Journal of Experimental Pharmacology

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Oyster Mushroom (*Pleurotus ostreatus*) Ethanolic Extract Inhibits Pparg Expression While Maintaining the Methylation of the *Pparg* Promoter During 3T3-L1 Adipocyte Differentiation

Eko Fuji Ariyanto[1,2], Anastasya Kania Farahana[3], Gabriella Sachiko Jannesha Sudirman[3], Erlina Widiarsih[4], Nurul Qomarilla[5], Nurul Setia Rahayu[4], Tenny Putri Wikayani[5], Henhen Heryaman[1], Dwi Wahyudha Wira[1], Rima Destya Triatin[1,2], Muhammad Hasan Bashari[1], Yunisa Pamela[1], Yuni Susanti Pratiwi[1], Mohammad Ghozali[1]

[1]Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Sumedang, West Java, Indonesia; [2]Research Center for Medical Genetics, Faculty of Medicine, Universitas Padjadjaran, Bandung, West Java, Indonesia; [3]Undergraduate Program of Medical Doctor, Faculty of Medicine, Universitas Padjadjaran, Sumedang, West Java, Indonesia; [4]Molecular Genetics Laboratory, Faculty of Medicine, Universitas Padjadjaran, Bandung, West Java, Indonesia; [5]Cell Culture Laboratory, Faculty of Medicine, Universitas Padjadjaran, Bandung, West Java, Indonesia

Correspondence: Eko Fuji Ariyanto, Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Sumedang, West Java, Indonesia, Tel +6281316099791, Email fuji@unpad.ac.id

Purpose: This study aims to provide new insights into the potential of oyster mushroom (*Pleurotus ostreatus*) ethanolic extract in preventing obesity through the inhibition of *Pparg* expression and modulation of methylation level on *Pparg* promoter during 3T3-L1 adipocyte differentiation.

Methods: This in vitro quantitative experimental study was conducted by treating the 3T3-L1 cell line differentiated using 0.5 mM methyl-isobutyl-xanthine, 1 µM dexamethasone, and 10 µg/mL insulin-containing medium with oyster mushroom ethanolic extract. The extract was obtained from 80 g of dried oyster mushroom powder extracted three times with 800 mL of ethanol, filtered, evaporated, and reconstituted in dimethyl sulfoxide (DMSO) to final concentrations of 0, 25, 50, and 100 µg/mL, with DMSO limited to 0.5% in all solutions. *Pparg* mRNA expression was quantified by qRT-PCR analysis and *Pparg* promoter methylation levels were measured quantitatively by pyrosequencing of bisulfite-treated DNA samples.

Results: The addition of 25 µg/mL oyster mushroom ethanolic extract significantly suppressed *Pparg* mRNA expression with no significant change in the *Pparg* promoter methylation levels.

Conclusion: Oyster mushroom ethanolic extract inhibited *Pparg* mRNA expression without altering *Pparg* promoter methylation, suggesting reduced adipocyte differentiation. This study emphasizes the potential of oyster mushroom in the prevention or treatment of obesity by inhibiting adipocyte differentiation.

Keywords: obesity, oyster mushroom, *Pparg* expression, *Pparg* methylation, 3T3-L1

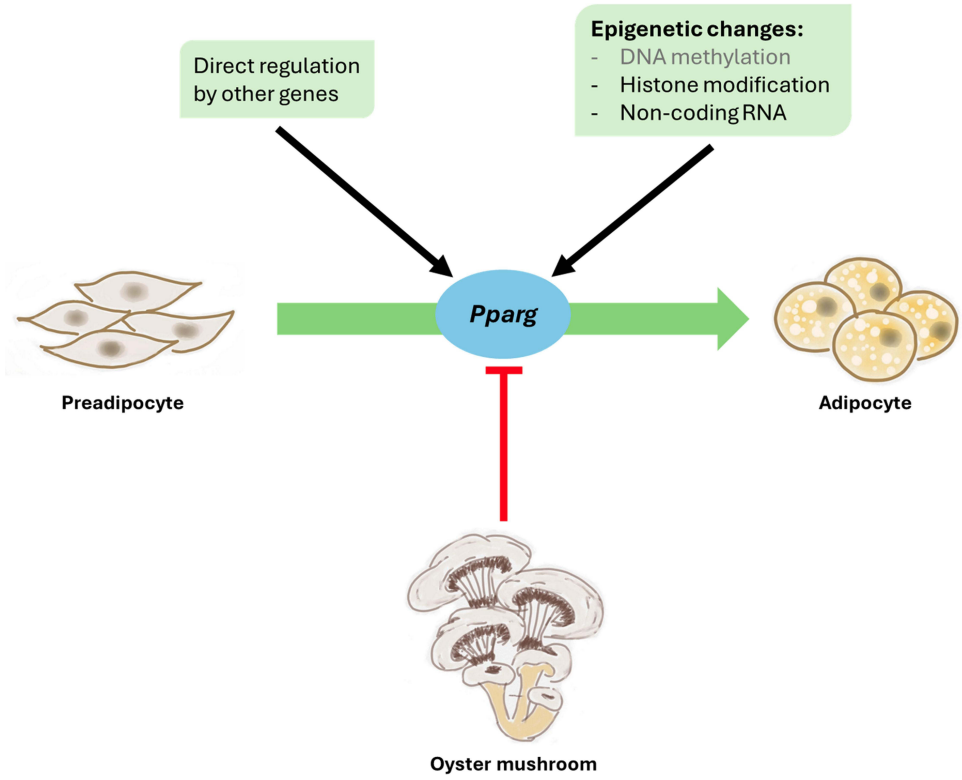
Introduction

Obesity is defined as an abnormal or excessive accumulation of adipocyte tissue.[1] According to the WHO, 2.5 billion adults worldwide are overweight with at least 890 million people being obese and the prevalence of obesity has increased by 200% since 1990.[2] The imbalance of daily energy intake and expenditure precipitates obesity,[1] a multifactorial disease characterized by abnormal accumulation of adipose tissue that might result in adipocyte dysfunction. An increased risk of adipocyte dysfunction is associated with genetic background, overconsumption habits, inadequate physical exercise, and

Received: 1 September 2024
Accepted: 12 December 2024
Published: 16 January 2025

© 2025 Ariyanto et al. This work is published and licensed by Dove Medical Press Limited. The full terms of this license are available at https://www.dovepress.com/terms.php and incorporate the Creative Commons Attribution – Non Commercial (unported, v3.0) License (http://creativecommons.org/licenses/by-nc/3.0/). By accessing the work you hereby accept the Terms. Non-commercial uses of the work are permitted without any further permission from Dove Medical Press Limited, provided the work is properly attributed. For permission for commercial use of this work, please see paragraphs 4.2 and 5 of our Terms (https://www.dovepress.com/terms.php).

Graphical Abstract



unhealthy lifestyles. The dysfunction leads to adipocyte tissue enlargement including elevated adipogenesis and increased adipocyte size.[3,4] Besides its concerning number of prevalence, obesity often leads to severe complications, mostly cardiovascular diseases and type 2 diabetes, which may result in disability or even death. These complications arise from ectopic fat accumulation in metabolic organs, which causes chronic inflammation.[5,6]

Adipogenesis is the term that describes the process by which mesenchymal stem cells (MSCs), particularly preadipocytes, undergo differentiation into adipocytes, the primary cell that constitutes adipocyte tissue.[5,6] Peroxisome proliferator-activated receptor gamma (PPARγ) and CCAAT/enhancer binding proteins alpha (C/EBPα) are the principal regulators of the transcription of various adipogenic genes in adipogenesis, including glucose transporter type 4 (*SLC2A4*), adipocyte fatty acid-binding protein 2 (*AP2*), and adiponectin (*ADIPOQ*).[7,8]

Pparg also plays a major role in the pathophysiology of obesity, specifically due to its regulatory role in fat deposition which is associated with the expansion of adipose tissue in obese individuals. A positive correlation has also been reported between upregulated *Pparg* mRNA expression in the serum of obese patients and triglycerides (TG), low-density lipoprotein (LDL), and BMI in increasing lipid accumulation.[9] Jayaprakash and Das confirmed the down-regulation of *Pparg* using the CRISPR CAS-9 tool following oyster mushroom treatment (0–300 µg/mL) to inhibit adipocyte lipogenesis.[10]

Recent studies indicate that various natural substances, including bee pollen and bee bread, curcumin, and ginger may act as potential remedies for obesity prevention. Bee pollen and bee bread are rich in bioactive compounds, such as flavonoid and polyphenols. Both bioactive compounds exhibit anti-oxidant activity and may reduce appetite by increasing leptin and reducing ghrelin hormone levels.[11] In contrast, curcumin does not act by suppressing appetite but instead increases basal metabolic rate, leading to enhanced energy expenditure. This effect is mediated through the browning of inguinal white adipose tissue (WAT) via the norepinephrine-β3 adrenergic receptor (β3AR) pathway.[12] Ginger, on the

other hand, contains an active compound, called 6-gingerol. This compound can reduce adipocyte size by downregulating adipogenic factors, including PPARγ, C/EBPα, and Fatty Acid Binding Protein 4 (FABP4).[13]

Similarly, the oyster mushroom (*Pleurotus ostreatus*) is widely cultivated in Asia[14–16] and has various health benefits including regulating the immune system, hypolipidemia, and hypoglycemia, as well as antioxidant and anti-inflammatory potential.[17–22] An in vitro study using rat liver tissue suggests that oyster mushroom ethanolic extract inhibits lipid peroxidation which is associated with obesity. Furthermore, it is low-fat, low-energy, and easily consumed, therefore suitable for human consumption to prevent obesity.[23] Additionally, some bioactive compounds in oyster mushrooms, such as alkaloids and flavonoids, modulate the expression of *Pparg* and *Cebpa*, as well as lipid formation.[10]

Various models have been developed to study the molecular pathway of *Pparg* in adipogenesis, for instance using 3T3-L1 cell line for in vitro study. The 3T3-L1 cell line is murine embryonic fibroblast and one of the most commonly used models to study the cellular mechanism of obesity.[24,25] It has also been used in transcriptional studies including the expression of C/EBP and PPAR in promoting adipogenesis.[26,27] It expresses *Pparg* and *Cebpa* when induced by the MDI differentiation cocktail, initiating the adipogenic differentiation phase.[28] The increase in glucose absorption and triglyceride synthesis causes 3T3-L1 to undergo lipid accumulation.[29]

The oyster mushroom *Pleurotus eryngii* increases DNA methylation, inducing hypermethylation to reduce gene expression.[30] It has been reported that hypermethylation of the CpG island on the *Pparg* promoter downregulates *Pparg* expression.[31,32] Therefore, this study was designed to explore the effect of an oyster mushroom ethanolic extract on *Pparg* promoter methylation during adipocyte differentiation using the 3T3-L1 cell line. This cell line was used due to its widespread usage as an in vitro adipogenesis model to depict human preadipocytes because of the similarities in the adipogenesis pathway.[33] This study determined whether oyster mushroom ethanolic extract can increase *Pparg* promoter methylation, acting as an epigenetic mechanism to decrease *Pparg* expression during adipocyte differentiation using the 3T3-L1 cell line.

Materials and Methods

Research Design

This in vitro quantitative experimental analytical study used the 3T3-L1 cell line and was conducted in the Cell Culture and Cytogenetics Laboratory and Molecular Genetics Laboratory, Faculty of Medicine, Universitas Padjadjaran. *Pparg* mRNA expression was quantified by qRT-PCR and pyrosequencing was performed to assess *Pparg* promoter methylation levels. The experiments were performed in triplicate to ensure the study's reliability.

Materials

The oyster mushrooms were obtained from an oyster mushroom farm in Lembang, West Java. It was formally identified by Joko Kusmoro from the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Indonesia (identification number: 465/LBM/IT/XI/2024) and the collection number was 6–13-63-P-1 (which was referred to University of Michigan Herbarium Catalog Collection). The 3T3-L1 cell line was a gift from Dr. Afiat Berbudi (Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Indonesia), and the utilization of this cell line in this study was approved by The Research Ethics Committee of Universitas Padjadjaran (number 1186/UN6.KEP/EC/2024). Other materials used included MDI cocktail medium (Sigma, USA), fetal bovine serum (FBS) (Gibco, USA, cat no. 10270–106), phosphate buffer saline (PBS) (Gibco, cat no. 70011004), Quick-DNA™ Miniprep Plus DNA Isolation Kit (Zymo Research, USA), EZ DNA Methylation-Lightning™ DNA Bisulfite Conversion Kit (Zymo Research), Quick-RNA™ cDNA Synthesis Kit (Bioline Reagents Ltd., UK), SensiFast™ SYBR® No-ROX Kit (Bioline Reagents Ltd)., PyroMark PCR Kit (Qiagen, Hilden, Germany), and Pyrosequencing Kit (Qiagen).

Oyster Mushroom Ethanolic Extract

The oyster mushroom ethanolic extract was derived from 80 g of oyster mushroom dry powder extracted thrice in 800 mL ethanol for 4 hours each. The extract was filtered through Advantec Grade No. 5C filter paper and evaporated.

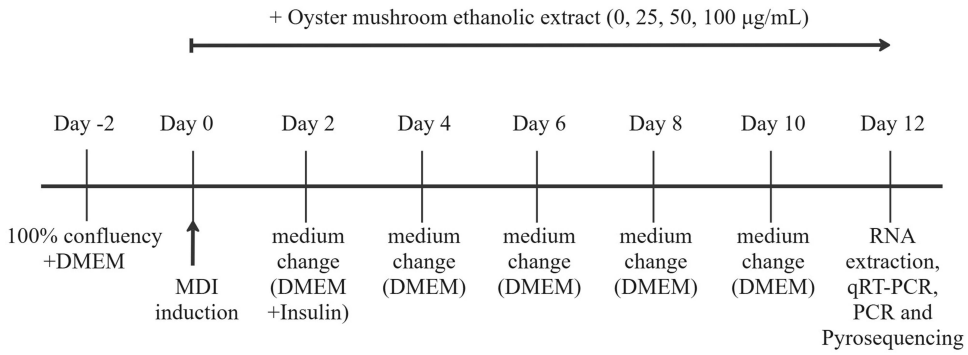


Figure 1 Culture and differentiation of the 3T3-L1 cell line.
Abbreviations: DMEM, dulbecco's modified eagle medium; MDI, methylisobutylxanthine, dexamethasone, and insulin; RNA, ribonucleic acid; qRT-PCR, quantitative reverse transcription polymerase chain reaction; PCR, polymerase chain reaction.

The oily residue (1.68 g) was obtained diluted in dimethyl sulfoxide (DMSO, Sigma, USA, cat no. D8418) to 25, 50, and 100 µg/mL concentrations. The DMSO concentration in the solution was limited to 0.5% for all experiments.

Culture and Differentiation of 3T3-L1 Adipocytes

The 3T3-L1 cell line was cultured in 10% DMEM (Gibco, cat no. 11995065) containing 1% penicillin/streptomycin at 37°C in 5% CO_2 (Figure 1). The cells were seeded into 12-well plates at a density of 12×10^4 cells/well and grown until 100% confluent. The confluent cells were induced using an MDI cocktail containing 0.5mM methyl-isobutyl-xanthine (Sigma, cat no. I5879), 1 µM dexamethasone (Sigma, cat no. D4902), and 10 µg/mL insulin (Sigma cat no. I0516) to commence adipocyte differentiation.[31] The medium was replaced with 10 µg/mL insulin and DMEM after 48 hours of cell culture for 48 hours and then medium was changed with DMEM every 48 hours from day 4 until day 12 to maximize glucose absorption and lipogenesis. The cells were treated with the oyster mushroom ethanolic extract (0, 25, 50, and 100 µg/mL) 48 hours after cell seeding until day 12. DNA was isolated on day 12 for PCR analysis and pyrosequencing.[32]

Quantitative Reverse Transcription PCR (qRT-PCR)

Total RNA was extracted from the cells using the Quick-RNA™ cDNA Synthesis Kit for qRT-PCR performed using the SensiFast™ SYBR® No-ROX Kit according to the manufacturer's instructions and gene-specific primers (Table 1). The cycling conditions were 95°C for 2 minutes, followed by 40 cycles of 5 seconds of denaturation at 95°C, and annealing or extension for 20 seconds at 60–65°C. All mRNA expression was normalized to the housekeeping gene, *Gapdh. Pparg* mRNA expression data was quantified using the Livak method formula of $2^{(-\Delta\Delta Ct)}$.[34]

Pyrosequencing

The DNA was isolated from cells using the *Quick-DNA*™ Miniprep Kit according to the manufacturer's protocol.[35] DNA bisulfite conversion was performed using the *EZ DNA* Methylation-Lightning™ *Kit*[36] and the DNA template was amplified for pyrosequencing using the PyroMark PCR Kit.[37] The amplification was initiated with an initial denaturation

Table 1 Primer Sequences for qRT-PCR

No.	Primer Name	Sequences
1	*Pparg*-Forward	5′-CAA GAA TAC CAA AGT GCG ATC AA-3′
2	*Pparg*-Reverse	5′-GAG CTG GGT CTT TTC AGA ATA ATA AG-3′
3	*Gapdh*-Forward	5′-CAT CAG CAA TGC CTC CTG C-3′
4	*Gapdh*-Reverse	5′-ATG GAC TGT GGT CAT GAG TCC-3′

Abbreviations: qRT-PCR, quantitative reverse transcription polymerase chain reaction; *Pparg*, peroxisome proliferator-activated receptor gamma; *Gapdh*, glyceraldehyde-3-phosphate dehydrogenase.

Table 2 Primer Sequences for Pyrosequencing

No.	Primer Name	Sequences
1	PCR-Forward	5′-TGGTTATTTTAGGAGGTGTGT-3′
2	PCR-Reverse	5′-TGGTTATTTTAGGAGGTGTGT-3′
3	Sequencing	5′-TATTTGGTATATATAGATAGTGGA-3′
4	Sequence to analyze	5′-ATATTATTYGGTTATTTAAAAAGATAGAGGTATTTATTATAA-3′

Abbreviation: PCR, polymerase chain reaction.

(95°C for 5 minutes), denaturation (45 cycles of 95°C for 30 seconds), annealing (58°C for 30 seconds), extension (72°C for 30 seconds), and final extension (72°C for 10 minutes). Then, the DNA products were subjected to 2% agarose gel electrophoresis to verify the PCR products. The biotinylated PCR products (10 µL) were incorporated with streptavidin-coated Sepharose beads (1 µL) and PyroMark binding buffer (40 µL) before purification using the PyroMark Vacuum Workstation and then combined with an annealing buffer containing the sequencing primer. The software then produced a controlled dispensation sequence and deoxynucleotide triphosphate (dNTP) dispensation order according to the sequence to be analyzed. Controls were also integrated in this order and all runs included a no-template control to assess reaction performance. The methylation levels of the *Pparg* promoter region in chromosome 6 at 115,337,600–-115,339,801 nitrogen bases were analyzed using the PyroMark software (v.2.0: Qiagen, Germantown, MD, USA). One of the primers (Table 2) was biotinylated to permit the immobilization of the streptavidin-coated beads.[38] Finally, pyrosequencing was performed using the PyroMark® Q96 ID according to the manufacturer's protocol.[39]

Statistical Analysis

The data were analyzed using SPSS Statistics version 27 (IBM software, New York) and the graphs were prepared using GraphPad Prism version 9 (GraphPad software, Inc., San Diego, CA). The Kruskal Wallis Test and Dunn's post hoc test were applied to analyze the differences in *Pparg* expression levels between groups. Differences in *Pparg* methylation levels between groups were evaluated by one-way ANOVA followed by Tukey's post hoc test. A p-value <0.05 was considered significant. The Kruskal–Wallis and one-way ANOVA tests were used as the study consists of more than 2 groups. Given that the data for *Pparg* expression levels did not follow a normal distribution, the Kruskal–Wallis test was applied. Conversely, the data for *Pparg* methylation levels were normally distributed, justifying the use of the one-way ANOVA test. Following post hoc tests were conducted to identify specific group differences.

Results

The oyster mushroom ethanolic extract (25 µg/mL, 50 µg/mL, and 100 µg/mL) reduced the median *Pparg* mRNA relative expression compared to the negative control (0 µg/mL), though not in a dose-dependent manner. The strongest reduction was observed after treatment of 25 µg/mL, followed by 50 µg/mL and 100 µg/mL that produced the weakest effect. Significant decrease in the median of relative *Pparg* mRNA expression between the concentration of 25 µg/mL of oyster mushroom ethanolic extract and the negative control was confirmed statistically (Figure 2).

The methylation level on the *Pparg* promoter slightly increased and aligned with the increased oyster mushroom ethanolic extract concentrations. There was a slight increase in the mean *Pparg* promoter methylation level in the samples induced with an MDI cocktail and treated with the 50 µg/mL of oyster mushroom ethanolic extract compared to those treated with 25 µg/mL (Figure 3). However, there were no significant differences of mean *Pparg* promoter methylation level between groups. The data was presented as the mean ± SD (Figure 3).

Discussion

Adipogenesis refers to the differentiation and proliferation of adipocyte precursor cells to mature adipocytes, targeted towards increasing adipocyte number for lipid storage.[5,6] This process results in adipose tissue remodeling that may result in adipocyte dysfunction,[7] inducing obesity and associated complications such as metabolic disorders including diabetes mellitus and hypertension. During adipocyte dysfunction, *Pparg*, the principal regulator that activates

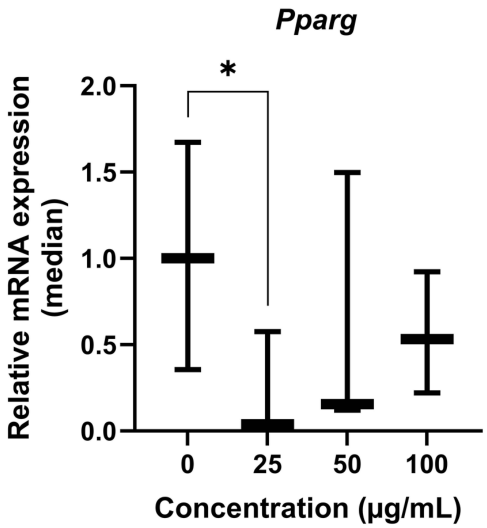


Figure 2 Relative *Pparg* mRNA expression on day 12 of 3T3-L1 cell line treated with oyster mushroom ethanolic extract. Note: *Pparg* mRNA expression was normalized to *Gapdh* and quantified by qRT-PCR. The experiment was conducted in hexaplicates and the data are presented as median (min-max). *$p<0.05$.
Abbreviations: mRNA, messenger ribonucleic acid; *Pparg*, peroxisome proliferator-activated receptor gamma; *Gapdh*, glyceraldehyde-3-phosphate dehydrogenase.

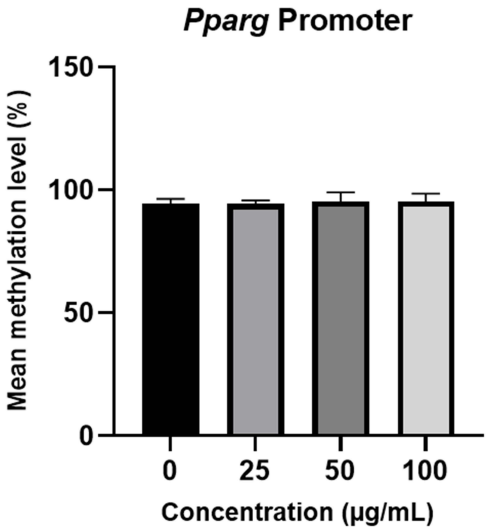


Figure 3 Methylation of the *Pparg* promoter of 3T3-L1 cell line treated with oyster mushroom ethanolic extract. The data is presented as mean ± SD.
Abbreviations: *Pparg*, peroxisome proliferator-activated receptor gamma; SD, standard deviation.

adipogenesis, is stimulated and leads to obesity.[5] Darwish et al demonstrated that serum samples from obese patients had elevated levels of *Pparg* compared to normal-weight patients. Higher levels of *Pparg* positively correlated with elevated triglyceride levels as *Pparg* also regulates the transcription of genes which enhance triglyceride synthesis and cause elevated lipid accumulation.[9,40,41] This finding supports that obesity leads to *Pparg* dysregulation, and it plays a crucial role in the pathogenesis of obesity. Therefore, the downregulation of *Pparg* is a target to decrease the rate of adipogenesis.

The oyster mushroom ethanolic extract reduces relative *Pparg* mRNA expression in the 3T3-L1 cell line. It is known that oyster mushrooms contain bioactive compounds, namely alkaloids, flavonoids, terpenoids, and polysaccharides.[10] It has been suggested that alkaloids significantly inhibited adipogenesis of 3T3-L1 cells by suppressing *Pparg* and *Cebpa* expression. The alkaloid-mediated inhibition of these genes during early or intermediate stages suppressed adipocyte-specific genes and the formation of lipids at a later stage of adipogenesis.[42] A statistically significant inhibition of *Pparg* mRNA relative expression was also noted in the present study at the concentration of 25 µg/mL. This therapeutic effect

of low doses is known as hormesis and occurs by direct stimulation or following an initial disruption in homeostasis and a mild overcompensation response, or as a "pre-" or "post-conditioning" strategy for the hormetic agent to block the toxic effects of the harmful substance.[43] Many studies have reported that flavonoids, also found in oyster mushrooms, frequently cause hormetic dose responses in a wide range of biological models and outcomes.[44]

The results also revealed that the mean of *Pparg* methylation levels tended to increase even though it was statistically insignificant. Lim et al reported that DNA methylation is associated with adipogenesis and obesity because it results in a decline or rise in the regulation of gene expression. DNA methylation level was higher in the lean population's adipogenesis than in the obese population and hypomethylation of the *Pparg* DNA promoter increases *Pparg* expression.[45] *Pparg* hypermethylation also reduces *Pparg* regulation, leading to a slower rate of adipogenic differentiation, thus resulting in longer adipogenesis.[46] One possible mechanism that can explain this phenomenon is the ability of oyster mushrooms to induce overexpression of the *Dnmt3a* gene, a gene crucial for regulating the production of the DNMT3A enzyme, which plays a significant role in DNA methylation. Consequently, *Dnmt3a* overexpression leads to hypermethylation of *Pparg*, resulting in decreased *Pparg* expression, thus slowing the rate of adipogenic differentiation.[46,47]

Reduced *Pparg* expression may result from various epigenetic mechanisms, including post-translational histone modifications and non-coding RNS (ncRNA).[48] Nucleosome, the fundamental unit of chromatin, comprises DNA base pairs wrapped around a histone octamer. The N- and C-terminal tails of these histone proteins undergo post-translational modifications through acetylation, methylation, phosphorylation, SUMOylation, and ubiquitylation, which influence chromatin structure and gene expression.[49] Acetylation and methylation are the most extensively studied histone modifications. Histone acetylation allows the chromatin to be a looser and more accessible conformation for transcription, while histone methylation often results in gene silencing.[48] Malodobra-Mazur et al *reported* that lower histone methylation and acetylation are correlated with reduced *Pparg* expression.[50]

Recent reports revealed that microRNAs (miRNAs) influence a variety of biological processes including adipogenesis.[51] This class of endogenous short ncRNAs with an average length of 19–23 nucleotides may be crucial in controlling posttranscriptional gene expression of *Pparg*.[52] For instance, *Pparg* mRNA and protein expression were decreased by miR-128-3p overexpression which also inhibited the expression of adipogenic marker genes, hence suppressing 3T3-L1 preadipocyte differentiation and lipid accumulation. These results suggest that miR-128-3p acts as a negative regulator during preadipocyte differentiation and adipogenesis, thus it may play a role in the inhibition of *Pparg* expression.[53]

There might be other molecular pathways besides DNA methylation that could regulate *Pparg* mRNA expression in this system. Oyster mushroom extract is thought to initiate the activation of adenosine monophosphate-activated protein kinase (AMPK)[10] which inhibits the synthesis of fatty acid via phosphorylation of acetyl-CoA carboxylase and downregulation of lipogenic enzyme gene transcription.[54] A study has proven that 3T3-L1 cells treated with flavonoids, compounds found in oyster mushrooms, showed significantly high AMPK phosphorylation levels and downregulation of adipogenic key transcription factors including *Cebpa, Cebpb*, and *Pparg*.[54,55]

The findings of this study align with a similar study conducted by Li et al using *Agaricus bisporus* mushroom. β-glucan that was derived from the mushroom showed the ability to inhibit *Pparg* expression which allow adipogenesis inhibition.[56] Jayaprakash and Das also performed a similar study using oyster mushroom. One of its bioactive compounds, anthraquinone, downregulated *Pparg* expression, leading to reduced adipocyte differentiation. Anthraquinone also stimulates AMPK, causing enhanced glucose uptake and inhibits adipocyte differentiation. However, those studies did not examine the methylation level on *Pparg* promoter.[10]

Despite the beneficial effects of oyster mushrooms, they may contain toxic compounds, such as ostreolysin and orellanine.[57] Ostreolysin is an acidic protein that may cause a temporary increase in the arterial blood pressure followed by a gradual decrease to mid-circulatory pressure, along with bradycardia, ventricular extrasystole, and myocardial ischemia. Hyperkalemia may also be seen which resulted from the hemolytic activity of this toxic compound. Orellanine is a bipyridine N-oxide that can produce oxygen radicals and inhibit protein synthesis.[58] However, the adverse effects of both compounds have only been demonstrated through in vitro and in vivo studies, with no reported cases in human studies. A study by Rahimah et al evaluated a wide range of doses of oyster mushroom ethanolic extract, from 10 mg/kg

to 5000 mg/kg, in mice. None of the mice exhibited any signs of intoxication or adverse effects following administration of the extract. Notably, the maximum tested dose of 5000 mg/kg, equivalent to 5000 µg/mL, was well-tolerated, supporting the safety of the extract at this dose level.[59]

Conclusion

Oyster mushroom ethanolic extract inhibited *Pparg* mRNA expression without altering the methylation of the *Pparg* promoter, suggesting reduced differentiation of adipocytes. Therefore, oyster mushroom ethanolic extract has the potential to combat obesity by inhibiting adipocyte differentiation.

Acknowledgments

This study was conducted at the Cell Culture and Cytogenetics Laboratory and Molecular Genetics Laboratory, Faculty of Medicine, Universitas Padjadjaran, Indonesia.

Funding

This study was funded by Universitas Padjadjaran research grant number 1577/UN6.3.1/PT.00/2024 for EFA and Ministry of Education, Culture, Research and Technology of Indonesia research grant number 3866/UN6.3.1/PT.00/2024 for EFA.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Panuganti KK, Nguyen M, Kshirsagar RK. Obesity. StatPearls [Internet]. 2023 [cited 2023 Oct 29]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK459357/. Accessed January 06, 2025.
2. Obesity and overweight [Internet]. [cited 2024 May 8]. Available from: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight. Accessed January 06, 2025.
3. Valli V, Heilmann K, Danesi F, Bordoni A, Gerhäuser C. Modulation of adipocyte differentiation and proadipogenic gene expression by sulforaphane, genistein, and docosahexaenoic acid as a first step to counteract obesity. *Oxid Med Cell Longev*. 2018;2018(1). doi:10.1155/2018/1617202
4. Zhao J, Zhou A, Qi W. The potential to fight obesity with adipogenesis modulating compounds. *Int J Mol Sci*. 2022;23(4):2299.
5. Jakab J, Miškić B, Mikšić Š, et al. Adipogenesis as a potential anti-obesity target: a review of pharmacological treatment and natural products. *Diabetes Metab Syndr Obes*. 2021;14:67–83. doi:10.2147/DMSO.S281186
6. Ghaben AL, Scherer PE. Adipogenesis and metabolic health. *Nat Rev Mol Cell Biol*. 2019;20(4):242–258. doi:10.1038/s41580-018-0093-z
7. Longo M, Zatterale F, Naderi J, et al. Adipose tissue dysfunction as determinant of obesity-associated metabolic complications. *Int J Mol Sci*. 2019;20(9):2358. doi:10.3390/ijms20092358
8. Kang MJ, Kim KK, Son BY, Nam SW, Shin PG, Do KG. The anti-adipogenic activity of a new cultivar, Pleurotus eryngii var. Ferulae 'Beesan no. 2', through down-regulation of PPAR γ and C/EBP α in 3T3-L1 cells. *J Microbiol Biotechnol*. 2016;26(11):1836–1844. doi:10.4014/jmb.1606.06049
9. Wagner N, Wagner KD. The role of PPARs in disease. *Cells*. 2020;9(11):2367. doi:10.3390/cells9112367
10. Jayaprakash B, Das A. An edible fungi Pleurotus ostreatus inhibits adipogenesis via suppressing expression of PPAR γ and C/EBP α in 3T3-L1 cells: in vitro validation of gene knock out of RNAs in PPAR γ using CRISPR spcas9. *Biomed Pharmacother*. 2019;116:109030.
11. El-Seedi HR, El-Wahed AAA, Salama S, et al. Natural remedies and health; a review of bee pollen and bee bread impact on combating diabetes and obesity. *Curr Nutr Rep*. 2024;13(4):751–767. doi:10.1007/s13668-024-00567-3
12. Wang S, Wang X, Ye Z, et al. Curcumin promotes browning of white adipose tissue in a norepinephrine-dependent way. *Biochem Biophys Res Commun*. 2024;466(2):247–253. doi:10.1016/j.bbrc.2015.09.018
13. Cheng Z, Xiong X, Zhou Y, et al. 6-gingerol ameliorates metabolic disorders by inhibiting hypertrophy and hyperplasia of adipocytes in high-fat-diet induced obese mice. *Biomed. Pharmacother.* 2022;146(December 2021):112491. doi:10.1016/j.biopha.2021.112491
14. Egra S, Kusuma IRAWANWIJAYA, Arung ENOSTANGKE, Kuspradini HARLINDA. The potential of white-oyster mushroom (Pleurotus ostreatus) as antimicrobial and natural antioxidant. *Biofarmasi J Nat Product Biochem*. 2019 17. 1. doi:10.13057/biofar/f170102
15. Ezealisiji KM. Formulation and evaluation of the effectiveness of a novel hand sanitizer using pleurotus ostreatus oyster mushroom extract [Internet]. Available from: https://www.researchgate.net/publication/314245369. Accessed January 06, 2025.
16. Kotadiya U, Talaviya J, Shah K, Lathiya S. Morphological and molecular identification of oyster mushroom [Pleurotus Ostreatus (Jacq.) P. Kumm]. 2021. doi:10.21203/rs.3.rs-934342/v1
17. Zhao Q, Liu X, Cui L, Ma C. Extraction and bioactivities of the chemical composition from Pleurotus ostreatus: a review. *J Fut Foods Beijing Academy Food Sci*. 2024;4(2):111–118. doi:10.1016/j.jfutfo.2023.06.001
18. Zhang Y, Hu T, Zhou H, Zhang Y, Jin G, Yang Y. Antidiabetic effect of polysaccharides from Pleurotus ostreatus in streptozotocin-induced diabetic rats. *Int J Biol Macromol*. 83:126–132. 2016. doi:10.1016/j.ijbiomac.2015.11.045

19. Wisbeck E, Facchini JM, Alves EP, et al. A polysaccharide fraction extracted from pleurotus ostreatus mycelial biomass inhibit sarcoma 180 tumor. *An Acad Bras Cienc*. 2017;89(3):2013–2020. doi:10.1590/0001-3765201720150635
20. Randhawa K, Singh V, Kaur S, Kaur R, Kumar S, Shri R. Isolation of pleurotus florida derived acetylcholinesterase inhibitor for the treatment of cognitive dysfunction in mice. *Food Science and Human Wellness*. 2021.10 4:490–496. doi: 10.1016/j.fshw.2021.04.011
21. Barbosa JR, Maurício MM, Oliveira LC, et al. Obtaining extracts rich in antioxidant polysaccharides from the edible mushroom pleurotus ostreatus using binary system with hot water and supercritical CO2. *Food Chem*. 2020 330:127173. doi:10.1016/j.foodchem.2020.127173
22. Othman A, Elshafei A, Hassan M, Haroun B, Elsayed M, Farrag A. Purification, biochemical characterization and applications of pleurotus ostreatus ARC280 laccase. *Br Microbiol Res J*. 2014;4(12):1418–1439. doi:10.9734/BMRJ/2014/11218
23. Jayakumar T, Thomas PA, Geraldine P. In-vitro antioxidant activities of an ethanolic extract of the oyster mushroom, Pleurotus ostreatus. *Innovative Food Sci Emerg Technol*. 2009;10(2):228–234. doi:10.1016/j.ifset.2008.07.002
24. Sarjeant K, Stephens JM. Adipogenesis. *Cold Spring Harb Perspect Biol*. 2012;4(9):a008417–a008417. doi:10.1101/cshperspect.a008417
25. Etesami B, Ghaseminezhad S, Nowrouzi A, Rashidipour M. Investigation of 3T3-L1 cell differentiation to adipocyte, affected by aqueous seed extract of phoenix dactylifera L [Internet]. *Rep Biochem Molecul Biol*. 2020;9:14.
26. Ariyanto EF, Wirajati F, Rahman PH, Berbudi A, Rohmawaty E. Mechanism of action of Indonesian medicinal plants in inhibiting 3T3-L1 adipocyte differentiation: a review. *J Appl Pharm Sci*. 2023. 13(5):50–57.
27. Bahmad HF, Daouk R, Azar J, et al. Modeling adipogenesis: current and future perspective. *Cells*. 2020;9(10):2326. doi:10.3390/cells9102326
28. Ambele MA, Dhanraj P, Giles R, Pepper MS. Adipogenesis: a complex interplay of multiple molecular determinants and pathways. *Int J Mol Sci*. 2020;21:1–27.
29. Pantoja C, Huff JT, Yamamoto KR. Glucocorticoid signaling defines a novel commitment state during adipogenesis in vitro. *Mol Biol Cell*. 2008;19 (10):4032–4041. doi:10.1091/mbc.e08-04-0420
30. Boulaka A, Mantellou P, Stanc GM, et al. Genoprotective activity of the Pleurotus eryngii mushrooms following their in vitro and in vivo fermentation by fecal microbiota. *Front Nutr*. 2022;9(August):1–15. doi:10.3389/fnut.2022.988517
31. Zhao X, Hu H, Wang C, et al. A comparison of methods for effective differentiation of the frozen-thawed 3T3-L1 cells. *Anal Biochem*. 2019 568:57–64. doi:10.1016/j.ab.2018.12.020
32. Ariyanto EF, Odaiyappan LS, Lidyana L, et al. Seeding cell number required for optimal lipid accumulation during adipocyte differentiation using 3T3-L1 cell line. *Wnofns*. 2019;25:220–226.
33. Cave E, Crowther NJ. The use of 3T3-L1 murine preadipocytes as a model of adipogenesis. *Methods Mol Biol*. 2019;1916:263–272.
34. Ariyanto EF, Shalannandia WA, Lantika UA, et al. Anthocyanin-containing purple sweet potato (Ipomoea batatas L.) synbiotic yogurt inhibited 3T3-L1 adipogenesis by suppressing white adipocyte-specific genes. *J Exp Pharmacol*. 2023;15:217–230. doi:10.2147/JEP.S405433
35. Zymo Research. *Quick -DNA ™ Miniprep Plus Kit*. 2023:1–25
36. Zymo-Reseach. EZ DNA methylation-lightning kit protocol. *Cat No D5030T*. 2003;1–183.
37. Pcr F. PyroMark PCR handbook for PCR amplification of template DNA sample & assay technologies QIAGEN sample and assay technologies. 2009.
38. Hajri T, Zaiou M, Fungwe TV, Ouguerram K, Besong S. Epigenetic regulation of peroxisome proliferator-activated receptor gamma mediates high-fat diet-induced non-alcoholic fatty liver disease. *Cells*. 2021.10 6:1355. doi: 10.3390/cells10061355
39. QIAGEN. Version 5 January 2016 PyroMark ® Q96 ID user manual sample & assay technologies. 2016.
40. Darwish NM, Gouda W, Almutairi SM, Elshikh MS, Morcos GNB. PPARG expression patterns and correlations in obesity. *J King Saud Univ Sci*. 2022;34(6):102116. doi:10.1016/j.jksus.2022.102116
41. El Akoum S. PPAR gamma at the crossroads of health and disease: a masterchef in metabolic homeostasis. *Endocrinol Metabolic Syndrome*. 2014;03(01):1–12. doi:10.4172/2161-1017.1000126
42. Choi JS, Kim JH, Ali MY, Min BS, Do KG, Jung HA. Coptis chinensis alkaloids exert anti-adipogenic activity on 3T3-L1 adipocytes by downregulating C/EBP-α and PPAR-γ. *Fitoterapia*. 2014;98:199–208. doi:10.1016/j.fitote.2014.08.006
43. Calabrese EJ, Dhawan G, Kapoor R, Agathokleous E, Calabrese V. Rhodiola rosea and salidroside commonly induce hormesis, with particular focus on longevity and neuroprotection. *Chem. Biol. Interact.* 2023;380:110540. doi:10.1016/j.cbi.2023.110540
44. Calabrese EJ, Hayes AW, Pressman P, et al. Flavonoids commonly induce hormetic responses. *Arch. Toxicol.* 2024;98:1237–1240. doi:10.1007/s00204-024-03684-8
45. Lim J, Heo Y, Choi SS. Investigation of changes in DNA methylation associated with alterations in gene expression resulting in differences between lean and obese adipogenesis. *Genomics*. 2023;115(3):110623. doi:10.1016/j.ygeno.2023.110623
46. Jones PA, Baylin SB. The epigenomics of cancer. *Cell*. 2007;128(4):683–692. doi:10.1016/j.cell.2007.01.029
47. Qimuge N, He Z, Qin J, et al. Overexpression of DNMT3A promotes proliferation and inhibits differentiation of porcine intramuscular preadipocytes by methylating p21 and PPARg promoters. *Gene*. 2019;696:54–62. doi:10.1016/j.gene.2019.02.029
48. Porcuna J, Mínguez-Martínez J, Ricote M. The PPARα and PPARγ epigenetic landscape in cancer and immune and metabolic disorders. *Int J Molecul Sci*. 2021;22:1.
49. Crusio WE, Dong H, Radeke HH. Histone Mutations and Cancer [Internet]. *Advances in Experimental Medicine and Biology*. Available from: http://www.springer.com/series/5584. Accessed January 06, 2025.
50. Małodobra-Mazur M, Cierzniak A, Myszczyszyn A, Kaliszewski K, Dobosz T. Histone modifications influence the insulin-signaling genes and are related to insulin resistance in human adipocytes. *Int J Biochem Biotechnol*. 137:106031. 2021. doi:10.1016/j.biocel.2021.106031
51. Chen L, Song J, Cui J, et al. microRNAs regulate adipocyte differentiation. *Cell Biol Int*. 2013;37(6):533–546. doi:10.1002/cbin.10063
52. Mallanna SK, Rizzino A. Emerging roles of microRNAs in the control of embryonic stem cells and the generation of induced pluripotent stem cells. *Dev. Biol.* 2010;344:16–25. doi:10.1016/j.ydbio.2010.05.014
53. Chen C, Deng Y, Hu X, et al. miR-128-3p regulates 3T3-L1 adipogenesis and lipolysis by targeting Pparg and Sertad2. *J Physiol Biochem*. 2018;74 (3):381–393. doi:10.1007/s13105-018-0625-1
54. Moseti D, Regassa A, Kim WK. Molecular regulation of adipogenesis and potential anti-adipogenic bioactive molecules. *Int J Mol Sci*. 2016;17:124.
55. Kim JH, Lee S, Cho EJ, Flavonoids from acer okamotoanum inhibit adipocyte differentiation and promote lipolysis in the 3T3-L1 cells. *Molecules*. 2020. 25(8):1920.

56. Li X, Xue Y, Pang L, et al. Agaricus bisporus-derived β-glucan prevents obesity through PPAR γ downregulation and autophagy induction in zebrafish fed by chicken egg yolk. *Int J Biol Macromol*. 2019;125:820–828. doi:10.1016/j.ijbiomac.2018.12.122
57. Nieminen P, Mustonen AM. Toxic potential of traditionally consumed mushroom species-a controversial continuum with many unanswered questions. *Toxins*. 2020;12(10):639. doi:10.3390/toxins12100639
58. Jo WS, Hossain MA, Park SC. Toxicological profiles of poisonous, edible, and medicinal mushrooms. *Mycobiology*. 2014;42(3):215–220. doi:10.5941/MYCO.2014.42.3.215
59. Rahimah SB, Kharisma Y, Dewi MK, Hartati J, Maharani W. Acute toxicity test for the ethanolic extract of the white oyster mushroom. Medical Technology and Environmental Health [Internet]. 2020 [cited 2024 Dec 4]. Available from: https://www.taylorfrancis.com/chapters/edit/10.1201/9781003016700-9/acute-toxicity-test-ethanolic-extract-white-oyster-mushroom-rahimah-kharisma-dewi-hartati-maharani. Accessed January 06, 2025.

Journal of Experimental Pharmacology

Dovepress
Taylor & Francis Group

Publish your work in this journal

The Journal of Experimental Pharmacology is an international, peer-reviewed, open access journal publishing original research, reports, reviews and commentaries on all areas of laboratory and experimental pharmacology. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit http://www.dovepress.com/testimonials.php to read real quotes from published authors.

Submit your manuscript here: https://www.dovepress.com/journal-of-experimental-pharmacology-journal