*Garcinia mangostana* L.) peel extract contains xanthones and it is potentially used as alternative medicine for obesity. This research was done to determine the anti-obesity characteristics of mangosteen peel extract (MPE) and xanthones (α-mangostin (AM) and γ-mangostin (GM) on 3T3L1 cell line.Anti-obesity effects of MPE and xanthones were investigated using differentiated-3T3L1 preadipocyte cells. Inhibitory activity of the extract and compounds on the production of triglyceride (TG), cholesterol (CHOL), glucose-6-phosphate dehydrogenase (G6PDH) activity, and lipid droplets were examined. MPE and its compound were capable to inhibit the production of TG, CHOL, G6PDH, lipid droplets. MPE 50 μg/mL and GM 75 μM were the most active to lower TG 57.95% and 59.72%, CHOL 33.33% and 31.68%, G6PDH 52.90%, 41.95%, lipid droplets 72.99% and 70.07% respectively. In conclusion mangosteen peel extract, γ-mangostinare the most active antiobesity compared to α-mangostin.

Keywords: Glucose-6-phosphate dehydrogenase, mangosteen, obesity, xanthones, 3T3-L1 cells

1. Introduction

Obesity is a complex disorder that has effects on the normal functions of the body. Obesity becomes a threat in worldwide public health, as obesity involved in various diseases, such as hypertension, coronary heart disease, aosteoarthritis, cancer, type 2 diabetes, and many more[1].In the first half of 21st century, obesity has become one of a great challenge in public health[2]. Many studies to prevent and treat obesity have been conducted[3]. There are many strategies known for effective obesity therapy, some of them areinhibiton of adipocyte differentiation, stimulation of energy expediture, suppression of food intake, regulation on lipid metabolism, and lipase inhibition[4].

Plants and their active chemical compounds are known to posses activity that can be used in the treatment of obesity. This approach is considered effective in which herbal medicine has less toxicity and side effect compared to chemical drugs[5-9]. Mangosteen (*Garcinia mangostana* Linn.) is a tropical tree and mostly found in India, Malaysia, Myanmar Philippines, Thailand, and other tropical countries.The fruit hull has been used for treatment of many medical conditions and diseases such as skin infection, wounds, and amobeic dysentery in Southeast Asia for hundreds of years [10]. The bioactive metabolites that are mostly found in *G.mangostana* are xanthone derivates including γ-mangostin,α-mangostin, garcinone-D (Gar-D), garcinone-C (Gar-C)[11], 8-deoxygartanin, cudraxanthone G, garcimangosone B, gartanin, 1-isomangostin, garcinone E, smeathxanthone A, tovophyllin A, and mangostinone[12]. Xanthones were reported to have their numerous and varied pharmacological effects, such asantioxidant, anti-bacteria, antifungal, anti-inflammatory, antihistamine, cytotoxic, anti-

inflammatory, central nervous system (CNS) depressant or stimulant, anti-HIV, anticancer, antihypertensive, antidiabetic, hepatoprotective, and/or immunomodulation properties having antioxidant, antimicrobial, central nervous system (CNS) depressant or stimulant and other activities[10, 13-15]. The dominant xanthone found from the *G.mangostana* L.'s fruit halls, α-mangostin, has been demonstrated by previous pharmacological studies to have antibacterial, antioxidant, anti-inflammatory, renoprotective and antitumor activities[16-21, 14]. Therefore, this study aimed to evaluate the inhibitory potential of mangosteen peel extract (MPE), α-mangostin (AM), γ-mangostin (GM) in adipogenesis cells model (3T3L1) by analyzing the level of cholesterol (CHOL), tryglyceride (TG), lipid, and glucose–6 - phosphate dehydrogenase(G6PDH) which plays a role in lipid metabolism.

2. Materials and Methods

2.1. Mangosteen Peel Extraction

The mangosteen (*G. mangostana* L.) fruit obtained from Indonesian farms in Cisalak- Subang, West Java, Indonesia. The plant was identified by a herbarium staff from Departement of Biology in School of Life Science and Technology, Bandung Institute of Technology, Bandung, Indonesia. The peel was collected from mangosteen fruit, then dried and then extracted using maceration indistilled ethanol 70% as solvents. The filtrate was collected after 24 h, this method was repeated until the filtrate becomes colorless. The filtrate was then evaporated with a rotatory evaporator 40°C, resulted in mangosteen peel extract (MPE), and thern it was stored in -20 ^{0} C [11,14,15].

2.2. Cell Cultures and Adipogenesis Induction

The 3T3-L1 cells (ATCC®CL173) were obtained from Aretha Medika Utama, Bimolecular and Biomedical Research Center, Bandung, Indonesia. The cellswere grown and then maintained in Dulbecco's Modified Eagle Medum (DMEM) (Gibco, 11995065) which is supplemented with 10% of fetal bovine serum (FBS) (Gibco, 26140079) and 1% antibiotic/antimycotic (ABAM) (Gibco, 15240-062) then incubated for 24 hours at 37ºC and 5% CO_2. After cells confluence reached 60-70%, cells differentiation was induced using Millipore ECM 950 kit.The medium was replaced by initiation medium (DMEM contained Fetal Calf Serum (FCS) 10% , IBMX (1:1000) dexamethasone (1:10000), 1% ABAM), and then incubated for 48 hours. The initiation medium was then replaced with progression medium (DMEM supplemented FBS 10% and insulin (1 : 1000), 1% ABAM) and incubated for 48 hours. The medium used was replaced again with the maintenance medium (DMEM supplemented with 10% FCS (Biowest, S0400-500) and then incubated for 4-5 days in 37ºC incubator. On the fifth day after differentiation induction, medium was refreshed with DMEM containing 10% FBS, 1% ABAM [22, 23].

2.3. Measurement of Lipid Acummulation

Measurement of lipid accumulation was done with Adipogenesis Assay Kit (Merck, ECM950). Differentiated cells were treated with MPE (25 and 50 μg/ml), AM (25 and 50 μM), and GM (50 and 75 μM), and incubated for as long as 24 h.The medium was discarded, washed with PBS, then added with Oil-red O (Merck, 90358)

500 µl, incubated for 15-30 minutes. Oil-red O was removed, and cells were washed with Wash Solution (Merck, 90360). The cells were observed with the inverted light Olympus microscope (Olympus Inverted Microscope CKX41-F32FL). Cells were extracted with Dye Extraction (Merck, 90359) 500 µl, incubated in orbital shaker (Labnet, S0600) for 15-30 min. Dye extraction was transferred into 96-well plate, and absorbance was read at 490 nmof wavelength (Multiskan™ GO Microplate Spectrophotometer) [22-24].

2.4. Triglyceride (TG) Assay

The 3T3-L1 adipocytes were harvested 5 days after the initiation of differentiation. The cells were washed twice with cold PBS, collected, and lysed in lysis buffer (1% Triton X-100 in PBS). The total TG content in the cells was determined with a colorimetric enzymatic test using glycerol-3-phospate-oxidase (GPO) (DiaSys 1 5760 99 10 023). 500 µl mixed reaction contained 450 µl reagent with five microlitre sample (cell lysate) was incubated in 37 ºC for 5 minutes. The absorbance was measured in 500 nm of wavelength[15,22].

2.5. Cholesterol (CHOL) Assay

The cholesterol level of lysed differentiated cells was measured withenzymatic photometric test (DiaSys 1 1300 99 10 021). Briefly, 5 µl sample (cell lysate after MPE, AM, GM treatment) was introduced into the sample well which contained 450 µl reagent, while 5 µl of ddH_2O was used as blank sample, incubated at temperature 37 ºC for 10 min. The absorbance was measured with 500 nm of wavelength[15,22].

2.6. Glucose-6-Phosphate Dehydrogenase Activity Assay

The differentiated cells were added in 96 well plates (5 x 10^3 cells/ well) in 100 µl medium (DMEM containing 10% FBS and 1% ABAM) for 24 h at 37ºC and 5% CO_2, then assayed using G6PDH kit (Abcam, AB102529). Briefly 50 µlreaction mixwere added into positive control and sample wells(MPE, AM, GM treatment), while background mix was added into background control wells. Samples were incubated at 37 ºC in dark room. The absorbance of samples was read at 450 nm of wavelength after 30 min [22,24].

2.7. Statistical Data Analysis

Statistical analysis of the data was evaluated using Statistical Package for the Social Sciences statistics version 17.0 software. Statistical analysis was evaluated by One-way analysis of variance (ANOVA). And then analysis was followed by Duncan post-hoc test and was considered to be significant ($p<0.05$). Data are presented as mean ± Standard Deviation.

3. Results

3.1. Effect of MPE, AM, GM on Lipid Accumulation in 3T3-L1 Adipocytes

Obesity is a disorder of lipid metabolism[25].Cell line that is well-characterized and often used to study the adipocytes differentiation is 3T3-L1, also 3T3-L1 cells have been often used to analyze insulin-induced glucose uptake and the obesity development mechanisms. This model system has greatly advanced the understanding of the

molecular basis and signaling pathways of adipogenesis. Hence, 3T3-L1 cells were induced as adipogenesis model in this study, and measured its lipid

To measure the lipid accumulation in obesity model in 3T3-L1, quantified by measuring the optical density (OD) at 490 nm[26]. Lipid accumulation is associated with the development and occurrence of obesity. Lipid accumulation in the adipocytes is a result of a hyperplasia and hypertrophy of adipocyte cells. Inhibition and prevention of accumulation of cytoplasmic lipid droplets and adipogenesis in 3T3-L1 cells that were treated at the differentiation and maintenance stages are shown to reduce lipid accumulation [24].

(Figure 1. The lipid measurement in 3T3-L1 cells stained after treatment of MPE, AM and GM)

As shown in the Figure 1, treatment of MPE, AM, and GM reduced the lipid accumulation as indicated by lipid droplet compared to differentiated cells (0.7968). MPE of 50 µg/ml showed highest decrease among treatments. This was also supported with the results of quantitative lipid measurement in which treatment of MPE 50 µg/ml showed the lowest lipid level (0.2153) among treatments (Table 1). Negative control (without treatment) showed no differentiation as indicated by the lipid droplet formation (Figure 1) and lowest of absorbance value (Table 1).

(Table 1. Level of lipid in adipocytes (3T3-L1) treated with MPE, AMP, GMP)

3.2. Effect of MPE, AM, GM on Cholesterol Level in 3T3-L1 Adipocytes

Consumption of long-chain saturated fatty acids increases cholesterol level[25]. High level of cholesterol is associated with both degree and distribution of obesity[27]. In previous study of a it was reported that cholesterol level was reduced in the differentiated 3T3-L1 adipocytes cells that was treated with ginsenoside Rb2 by upregulating expression of SREBPs (Sterol Regulatory Element-Binding Proteins) mRNA in mammals and affecting expression of genes that modify lipid metabolism. Induced expression of lipoprotein lipase (LPL) and leptin also play roles in cholesterol reduction[28]. Effect of MPE and xanthones treatment on CHOL level of adipocytes (3T3-L1) is presented in Table 2.

(**Table 2. Level of cholesterol in adipocytes (3T3-L1) treated with MPE, AM, GM)**

MPE of 50 µg/ml showed the highest inhibitory activity among treatments (33.33%) with cholesterol level of 177.42 mg/dl. The inhibition activity of MPE was comparable with negative control (29.43%). However, MPE 50 µg/ml and GM 75 µM, have antiobesity potency due to CHOL inhibitory activity (Table 2).

3.3. Effect of MPE, AM, GM on Triglyceride Level in 3T3-L1 Adipocytes

Obesity is correlated with high level of TG[29].TG in adipose tissue acts as a major energy storage form. Obesity that is associated with adipocyte hypertrophy occurs when TG synthesis exceeds TG breakdown and resulting in elevated TG storage[30]. In recent studies, it was shown that lypolisis is one of the mechanism that can reduce adipose tissue mass by breaking down the TG in 3T3-L1 adipocyte cells. It was also stated that

3T3-L1 adipocyte cells that is treated with lypolitic substance at differentiation stage[24]. Effect MPE and xanthones treatment on TG level of adipocytes (3T3-L1) can be seen in Table 3.

(Table 3. Level of triglyceride in adipocytes (3T3-L1) treated with MPE, AM, GM)

Based on Table 3, GM 75 µM has the lowest TG level (201.90mg/dl), GM 50 µM (227.55 mg/dl), MPE 50 µg/ml (210.76 mg/dl) were comparable with negative control (195.37 mg/dl), this data is in line with inhibitory activity of TG of GM 75 µM (59.72%), GM 50 µM (54.60 %), MPE 50 µg/ml (57.95 %). However, MPE 50µg/ml,GM 75 µM, GM 50 µM has antiobesity potency due to TG inhibitory activity (Table 3).

3.4. Effect MPE, AM, GM on G6PDH Level in 3T3-L1 Adipocytes

G6PDHis responsible in adipogenesis by generating ligand peroxisome proliferator-activated receptor γ (PPARγ) activating adipocyte-specific gene expression and differentiation, as well as regulating adipose tissue mass which is associated with obesity development[31]. Effect MPE and xanthones treatment on G6PDH level of adipocytes (3T3-L1) can be seen Table 4.

(Table 4. Level of G6PDH in adipocytes (3T3-L1) treated with MPE, AM, GM)

In G6PDH level, MPE 50 µg/ml showed the lowest level (0.37 nmol/min/ml), this result was comparable with negative control (0.33 nmol/min/mL). MPE 50 µg/ml also showed the highest inhibitory activity of G6PDH among treatments (52.90%), and this data was comparable with negative control (57.84%). However, MPE of 50 µg/ml has antiobesity potency due to G6PDH inhibitory activity (Table 4).

4. Discussion

Obesity is a disorder which involves lipid metabolism and the enzymes that are involved in this process can be targeted to develop various antiobesity drugs. Xanthones from *G. mangostana* have antiobesity activity through anti-adipogenic, anti-inflammatory, antioxidant activities[29]. α-mangostin attenuated TNF-α and IL-8 secretion by the various cell lines activated macrophage including THP-1, hepatic HepG2, enterocyte-like Caco-2, and colon HT-29 primary human monocyte-derived macrophages (MDM)[32], mangosteen peel extract and its compound α-mangostin, and γ-mangostin possess the anti-inflammatory effect by reducing COX-2, IL-6, IL-1β, and NO production in LPS-induces RAW 264.7cells[14]. Mangosteen peel and its compound have high antioxidant activities[33]. α-mangostin compound can suppress intracellular lipid accumulation in differentiating adipocytes and stimulated lipolysis in mature adipocytes; inhibit fatty acid synthase (FAS)[34]. Mangosteen peel extract and its compound have antiinflammatory, antioxidant, antiadipogenesis which these mechanism usefulness in treating or preventing obesity[34].

Lipid-lowering activity of MPE, and its compounds (Table 1), is caused by the inhibition of the transcriptional regulation of lipid synthesis and/or stimulation of lipolysis in 3T3-L1 adipocytes[35]. The differentiation of preadipocytes into adipocytes is regulated by a complex network of transcription factors. After differentiation, C/EBPβ

was induced immediately, while C/EBPα and PPARγ are master regulators of adipogenesis; their maintenance is critical to the progression of the final stages of adipocyte differentiation[36,37]. Adipocyte differentiation and fat accumulation are associated with the occurrence and development of obesity[38]. Increase in the number of fat cells and adipose tissue mass further cause obesity.

Theoretically, the higher the lipid droplets formation the higher the optical density and therefore, the plants with more formation of lipid droplets results higher absorbance which would be effective in the induction of differentiation of pre-adipocyte to adipocyte. Control (without treatment) showed no differentiation as indicated by the lipid droplet formation and absorbance reading. There was significant difference for undifferentiated cells compared with the control (differentiated cells) [39].

In this study, MPE and its compound decreased CHOL level compared to positive control (Table 2). The consumption of long-chain saturated fatty acids (C>10) has led to increase in TG and CHOL levels[25,40]. Furthermore, high triglyceride level leads to increase in very low density lipoprotein (VLDL) and chylomicron levels, as transporters of triglycerides. LDL is the last stage of VLDL catabolism, therefore raised VLDL levels also increase LDL levels. LDL is responsible for transporting the cholesterol to peripheral tissues for oxidation or to adipose tissues for storage [41].

High plasma TG is associated with obesity[29]. In the current study,MPE reduced TG level in 3T3-L1. Metabolism of TG is activated by the expression of adipocyte-specific fatty acid binding protein (aP2), fatty acid synthase (FAS), and Acetyl-CoA carboxylase (ACC) genes. The decrease of TG content may be resulted from decreasing lipid synthesis. MPE also decreased level of G6PDH which plays role in adipogenesis through generating ligand peroxisome proliferator-activated receptor γ (PPARγ) which contributes to activate adipocyte-specific gene expression and differentiation and controls energy accumulation in the form of adipose tissue mass which is associated with obesity development [31].These results suggest that MPE has anti-adipogenesis effect. MPE caused the lowest body weight gain percentage as well as the lowest FAS concentration in adipose tissue and serum of experimental rats. Antiobesity potency of MPE might strongly relate to its α-mangostin content (29.13%) based on HPLC assay [42]. Based on study Adnyana et al. (2015), MPE has antiobesity activityhigherthanAM due to α-amylase and pancreatic lipase inhibitory activities[43.]

In this study, MPE showed good activity compared to marker compounds, AM and GM. Many studies have reported beneficial properties of MPE toward the lipid profile. A study carried by Adiputro et al. (2013) revealed that the ethanolic extract of mangosteen pericarp reduced total CHOL, TG, and low-density lipoprotein (LDL) levels along with increased high-density lipoprotein (HDL) levels in rats fed high-lipid diet. There were several compounds of xanthones involved in the stimulation of adipolysis in differentiated 3T3-L1 and primary human adipocytes. Several studies reported that α-mangostin plays a role in reducing lipid accumulation with decreased peroxisome proliferator activated PPARγ expression along with stimulation of the glucose uptake and free fatty acid release from 3T3-L1 adipocytes via GLUT4 and leptin expression[44]. Mangosteen and its xanthones have good potential to control and modify the metabolic syndrome and its related disorders such as obesity, disrupted lipid profile, diabetes and its complications[45].

5. Conclusion

Mangosteen peel extract, and its compounds reduced level of lipid, cholesterol, triglyceride, and G6PDH which makes it as promising antiobesity agent.

6. Acknowledgment

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Figures

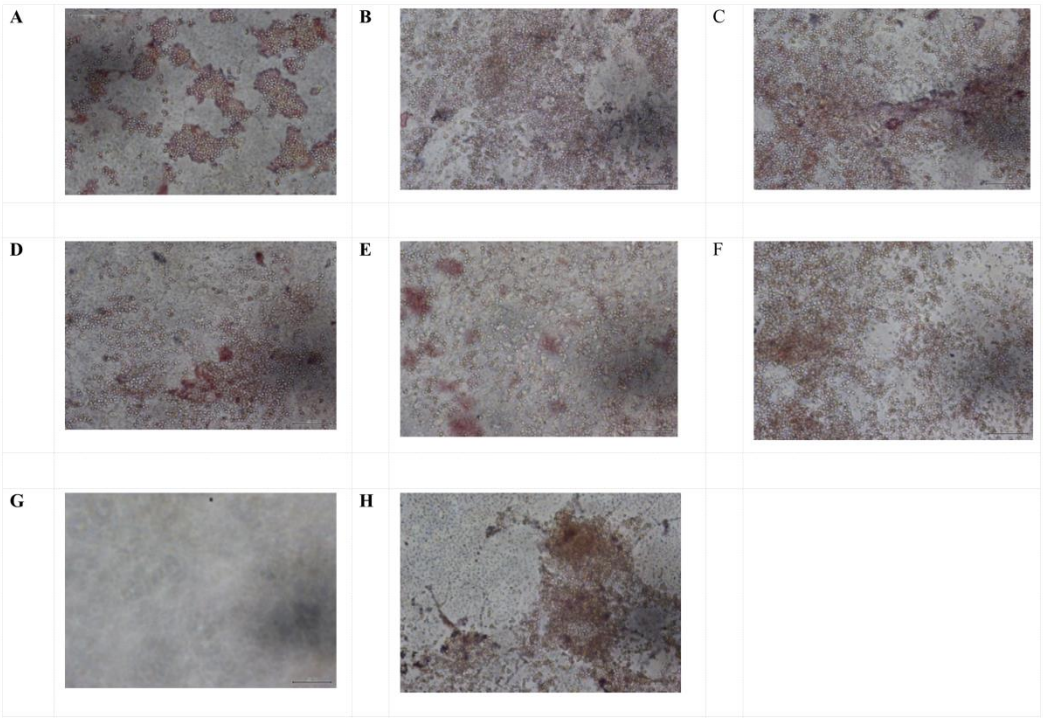

Figure 1. The lipid measurement in 3T3-L1 cells stained after treatment of MPE, AM and GM.

A) MPE 25 µg/ml, lipid accumulation was lower than positive control; B) MPE 50 µg/mL, lipid accumulation was lower than positive control; C) AM mangostin 25 µm; D) AM 50 µM, lipid accumulation was lower than positive control; E) GM 50 µm, lipid accumulation was lower than positive control; F) GM 75 µM, lipid accumulation was lower than positive control; G) Non-differentiated 3T3-L1 cells were not stained by Oil Red O Solution; H). Differentiated cells (positive control). Most preadipocytes were differentiated 5 days after weaning the cells from induction medium to insulin medium. Lipid accumulation in the differentiated cells can be visualized by Oil Red O Solution staining

Tables

Table 1. Level of lipid in adipocytes (3T3-L1) treated with MPE, AMP, GMP

Samples	Level of lipid
Positive control (Differentiated)	0.7968 ± 0.0368^{e}
Negative control (Un-differentiated)	0.1326 ± 0.0182^{a}
MPE 25 μg/ml	0.3172 ± 0.0009^{abc}
MPE 50 μg/ml	0.2153 ± 0.0549^{ab}
AM 25 μM	0.5704 ± 0.09820^{d}
AM 50 μM	0.4316 ± 0.0682^{cd}
GM 50 μM	0.3951 ± 0.0770^{bcd}
GM 75 μM	0.2385 ± 0.0103^{abc}

*Data are presented as Mean±Standard Deviation. Different supercript letters (a,ab,abc,bcd,cd,d,e) show significant difference ($p<0.05$) among treatments (un-differentiated, differentiated cells, MPE, AM, GM treatment)the data was analyzed with Anova and Duncan pos hoc test.

Table 2. Level of cholesterol in adipocytes (3T3-L1) treated with MPE, AM, GM

Samples	CHOL (mg/dl)	Inhibition CHOL level over positive control (%)
Positive control (Differentiated)	266.12±37.95^{b}	0.00±14.26^{a}
Negative control (Un-differentiated)	187.80±37.38^{a}	29.43±14.05^{b}
MPE 25 μg/ml	220.17±8.67^{ab}	17.27±3.26^{ab}
MPE 50 μg/ml	177.42±32.75^{a}	33.33±12.31^{b}
AM 25 μM	245.34±34.79^{ab}	7.81±13.07^{ab}
AM 50 μM	218.57±19.95^{ab}	17.87±7.50^{ab}
GM 50 μM	222.97±15.27^{ab}	16.22±5.74^{ab}
GM 75 μM	181.81±9.08^{a}	31.68±3.41^{b}

*Data are presented as Mean±Standard Deviation. Different superscript letters (a,ab,b) show significant difference ($p<0.05$) among treatments (un-differentiated, differentiated cells, MPE, AM, GM treatment)in CHOL level and inhibition CHOL level over positive control show significant difference, the data wasanalyzed with Anova and Duncan pos hoc test.

Table 3. Level of triglyceride in adipocytes (3T3-L1) treated with MPE, AM, GM

Samples	TG (mg/dl)	Inhibition TG level over positive control (%)
Positive control (Differentiated)	501.26±35.69^{c}	0.00±7.12^{a}
Negative control (Un-differentiated)	195.37±15.41^{a}	61.02±3.07^{c}
MPE 25 μg/ml	286.30±10.68^{b}	42.88±2.13^{b}
MPE 50 μg/ml	210.76±24.12^{a}	57.95±4.81^{c}
AM 25 μM	316.14±17.27^{b}	36.93±3.44^{b}
AM 50 μM	296.09±14.56^{b}	40.93±2.90^{b}
GM 50 μM	227.55±24.64^{a}	54.60±4.92^{c}
GM 75 μM	201.90±4.85^{a}	59.72±0.97^{c}

*Data are presented as Mean±Standard Deviation. Different superscript letters (a,b,c) show significant difference ($p<0.05$) among treatments (un-differentiated, differentiated cells, MPE, AM, GM treatment)in TG level and inhibition TG level over positive control show significant difference, the data wasanalyzed with Anova and Duncan pos hoc test.

Table 4. Level of G6PDH in adipocytes (3T3-L1) treated with MPE, AM, GM

Samples	G6PDH level (nmol/min/ml)	Inhibition G6PDH over positive control (%)
Positive control (Differentiated)	0.79 ± 0.03^{f}	0.00 ± 4.41^{a}
Negative control (Undifferentiated)	0.33 ± 0.02^{a}	57.84 ± 2.00^{f}
MPE 25 µg/ml	0.53 ± 0.03^{cde}	32.69 ± 3.45^{bcd}
MPE 50 µg/ml	0.37 ± 0.06^{ab}	52.90 ± 8.09^{de}
AM 25 µM	0.63 ± 0.02^{e}	20.28 ± 3.10^{b}
AM 50 µM	0.52 ± 0.01^{cd}	34.46 ± 1.83^{cd}
GM 50 µM	0.59 ± 0.06^{de}	25.24 ± 7.10^{bc}
GM 75 µM	0.46 ± 0.05^{bc}	41.95 ± 5.71^{de}

434 ⁎Data are presented as Mean±Standard Deviation. Different superscript letters (a,ab,bc,cd,cde,de,e,f) show significant difference
435 ($p<0.05$) among treatments (un-differentiated, differentiated cells, MPE, AM, GM treatment)in G6PDH level and inhibition
436 G6PDH level over positive control show significant difference, the data wasanalyzed with Anova and Duncan pos hoc test.

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