CHAPTER 3

Results

3.1 Effect of rice extracts on 3T3-L1 adipocyte cell viability

To evaluate the effects of rice extracts on 3T3-L1 cell growth, the extracted at 5-200 μg/mL were incubated for 24 h in preadipocyte and 72 h in mature adipocyte. The results demonstrate that all doses of rice extracts did not cause toxicity in both preadipocyte (figure 3.1) and mature adipocyte (figure 3.2). In preadipocyte, the present of the cell growth were induced to 10-30% by both of the dichloromethane and methanol extracts of rice varieties DSK, PYO and RD6. The dichloromethane extracts of NAN was significantly increased cell growth of 3T3-L1preadipocytes by 10-20%. On the other hand, the methanol extracts NAN at 200 μg/mL was significantly reduced cell growth of 3T3-L1 preadipocytes by 5%. In mature adipocyte, all of the dichloromethane extracts of rice were stimulated the cell growth better than the methanol extracts. The methanol extracts of purple rice cultivars PYO, NAN and RD6 were significantly increased by 5-10% of cell viability of 3T3-L1 mature adipocytes.

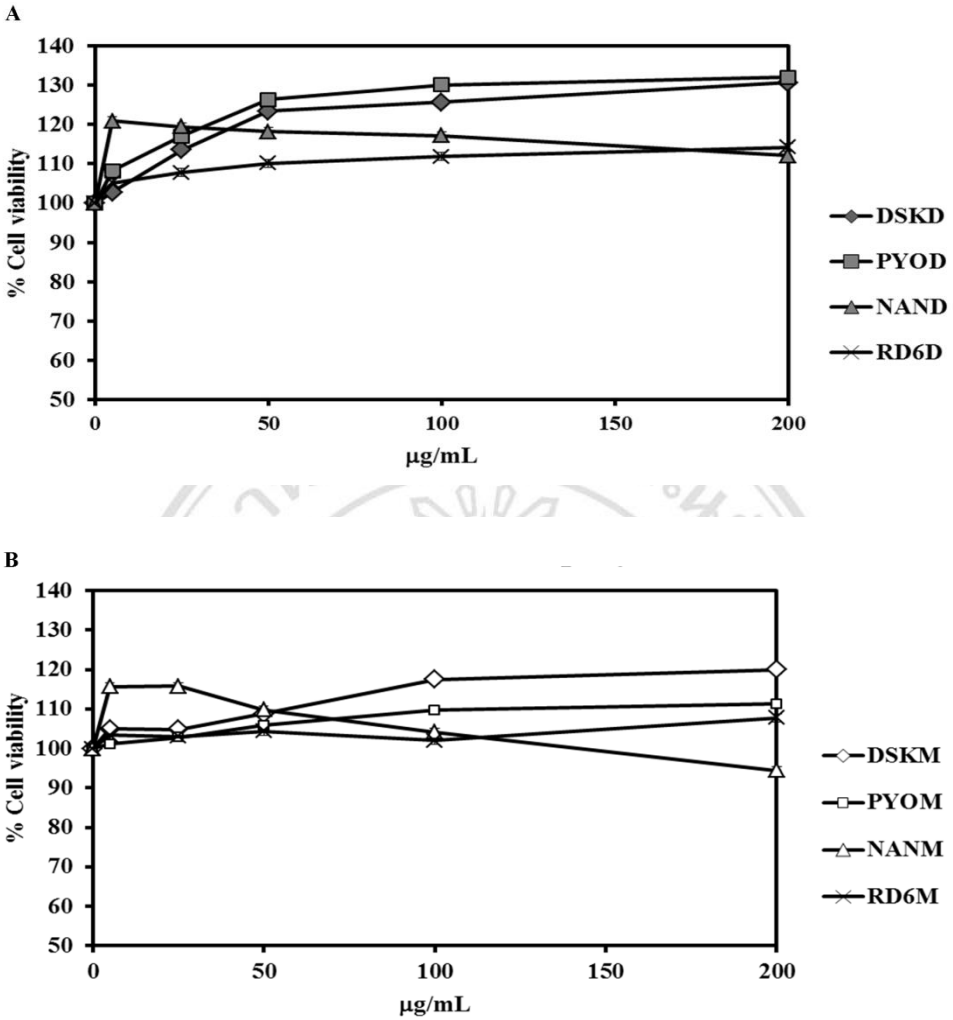


Figure 3.1 Effect of rice extracts on 3T3-L1 preadipocyte cell viability (A) Dichloromethane extracts (B) Methanol extracts. Cells were incubated with rice extracts at concentrations (5-200 µg/ml) for 24 h. Cell viability was determined by using WST-1 assay. Data are expressed as % growth rate of cells cultured in the presence of extracts compared with untreated control cells, taken as 100% (mean ± SD).

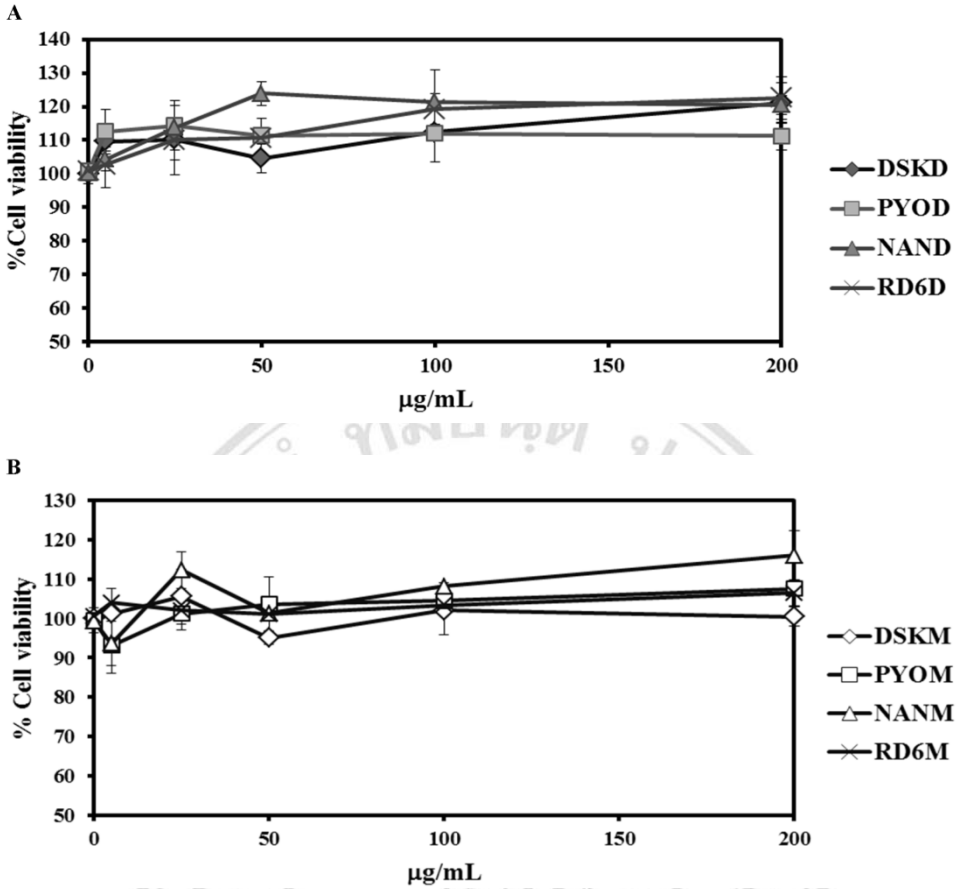
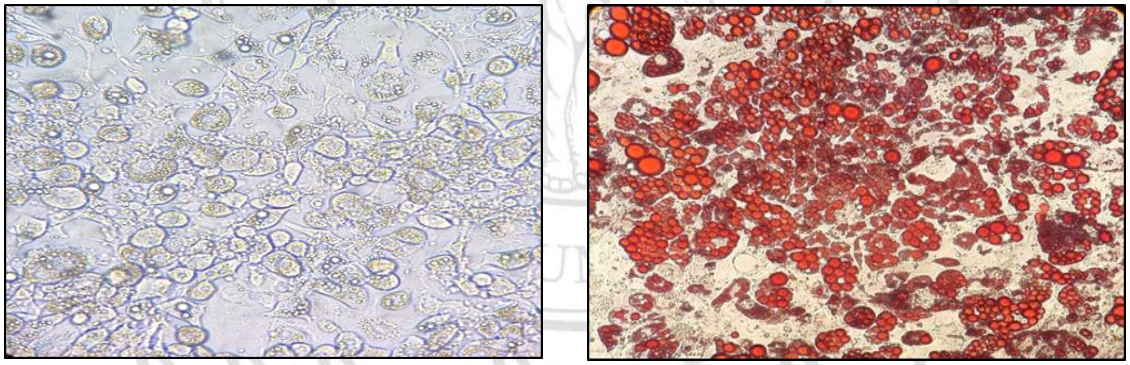


Figure 3.2 Effect of rice extracts on 3T3-L1 mature adipocyte cell viability (A) Dichloromethane extracts (B) Methanol extracts. After induce cell differentiation with induction media, cells were incubated with rice extracts at concentrations (5-200 µg/ml) for 72 h. Cell viability was determined by using WST-1 assay. Data are expressed as % growth rate of cells cultured in the presence of extracts compared with untreated control cells, taken as 100% (mean ± SD).

3.2 Adipocyte differentiation

3T3-L1 preadipocyte were seeded in 96 well plates and induced to differentiate 2 days post confluent using hormone mixture MDI media for 3 day and insulin media for 3 day after MDI media. Cells were then fed 10 % FBS containing insulin media every two day. Lipid droplets, the indicator of adipocyte differentiation were appeared within 4-7 days after induced with differentiation media. An inverted microscope image of mature adipocyte on day 12 is shown in figure 3.3A. The cell morphology revealed the lipid accumulation in 3T3-L1 mature adipocyte. The lipid accumulation was detected by Oil red O staining. Oil red O staining of mature adipocyte on day 12 revealed the lipid accumulation and adipogenesis (figure 3.3B).



A **B**

Figure 3.3 The inverted microscope images of (A) mature adipocyte on day 12 and (B) mature adipocyte on day 12 with Oil red O staining.

3.3 Effect of rice extracts on differentiation of 3T3-L1 preadipocyte

3.3.1 Effect of rice extracts on adipocyte differentiation

The effect of rice extracts on lipid accumulation of 3T3-L1 was evaluated during the induction of adipogenesis by adipogenic hormone mixture. At day 3 of differentiation stage, cells were treated with 50-200 μg/mL of extracts for 72 hours and then incubated in the maintenance medium for 4 days. Oil red O staining of mature adipocytes at day 10 showed the significant cytoplasmic lipid accumulation in the hormone-induced differentiation and untreated adipocytes (data not shown but similar to figure 3.3B). Adipocyte differentiation revealed by Oil red O staining indicated that all of the dichloromethane extracts of rice reduced the differentiation into mature cell (table 3.1). DSKD and PYD slightly decreased the Oil red O staining by approximately 5 to 25%. The cell treated with NAND had a significantly lower lipid accumulation by 20-70%. The concentration which had a highest inhibitory effect is 50 μg/mL. Unpolished brown rice RD6D significantly reduced the differentiation of preadipocyte when compare with purple rice (figure 3.4). The inhibitory effect of RD6D was dose dependent. The differentiated cell treated with 200 μg/mL of RD6D had a significantly lowest number of Oil red O staining (3.3%). In methanol extracts (table 3.2), purple rice at concentration 50-100 μg/mL were reduced the differentiation of preadipocyte. However, 200 μg/mL of purple rice were induced the differentiation of preadipocyte compare with untreated adipocyte (figure 3.5). RD6M significantly reduced the preadipocyte differentiation in dose dependent manner and treating differentiated adipocyte with a 200 μg/mL of RD6M decreased lipid content by approximately 85% (figure 3.5).

Table 3.1 Effect of dichloromethane extracts of rice on adipocyte differentiation

Species	% Relative lipid accumulation			
	0 μg/mL	50 μg/mL	100 μg/mL	200 μg/mL
DSKD	100.00±2.60	73.60±2.60^{*bcd}	91.50±1.90^{*cd}	78.20±13.30^{*d}
PYOD	100.00±2.60	94.50±1.60^{acd}	72.80±10.10^{*d}	96.90±6.90^{cd}
NAND	100.00±2.60	26.50±4.30^{*ab}	76.20±0.70^{*ad}	68.40±8.30^{*bd}
RD6D	100.00±2.60	4.61±11.20^{*ab}	44.10±14.40^{*abc}	3.30±0.00^{*abc}

Data are expressed as % relative lipid accumulation of cells cultured in the presence of extracts compared with untreated control cells (0 μg/mL), taken as 100%. Results are the mean ± SD of three determinations. *P<0.05 relative to the untreated control, ^{a}P<0.05 relative to DSKD, ^{b}P<0.05 relative to PYOD, ^{c}P<0.05 relative to NAND, ^{d}P<0.05 relative to RD6D, respectively.

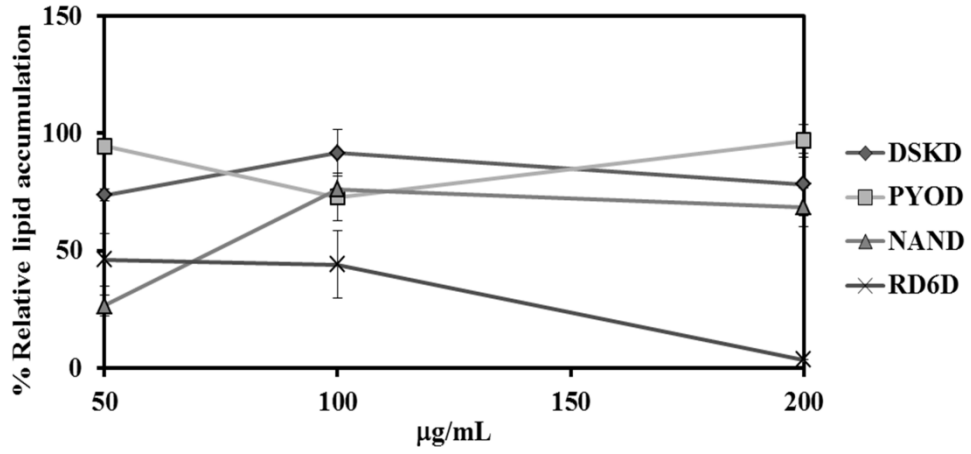


Figure 3.4 Effect of dichloromethane extracts of rice on adipocyte differentiation. Data are expressed as % relative lipid accumulation of cells cultured in the presence of extracts compared with untreated control cells (0 μg/mL), taken as 100%. Results are the mean ± SD of three determinations.

Table 3.2 Effect of methanol extracts of rice on adipocyte differentiation

Species	% Relative lipid accumulation			
	0 μg/mL	50 μg/mL	100 μg/mL	200 μg/mL
DSKM	100.00±2.60	90.10±7.90^{c}	60.60±9.90^{*c}	142.70±10.10^{*bcd}
PYOM	100.00±2.60	71.20±8.20^{*cd}	80.50±5.50^{*cd}	120.90±2.30^{*cd}
NANM	100.00±2.60	37.10±7.70^{*abd}	35.10±2.70^{*abd}	86.80±4.10^{*abd}
RD6M	100.00±2.60	101.50±10.10^{bc}	50.90±2.60^{*bc}	15.10±9.10^{*abc}

Data are expressed as % relative lipid accumulation of cells cultured in the presence of extracts compared with untreated control cells (0 μg/mL), taken as 100%. Results are the mean ± SD of three determinations. *P<0.05 relative to the untreated control, ^{a}P<0.05 relative to DSKM, ^{b}P<0.05 relative to PYOM, ^{c}P<0.05 relative to NANM, ^{d}P<0.05 relative to RD6M, respectively.

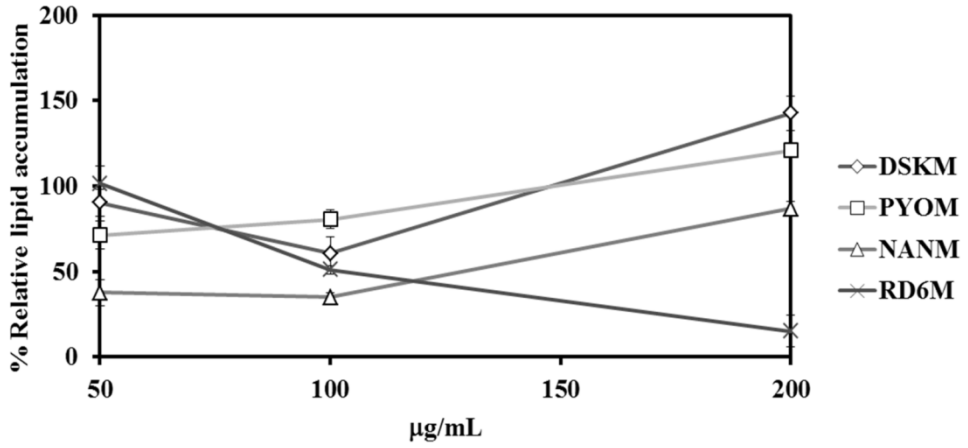


Figure 3.5 Effect of methanol extracts of rice on adipocyte differentiation.
Data are expressed as % relative lipid accumulation of cells cultured in the presence of extracts compared with untreated control cells (0 μg/mL), taken as 100%. Results are the mean ± SD of three determinations.

3.3.2 Effect of rice extracts on the expression of master regulators of adipocyte differentiation

We further evaluated the mRNA expression of CEBP-α and PPARγ which are a key adipogenic gene at various concentrations of rice extract. Studies have shown that it is the driving force for the differentiation process and decrease in its expression is consistent with inhibition of the adipogenic program. In dichloromethane extracts, all of rice significantly decreased CEBP-α gene expression in 3T3-L1 adipocytes (table 3.3). Especially, RD6D highly decreased the expression of CEBP-α gene in dose dependent manner (figure 3.6). NAND also decreased the CEBP-α gene expression in a dose dependent manner but less effect than RD6D did. In methanol extracts, all of rice highly decreased the expression of CEBP-α gene when compare with the untreated control (figure 3.7). The inhibitory effect of CEBP-α gene expression of the purple rice and unpolished brown rice were not significantly different at the concentration of 200 μg/mL of rice extract (table 3.4). Table 3.5, shows PPARγ mRNA expression at various concentrations of dichloromethane extracts of rice. All of purple rice significantly decreased PPAR-γ gene expression in 3T3-L1 adipocytes. The RD6D slightly decreased the expression of PPAR-γ gene but in dose dependent manner (figure 3.8). In methanol extract of rice (table 3.6), all of rice highly decreased the expression of PPAR-γ gene in dose dependent manner but only the concentration at 100 μg/mL of DSK increased the expression of PPAR-γ gene (figure 3.9).

Table 3.3 Effect of dichloromethane extracts of rice on the expression of CEBP-α

Species	Relative expression level			
	0 μg/mL	50 μg/mL	100 μg/mL	200 μg/mL
DSKD	1.00±0.05	0.57±0.01^{*bc}	0.29±0.06^{*bd}	0.22±0.09^{*d}
PYOD	1.00±0.05	0.21±0.08^{*a}	0.12±0.03^{*ad}	0.20±0.04^{*d}
NAND	1.00±0.05	0.22±0.02^{*a}	0.25±0.05^{*bd}	0.32±0.03^{*d}
RD6D	1.00±0.05	0.41±0.18*	0.04±0.01^{*abc}	0.02±0.00^{*abc}

Data are expressed as fold changes relative to the cells without treatment of rice. Results are the mean ± SD of three determinations. *P<0.05 relative to the untreated control (0 μg/mL), ^{a}P<0.05 relative to DSKD, ^{b}P<0.05 relative to PYOD, ^{c}P<0.05 relative to NAND, ^{d}P<0.05 relative to RD6D, respectively.

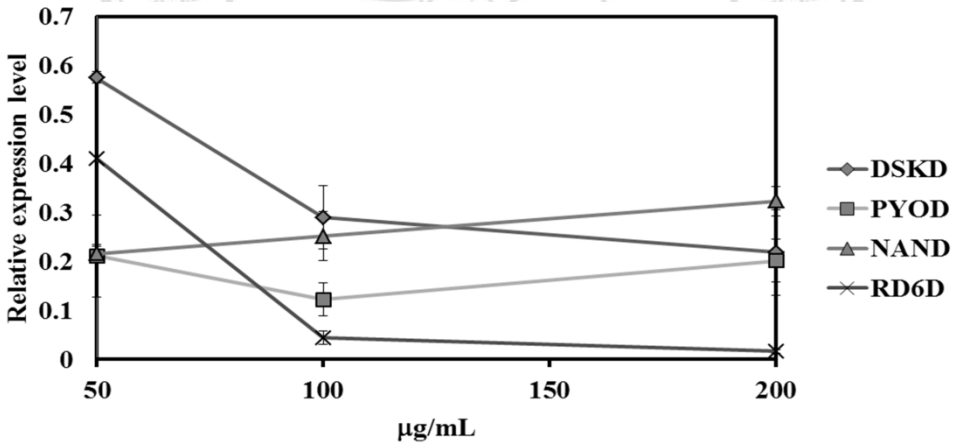


Figure 3.6 Effect of dichloromethane extracts of rice on the expression of CEBP-α. Data are expressed as fold changes relative to the cells without treatment of rice. Results are the mean ± SD of three determinations.

Table 3.4 Effect of methanol extracts of rice on the expression of CEBP-α

Species	Relative expression level			
	0 μg/mL	50 μg/mL	100 μg/mL	200 μg/mL
DSKM	1.00±0.14	0.42±0.14^{*c}	0.22±0.08^{*d}	0.28±0.14*
PYOM	1.00±0.14	0.34±0.02^{*cd}	0.32±0.06*	0.38±0.15*
NANM	1.00±0.14	0.10±0.03^{*ab}	0.28±0.05*	0.33±0.05*
RD6M	1.00±0.14	0.19±0.07^{*ab}	0.48±0.16*	0.23±0.04*

Data are expressed as fold changes relative to the cells without treatment of rice. Results are the mean ± SD of three determinations. *P<0.05 relative to the untreated control (0 μg/mL), ^{a}P<0.05 relative to DSKM, ^{b}P<0.05 relative to PYOM, ^{c}P<0.05 relative to NANM, ^{d}P<0.05 relative to RD6M, respectively.

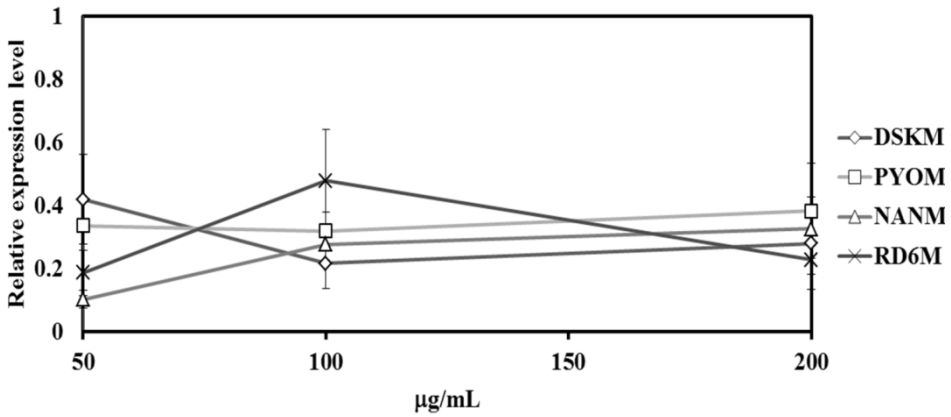


Figure 3.7 Effect of methanol extracts of rice on the expression of CEBP-α.

Data are expressed as fold changes relative to the cells without treatment of rice. Results are the mean ± SD of three determinations.

Table 3.5 Effect of dichloromethane extracts of rice on the expression of PPARγ

Species	Relative expression level			
	0 μg/mL	50 μg/mL	100 μg/mL	200 μg/mL
DSKD	1.00±0.02	0.80±0.15^{*bc}	0.77±0.07^{*d}	0.83±0.08^{*bd}
PYOD	1.00±0.02	0.47±0.05^{*ad}	0.58±0.24^{*d}	0.58±0.075^{*c}
NAND	1.00±0.02	0.51±0.21^{*ad}	0.64±0.25^{*d}	0.74±0.15^{*bd}
RD6D	1.00±0.02	1.03±0.14^{bc}	0.98±0.18^{abc}	0.60±0.05^{*ac}

Data are expressed as fold changes relative to the cells without treatment of rice. Results are the mean ± SD of three determinations. *P<0.05 relative to the untreated control (0 μg/mL), ^{a}P<0.05 relative to DSKD, ^{b}P<0.05 relative to PYOD, ^{c}P<0.05 relative to NAND, ^{d}P<0.05 relative to RD6D, respectively.

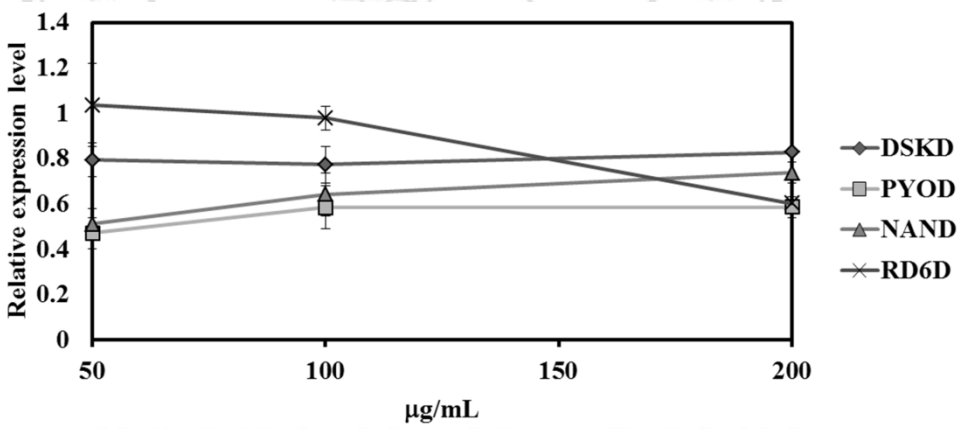


Figure 3.8 Effect of dichloromethane extracts of rice on the expression of PPARγ. Data are expressed as fold changes relative to the cells without treatment of rice. Results are the mean ± SD of three determinations.

Table 3.6 Effect of methanol extracts of rice on the expression of PPARγ

Species	Relative expression level			
	0 μg/mL	50 μg/mL	100 μg/mL	200 μg/mL
DSKM	1.00±0.07	0.63±0.19*	1.24±0.13^{bcd}	0.11±0.02*^{cd}
PYOM	1.00±0.07	0.81±0.17	0.64±0.05^{*a}	0.07±0.01^{*cd}
NANM	1.00±0.07	0.79± 0.06*^{d}	0.67± 0.07^{*a}	0.24±0.06*^{ab}
RD6M	1.00±0.07	0.63±0.07^{*c}	0.52±0.14^{*a}	0.17±0.04^{*ab}

Data are expressed as fold changes relative to the cells without treatment of rice. Results are the mean ± SD of three determinations. *P<0.05 relative to the untreated control (0 μg/mL), ^{a}P<0.05 relative to DSKM, ^{b}P<0.05 relative to PYOM, ^{c}P<0.05 relative to NANM, ^{d}P<0.05 relative to RD6M, respectively.

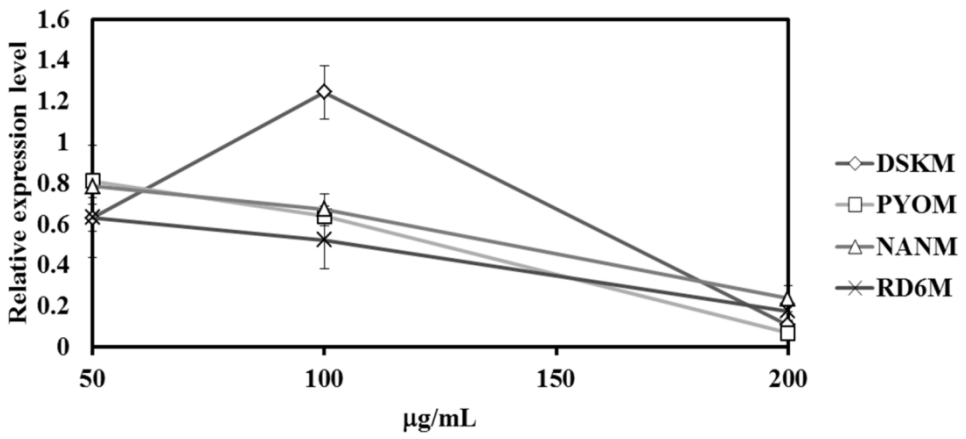


Figure 3.9 Effect of methanol extracts of rice on the expression of PPARγ.
Data are expressed as fold changes relative to the cells without treatment of rice. Results are the mean ± SD of three determinations.

3.4 Effect of rice extracts on lipid accumulation of preadipocyte without hormone induction

To evaluate the effects of rice extracts alone on lipogenesis of 3T3-L1 preadipocyte, all cells in the experiment were cultured in 10% calf serum non-induction media without any hormone stimulation. When compare with the untreated control, DSKD and PYOD stimulated the lipid accumulation in preadipocyte (figure 3.10). On the other hand, NAND and RD6D were significantly reduced the preadipocyte lipid content (table 3.7). The accumulation of lipid in preadipocyte was decreased nearly 20-40% by NAND treatment and 60-85% by RD6D treatment respectively (figure 3.10). In the methanol rice extracts (table 3.8), each rice has different effect to the lipid accumulation in preadipocyte. None of the concentration of DSKM affected preadipocyte lipid accumulation. All concentration of PYOM stimulated the lipid accumulation in preadipocyte by approximately 120 to 200%. A 100 μg/mL of NANM was significantly induced lipogenesis in adipocyte but not in the concentration at 50 or 200 μg/mL. RD6M were only extract significantly reduced the lipid accumulation in preadipocyte in a dose-dependent manner (60-85%) when compared with the untreated control (figure 3.11).

Table 3.7 Effect of dichloromethane extracts of rice on adipocyte lipogenesis

Species	% Relative lipid accumulation			
	0 μg/mL	**50 μg/mL**	**100 μg/mL**	**200 μg/mL**
DSKD	100.00±4.70	116.20±4.40^{*bcd}	147.10±7.00^{*cd}	215.50±12.90^{*bcd}
PYOD	100.00±4.70	159.5±14.40^{*acd}	149.00±6.70^{*cd}	130.5±8.30^{*acd}
NAND	100.00±4.70	59.00±7.20^{*ab}	58.30±5.20^{*ab}	80.20±7.00^{*abd}
RD6D	100.00±4.70	40.80±11.30^{*ab}	41.90±15.40^{*ab}	15.20±2.20^{*abc}

Data are expressed as % relative lipid accumulation of cells cultured in the presence of extracts compared with untreated control cells (0 μg/mL), taken as 100%. Results are the mean ± SD of three determinations. *P<0.05 relative to the untreated control, ^{a}P<0.05 relative to DSKD, ^{b}P<0.05 relative to PYOD, ^{c}P<0.05 relative to NAND, ^{d}P<0.05 relative to RD6D, respectively.

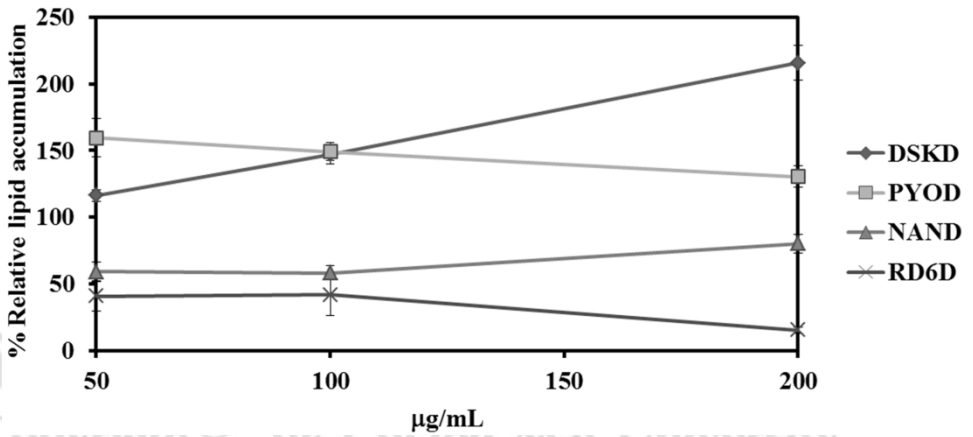


Figure 3.10 Effect of dichloromethane extracts of rice on adipocyte lipogenesis.
Data are expressed as % relative lipid accumulation of cells cultured in the presence of extracts compared with untreated control cells (0 μg/mL), taken as 100%. Results are the mean ± SD of three determinations.

Table 3.8 Effect of methanol extracts of rice on adipocyte lipogenesis

Species	% Relative lipid accumulation			
	0 μg/mL	50 μg/mL	100 μg/mL	200 μg/mL
DSKM	100.00±4.70	113.00±13.70 ^{bd}	127.80±20.50^{bcd}	117.50±15.80^{d}
PYOM	100.00±4.70	199.20±8.20^{*acd}	204.30±12.40^{*ad}	138.10±4.70^{*cd}
NANM	100.00±4.70	92.50±7.60^{bc}	202.30±00.90^{*ad}	102.2±1.10^{bd}
RD6M	100.00±4.70	43.80±3.20^{*abc}	37.20±3.00^{*abc}	22.40±2.40^{*abc}

Data are expressed as % relative lipid accumulation of cells cultured in the presence of extracts compared with untreated control cells (0 μg/mL), taken as 100%. Results are the mean ± SD of three determinations. *P<0.05 relative to the untreated control, ^{a}P<0.05 relative to DSKM, ^{b}P<0.05 relative to PYOM, ^{c}P<0.05 relative to NANM, ^{d}P<0.05 relative to RD6M, respectively.

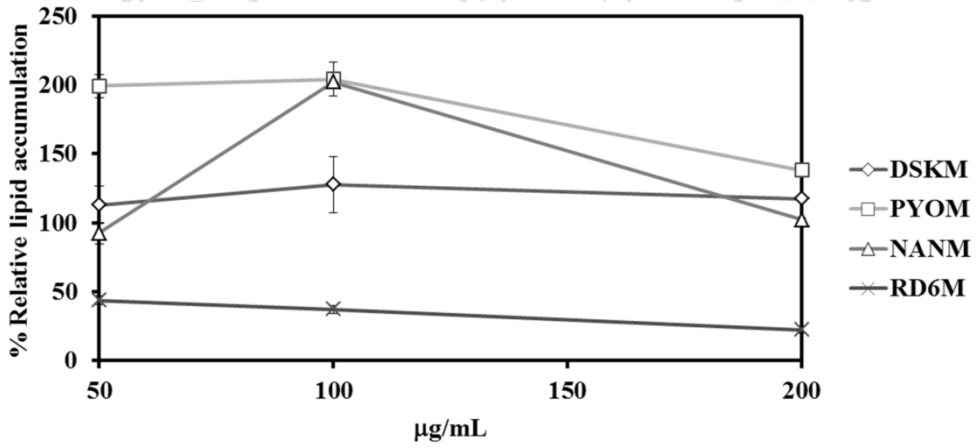


Figure 3.11 Effect of methanol extracts of rice on adipocyte lipogenesis.

Data are expressed as % relative lipid accumulation of cells cultured in the presence of extracts compared with untreated control cells (0 μg/mL), taken as 100%. Results are the mean ± SD of three determinations.

3.5 Effect of rice extracts on TNF-α-induced insulin resistance in adipocyte

The major function of insulin in adipocyte is to stimulate glucose uptake and also inhibit lipolysis. To determine the effect of rice extracts on insulin resistance in adipocyte, TNF-α was used to induce insulin resistant state in adipocyte. TNF-α induced insulin resistance would impair insulin-stimulated glucose uptake and inhibited lipolysis in adipocytes.

3.5.1 Effect of rice extracts on glucose uptake in TNF-α–induced insulin resistant adipocytes

As shown in table 3.9, insulin-stimulated glucose uptake in adipocyte was reduced by 55% after TNF-α treatment for 24 h. In the dichloromethane extracts of rice treatment (table 3.9), DSKD significantly increased glucose uptake in mature cell in dose dependent manner when compare with other purple rice (figure 3.12). NAND also significantly increased glucose uptake in the insulin resistant adipocyte at the concentration of 50 and 200 μg/mL but not at the concentration of 100 μg/mL. A 200 μg/mL of PYOD was significantly induced glucose uptake in the insulin resistant adipocyte but not the lower concentration at 50 and 100 μg/mL. RD6D also significantly improved the glucose uptake at the concentration of 100 and 200 μg/mL of the extracts. In the methanol extracts of rice treatment (table 3.10), all the rice extracts at both 100 and 200 μg/mL significantly improved the glucose uptake of TNF-α-treated-adipocyte cell. This improvement of the relative level of glucose uptake is increased by 1.3 to 5 times of the non TNF-α-treated adipocyte. RD6M and NANM at the concentration 100 ug/mL significantly increased the highest glucose uptake level of the TNF-α-treated adipocyte respectively (figure 3.13). Overall methanol extracts of rice are significantly improved the insulin-stimulated glucose uptake in adipocyte treated with TNF-α better than the dichloromethane extracts of rice.

Table 3.9 Effect of dichloromethane extracts of rice on glucose uptake in TNF-α–induced insulin resistant adipocytes

Species	% Relative glucose uptake			
	0 μg/mL	50 μg/mL	100 μg/mL	200 μg/mL
DSKD	45.30±8.60	49.40±8.80^{cd}	74.50±5.70^{*bd}	101.10±2.80^{*bd}
PYOD	45.30±8.60	38.10±2.40^{*c}	46.00±4.70^{ad}	77.60±13.90^{*a}
NAND	45.30±8.60	69.40±6.40^{*abd}	61.80±9.50^{a}	96.40±6.00*
RD6D	45.30±8.60	32.40±4.10^{*ac}	69.60±5.30^{*b}	80.40±7.90^{*a}

Data are expressed as % relative to the control without TNF-α treatment as 100%. Results are the mean ± SD of three determinations. *P<0.05 relative to the untreated control (0 μg/mL), ^{a}P<0.05 relative to DSKD, ^{b}P<0.05 relative to PYOD, ^{c}P<0.05 relative to NAND, ^{d}P<0.05 relative to RD6D, respectively.

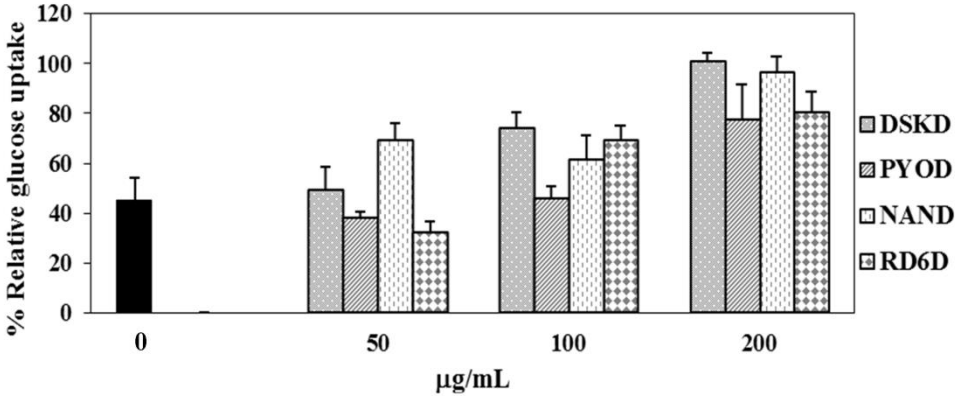


Figure 3.12 Effect of dichloromethane extracts of rice on glucose uptake in TNF-α–induced insulin resistant adipocytes. Insulin resistance of mature 3T3-L1 adipocytes were developed upon 24-hour-treatment with TNF-α. Next, cells were incubated with 50-200 μg/mL of rice extracts for additional 24 hours. Then, glucose uptake was performed using 2-NBDG and 100 nM insulin. The level of glucose uptake was relative to the control without TNF-α treatment (value 100). The results are presented as the mean ± S.D. of three-independent experiments in triplicate. *P<0.05 relative to the untreated control (0 μg/mL).

Table 3.10 Effect of methanol extracts of rice on glucose uptake in TNF-α–induced insulin resistant adipocytes

Species	% Relative glucose uptake			
	0 μg/mL	50 μg/mL	100 μg/mL	200 μg/mL
DSKM	45.30±8.60	52.40±5.20^{cd}	243.00±65.30^{*cd}	175.40±38.60^{*d}
PYOM	45.30±8.60	41.30±11.00^{cd}	136.70±32.00^{*cd}	172.60±34.80^{*d}
NANM	45.30±8.60	121.95±7.10^{*ab}	458.75±64.50^{*ab}	264.99±48.20*
RD6M	45.30±8.60	97.30±23.20^{*ab}	338.10±9.74^{*ab}	410.86±70.60^{*ab}

Data are expressed as % relative to the control without TNF-α treatment. Results are the mean ± SD of three determinations. *P<0.05 relative to the untreated control (0 μg/mL), ^{a}P<0.05 relative to DSKM, ^{b}P<0.05 relative to PYOM, ^{c}P<0.05 relative to NANM, ^{d}P<0.05 relative to RD6M, respectively.

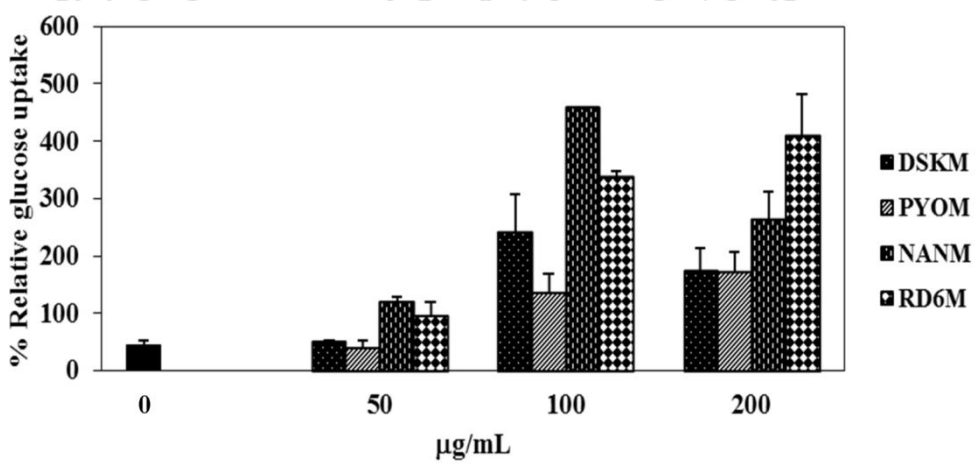


Figure 3.13 Effect of methanol extracts of rice on glucose uptake in TNF-α–induced insulin resistant adipocytes. Insulin resistance of mature 3T3-L1 adipocytes were developed upon 24-hour-treatment with TNF-α. Next, cells were incubated with 50-200 μg/mL of rice extracts for additional 24 hours. Then, glucose uptake was performed using 2-NBDG and 100 nM insulin. The level of glucose uptake was relative to the control without TNF-α treatment (value 100). The results are presented as the mean ± S.D. of three-independent experiments in triplicate.

3.5.2 Effect of rice extracts on lipolysis in mature adipocyte

Lipolysis or the releasing of fatty acids from triacylglycerol is also under control of insulin. The effect of rice extracts on lipolysis was examined by the amounts of free glycerol release of 3T3-L1 adipocytes. The result in table 3.11 showed that DSKD and RD6D significantly inhibited lipolysis in TNF-α-induced 3T3-L1 in dose dependent manner. Both of the highest dose of DSKD and RD6D (200 μg/mL) were nearly complete inhibit the release of glycerol (88% and 85% respectively) as compared with the TNF-α-treated control. The highest concentration of PYOD and NAND were significantly decreased the release of glycerol but not at 50 and 10 μg/mL (fig 3.14). In methanol extracts of rice treatment (table 3.12), DSKM, RD6M and NANM significantly decrease the glycerol content in dose dependent manner (figure 3.15). DSKM showed highest inhibitory activity in lipolysis than RD6M, NANM and PYOM respectively. Thus, these results clearly suggested the anti-insulin resistant activity of purple rice and unpolished brown rice in adipocytes.

Table 3.11 Effect of dichloromethane extracts of rice on lipolysis in TNF-α –induced insulin resistant adipocytes

Species	Glycerol content (μg/mL)			
	0 μg/mL	50 μg/mL	100 μg/mL	200 μg/mL
DSKD	7.47±0.01	7.07±0.13^{*bcd}	3.32±0.05^{*bcd}	0.86±0.00^{*bcd}
PYOD	7.47±0.01	8.38±0.12^{*ad}	7.99±0.11^{*ad}	4.74±0.11^{*acd}
NAND	7.47±0.01	8.41±0.04^{*ad}	7.92±0.06^{*ad}	3.32±0.01^{*abd}
RD6D	7.47±0.01	4.77±0.02^{*abc}	4.46±0.02^{*abc}	1.08±0.01^{*abc}

Results are the mean ± SD of three determinations. *P<0.05 relative to the untreated control (0 μg/mL), ^{a}P<0.05 relative to DSKD, ^{b}P<0.05 relative to PYOD, ^{c}P<0.05 relative to NAND, ^{d}P<0.05 relative to RD6D, respectively.

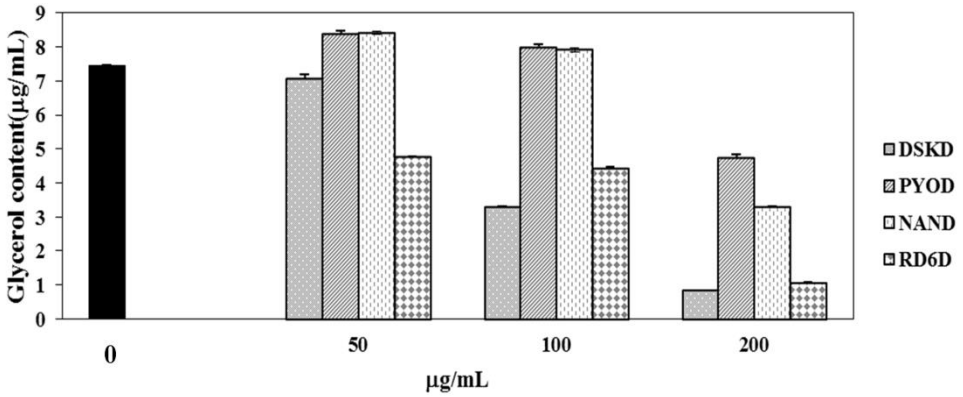


Figure 3.14 Effect of dichloromethane extracts of rice on lipolysis in TNF-α –induced insulin resistant adipocytes. Insulin resistance of mature 3T3-L1 adipocytes were developed upon 24-h-treatment with TNF-α. Next, cells were incubated with 50-200 μg/mL of rice extracts for additional 24 h. Free glycerol release was detected in the conditioned medium. The results are presented as the mean ± S.D. of three-independent experiments in triplicate.

Table 3.12 Effect of methanol extracts of rice on lipolysis in TNF-α –induced insulin resistant adipocytes

Species	Glycerol content (μg/mL)			
	0 μg/mL	50 μg/mL	100 μg/mL	200 μg/mL
DSKM	7.47±0.01	4.46±0.04^{*bcd}	2.35±0.01^{*bcd}	0.51±0.00^{*bcd}
PYOM	7.47±0.01	8.23±0.02^{*acd}	6.68±0.03^{*acd}	4.17±0.02^{*acd}
NANM	7.47±0.01	6.53±0.07^{*abd}	7.39±0.05^{*abd}	4.89±0.04^{*abd}
RD6M	7.47±0.01	5.84±0.03^{*abc}	3.25±0.00^{*abc}	1.30±0.00^{*abc}

Results are the mean ± SD of three determinations. *P<0.05 relative to the untreated control (0 μg/mL), ^{a}P<0.05 relative to DSKM, ^{b}P<0.05 relative to PYOM, ^{c}P<0.05 relative to NANM, ^{d}P<0.05 relative to RD6M, respectively.

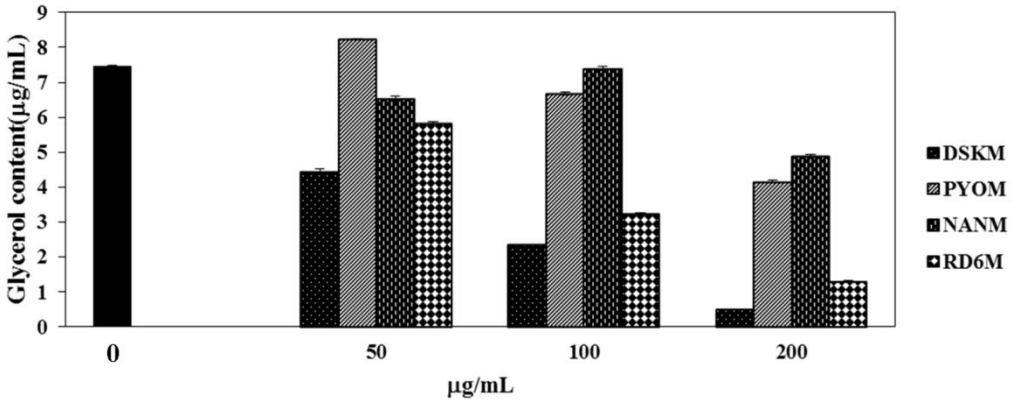


Figure 3.15 Effect of methanol extracts of rice on lipolysis in TNF-α–induced insulin resistant adipocytes. Insulin resistance of mature 3T3-L1 adipocytes were developed upon 24-h-treatment with TNF-α. Next, cells were incubated with 50-200 μg/mL of rice extracts for additional 24 h. Free glycerol release was detected in the conditioned medium. The results are presented as the mean ± S.D. of three-independent experiments in triplicate.