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Original Research Article

Hesperidin and Myricetin Attenuated Non-Alcoholic Fatty Liver Disease (NAFLD) in HepG2 Cells

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ABSTRACT

Flavonoids are natural compounds that might have beneficial effects on non-alcoholic fatty liver disease (NAFLD). This study determined the efficacy of flavonoids in an NAFLD cell model. HepG2 cells were treated for 24 h with either 1 mM oleic acid (OA) or palmitic acid (PA) and 10 µM of one of eleven flavonoids (4 flavones, 2 flavanones, and 5 flavonols) or fenofibrate. Cell morphology was examined by oil-red-O staining. Metabolic gene expression (PPAR α/γ, SREBP 1a/1c, ACC, ACOX, and FAS) was analyzed by RT/qPCR. All flavonoids and fenofibrate reduced intracellular lipid content. Hesperidin, myricetin, and all tested flavanones and flavonols prevented fatty acid-induced increases in metabolic gene expression but fenofibrate and some flavones did not suppress OA-induced FAS expression. Hesperidin and myricetin suppressed metabolic gene expression by more than fenofibrate indicating that they may provide potential pharmacological treatment options for regression of NAFLD status.

Keywords: Flavonoids, Oleic acid, Palmitic acid, PPARs, SREBPs, Fenofibrate.

Introduction

Non-alcoholic fatty liver disease (NAFLD) mostly develops as fat accumulation induced by fatty acids and is not caused by alcohol or viral effects. The worldwide prevalence of NAFLD has increased by over 30% within the last decade.[1] While the pathogenesis of this disease remains unclear,[2] oleic acid (OA) and palmitic acid (PA) are the most common dietary fatty acids and are the most abundant fatty acids in triglyceride (TG) stored in human adipose tissue in NAFLD.[3] Various studies have reported that nuclear receptors including peroxisome proliferator-activated receptors (PPARs) and sterol regulatory element binding proteins (SREBPs) play a main role in lipid metabolism and they have become a common indication for this disease. [4] Lifestyle modifications including exercise and dietary changes can be beneficial but they are dependent upon patient compliance. Fenofibrate is a fibrate drug known to be a PPAR-α agonist. Fenofibrate is recommended for reducing serum TG levels and may have beneficial effects in NAFLD although one study reported it to have no effect on steatosis grade in NAFLD.[5,6] Therefore research to identify novel NAFLD therapeutic agents is justified.
Flavonoids are phenolic plant secondary metabolites with a flavan nucleus that are ubiquitously found in nature.[2] Flavones, flavanones, and flavonols are major subclasses of flavonoids (Figure 1). Flavonoids are common in the diet and the use of flavonoids as food supplements and therapeutic agents is associated with various health benefits. For example, myricetin, a flavone that is abundant in citrus fruits, berries, teas, and vegetables, has anti-oxidation, anti-inflammation, anti-tumour, and anti-diabetic activities.[7] Hesperidin, a flavanone *β*-7-rutinoside of hesperetin present in citrus fruits such as lemon, orange, and grapefruit, has anti-oxidant, anti-inflammatory, and anti-carcinogenic properties.[8] Silymarin, a flavonol derived from thistle milk (*Silybum marianum*), has demonstrated antioxidant, antifibrotic, and antiviral properties.[9] The multifarious actions of flavonoids may suit the treatment of NAFLD.
Currently, the multi-hit hypothesis is used to explain the pathogenesis of NAFLD. It states that there are multiple hits involving insulin resistance, mitochondrial dysfunction, endoplasmic reticulum stress, adipose tissue dysfunction, and dietary factors that lead to lipid accumulation in the liver and the advancement and progression of NAFLD.[4] The most common hits that cause NAFLD are insulin resistance and mitochondrial dysfunction. Altered expression of PPAR-α and PPAR-γ, SREBP-1a and SREBP-1c, acetyl-CoA carboxylase (ACC), acyl-CoA oxidase (ACOX), and fatty acid synthase (FAS) metabolic genes are indicators of these two hits.[10] Altered expressions of these genes directly affect liver function and hepatic histomorphology. Hence, this study aimed at developing therapeutics from flavonoids. In addition, three subclasses of flavonoids (flavones, flavanones, and flavonols) were evaluated for their effects on hepatic cell histomorphology and the expression of PPAR-α/γ, SREBP-1a/1c, ACC, ACOX, and FAS in OA or PA-induced NAFLD using HepG2 cells.

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Materials and Methods

Chemicals and reagents

Dulbecco's modified Eagle medium (DMEM [+] 1g/L D-glucose, [+] L-glutamine, [+] 110 mg/mL sodium pyruvate), Dulbecco's modified Eagle medium F/12 (DMEM F/12 [+] L-glutamine), penicillin/streptomycin (10,000 U/mL), and fetal bovine serum (FBS)

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were obtained from Gibco® (Life TechnologiesTM, MA, USA). Oleic acid (OA, O1008), palmitic acid (PA, P0500), fenofibrate (F6020), and oil red O (ORO) were purchased from Sigma-Aldrich (Missouri, USA). ReverTra Ace® was a product of Toyobo Co., Ltd. (Osaka, Japan). Taq DNA polymerase was purchased from Vivantis® (Selangor, Malaysia). Flavonoids (apigenin cas no. 520-36-5, genistein cas no. 446-72-0, luteolin cas no. 491-70-3, myricetin cas no 529-44-2, hesperidin cas no. 520-26-3, naringenin cas no 480-41-1, galangin cas no. 548-83-4, morin cas no. 480-16-0, kaempferol cas no. 520-18-3, quercetin cas no 117-39-5, and silymarin cas no. 22888-70-6) with purity > 95% were procured from Chengdu Biopurify (Chengdu, China).

Experimental design and treatments
HepG2 cells (ATCC® HB-8065, Manassas, USA) were cultured in 10% FBS and 110 units/mL penicillin/streptomycin supplemented DMEM at 37ºC under 95% humidity and 5% CO_2. The cells were plated in 6-well plates at 5×10^5 cells/well and incubated until reaching 80% confluence. OA and PA stock solutions (1 M) were prepared in isopropanol and diluted with the supplemented DMEM F/12 to 1 mM while fenofibrate and flavonoid stock solutions (10 M) were dissolved in isopropanol and diluted with the medium to 10 μM. The cells were co-treated with either 1 mM OA or PA and 10 μM fenofibrate or flavonoids for 24 h and cells and media were collected for further analysis.

Determination of intracellular fat content using oil red O Staining
The assay was modified from a previous study.[11] ORO solution (0.18%) was freshly prepared in 60% isopropanol. The medium was removed, the cells were washed with phosphate buffered saline (PBS), fixed with 10% neutral-buffered-formalin, then washed twice with distilled water followed by 60% isopropanol. The fixed cells were stained with ORO. The background was adjusted via immersion in 60% isopropanol, followed by washing with distilled water. The hepatic histological features were assessed using 10× magnification of a Motic AE2000 inverted microscope (Motic, Kowloon, Hong Kong). The image was displayed and analyzed on screen using Motic image plus 3.0 software.

Quantitative reverse transcription and real-time polymerase chain reaction (RT/qPCR)
Total RNA was extracted by the guanidine-thiocyanate-phenol-chloroform method and the concentration was determined using a NanoDrop 2000c UV-spectrophotometer (NanoDrop Technologies, Inc., Thermo Fisher Scientific, MA, USA). Total RNA was converted to cDNA using ReverTra Ace® (Toyobo Co., Ltd., Osaka, Japan) at 25ºC for 10 min, 42ºC for 60 min, and 95ºC for 5 min. Expression of metabolic genes, i.e. PPAR-α, PPAR-γ, SREBP-1a, SREBP-1c, ACC, COX, and FAS and a reference *β*-actin gene were subjected to qPCR with specific primers (Table 1) under the manufacturers conditions (Applied Biosystems, Branchburg, NJ, USA) and calculated as previously described. [12] The mRNA levels were normalized with *β*-actin and expressed as the fold difference (ΔC_t calculation) to control. [13]

Statistical analysis
The data were analyzed using one-way ANOVA followed by Tukey's post hoc test using the Statistical Package for Social Sciences (SPSS) statistical program version 23 (Armonk, USA). $p < 0.05$ was considered statistically significant.

Results and Discussion

The current study examined the effect of flavonoids on the main indicators of NAFLD consisting of TG accumulation, which was defined by ORO staining, and the induction of metabolic genes including PPARs, SREBPs, FAS, ACC, and ACOX. PPARs and SREBPs are involved in the regulation of lipolysis and lipogenesis. These metabolic genes are main factors that cause NAFLD via 3 of the 5 hits of the multi-hit hypothesis.[4,14] The flavonoids used in this study were divided into three subtypes (Figure 1): flavones (apigenin, genistein, luteolin, and myricetin), flavanones (hesperidin and naringenin), and flavonols (galangin, morin, kaempferol, quercetin, and silymarin). The different structures of each subtype might offer different anti-steatosis mechanisms, for example by improving the antioxidant status by reducing expression of PPARs and SREBPs. [15] The treatment of flavonoids did not alter cell viability between each group (100.66 ± 2.84 %) but affected hepatocellular histomorphology.

Effect of flavones on histomorphology and the expression of metabolic genes in OA or PA-induced NAFLD in HepG2 cells.
OA-induced NAFLD in HepG2 cells.
The effects of flavones on HepG2 cell histomorphology in the OA-induced NAFLD are shown in Figure 2A. The control and the 0.5% isopropanol treated HepG2 cells exhibited regular histological characteristics. Treatment with 1 mM OA resulted in the accumulation of lipid droplets within the cells (red staining, Figure 2A), which is termed steatosis. Fenofibrate and flavones reduced lipid deposition in the cells without improvement in the histomorphological features. The expression of all tested metabolic genes (PPAR-α, PPAR-γ, SREBP-1a, SREBP-1c, ACC, ACOX, and FAS) was up regulated by OA (Fig. 2B). The OA-induced increases in PPAR-α, PPAR-γ, SREBP-1a, SREBP-1c, ACC, and ACOX expression were down regulated by fenofibrate, apigenin, genistein, and luteolin. Myricetin suppressed the OA-induced increases in PPAR-α, PPAR-γ, SREBP-1a, ACC and FAS expression. Notably, myricetin suppressed the OA-induced increase in FAS expression by more than fenofibrate (Figure 2B).

PA-induced NAFLD in HepG2 cells.
The effects of flavones on HepG2 cell histomorphology in the PA-induced NAFLD are shown in Figure 3A. Treatment with 1 mM PA led to the accumulation of lipid droplets and membrane condensation in the cells (red staining, Figure 3A). Fenofibrate and flavones reduced lipid deposition in the cells and myricetin improved the cell histomorphological features more than fenofibrate. The expression of all tested metabolic genes (PPAR-α, PPAR-γ, SREBP-1a, SREBP-1c, ACC, ACOX, and FAS) was up regulated by PA (Figure 3B).

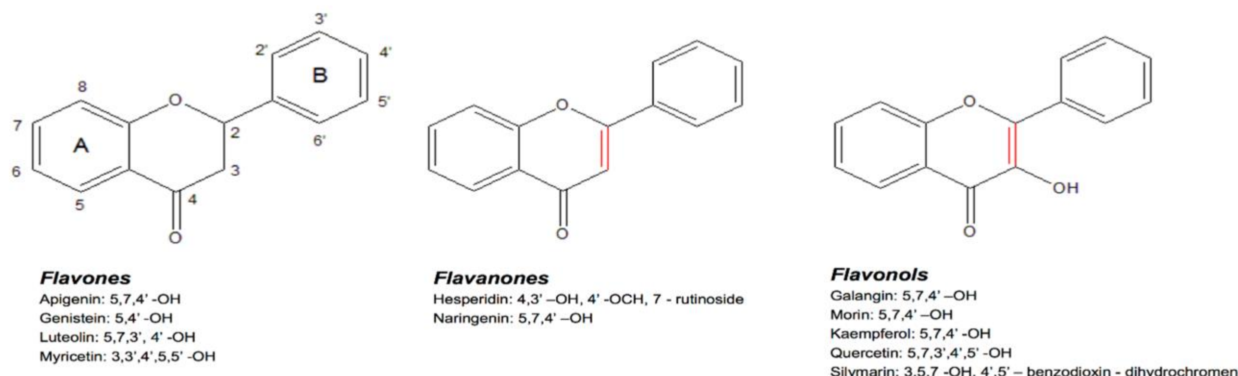

Figure 1: Structure of flavonoid subclasses

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Table 1: Forward and reverse primers for qPCR

Genes		Primers (5' → 3')	Annealing temperature (°C)
PPAR-α	*Forward*	GGT GGA CAC GGA AAG CCC AC	60.6
	Reverse	GGA CCA CAG GAT AAG TCA CC	
PPAR-γ	*Forward*	GGC GAG GGC GAT CTT GAC AGG	57.4
	Reverse	TGC GGA TGG CCA CCT CTT TGC	
SREBP-1a	*Forward*	TCA GCG AGG CGG CTT TGG AGC AG	57.4
	Reverse	CAT GTC TTC GAT GTC GGT CAG	
SREBP-1c	*Forward*	GGA GGG GTA GGG CCA ACG GCC T	57.4
	Reverse	CAT GTC TTC GAA AGT GCA ATC C	
ACC	*Forward*	GAA TGT TTG GGG ATA TTT CAG	60.7
	Reverse	TTC TGC TAT CAG TCT GTC CAG	
ACOX	*Forward*	GGG CAT GGC TAT TCT CAT TGC	60.2
	Reverse	CGA ACA AGG TCA ACA GAA GTT AGG	
FAS	*Forward*	CGG AAA CTG CAG GAG CTG TC	60.0
	Reverse	CAC GGA GTT GAG CCG CAT	
β-actin	*Forward*	TCC GCA AAG ACC TGT ACA CC	61.1
	Reverse	GAG TAC TTG CGC TTG GGA GG	

The PA-induced increases in PPAR-α, PPAR-γ, SREBP-1a, SREBP-1c, ACC, ACOX, and FAS expression were down regulated by fenofibrate, apigenin, genistein and luteolin. Myricetin suppressed the PA-induced increases in SREBP-1a, ACC, and ACOX expression by more than fenofibrate (Figure 3B).
According to the results of the gene expression analysis, flavones might prevent NAFLD by normalizing expression of PPARs, SREBPs, ACC, and ACOX, which corresponds to previous studies.[16,17] OA and PA are the main fatty acids in the high fat foods that induce steatosis, which correlates to a high fat diet-fed mice model.[18] This model demonstrated the formation of lipid vacuoles in the mouse livers, which was relieved by myricetin. Hence, myricetin might present an effective NAFLD treatment. [19] Myricetin was also reported as to be a superior cytoprotective agent compared to other flavones, exerting this activity via inhibition of PPARs, SREBPs, and elevation of nuclear factor erythroid 2-related factor 2 (NRF2), which is involved in cytoprotective regulation in the high fat diet-fed mouse model.[19]

Effect of flavanones on histomorphology and the expression of metabolic genes in OA or PA-induced NAFLD in HepG2 cells.
OA-induced NAFLD in HepG2 cells.
The effects of flavanones on HepG2 cell histomorphology in the OA-induced NAFLD are shown in Figure 4A. Treatment with 1 mM OA caused microvesicular steatosis in the cells (red staining, Figure 4A). Flavanones attenuated the lipid deposition in HepG2 cells and improved cell characteristics to the same extent as fenofibrate. OA up-regulated all of the tested metabolic genes while hesperidin and naringenin decreased the OA-induced increases in PPAR-α, PPAR-γ, SREBP-1a, SREBP-1c, ACC, ACOX, and FAS expression (Figure 4B). Notably, both flavanones suppressed the OA-induced increases in PPAR-α and FAS expression more than fenofibrate, hesperidin suppressed PPAR-α, ACC, and FAS expression greater than fenofibrate, and naringenin attenuated the increase in SREBP-1c and ACOX expression by more than fenofibrate.

PA-induced NAFLD in HepG2 cells.
The effects of flavanones on HepG2 cell histomorphology in the PA-induced NAFLD are shown in Figure 5A. Fenofibrate and the tested flavanones attenuated the amount of lipid deposition in the cells with the appearance of the hesperidin treated cells recovering to a condition nearly the same as the control (Figure 5A). For the metabolic gene expression results, PA increased the expression of all tested metabolic genes, which was suppressed by treatment with flavanones (Figure 5B). Hesperidin suppressed PPAR-α, ACC, and FAS superior to fenofibrate while naringenin suppressed SREBP-1c and ACOX more than fenofibrate. Regulation of PPAR-α, ACC, and FAS is relevant to lipolysis and lipogenesis synergistic mechanisms, which could slow the progression of NAFLD rather than only lipogenesis suppression. Previous studies have shown that flavanones are not only PPAR-α agonists but can also produce cardioprotective activity via PPAR-γ.[20] Furthermore, hesperidin prevented metabolic alterations in an obesogenic Wistar rat model.[21]

Effect of flavonols on histomorphology and the expression of metabolic genes in OA or PA-induced NAFLD in HepG2 cells.
OA-induced NAFLD in HepG2 cells.
Effects on the histomorphology of HepG2 cells in the OA-induced NAFLD by flavonols are shown in Figure 6A. Treatment with 1 mM OA caused lipid accumulation with microvesicular steatosis in the cells (red staining, Figure 6A). HepG2 cells treated with flavonols exhibited cell shrinkage and nuclear fragmentation, which was different to the reduction in lipid accumulation seen with fenofibrate. The expression of all tested metabolic genes was upregulated by OA (Figure 6B). The OA-induced increases in PPAR-α, PPAR-γ, SREBP-1a, SREBP-1c, ACC, and ACOX expression were down regulated by fenofibrate and all tested flavonols. The OA-induced increase in FAS expression was suppressed by flavonols to a greater degree than fenofibrate. Thus, flavonols provided anti-metabolic activity and the reductions seen in ACC, ACOX, and FAS expression could affect insulin resistance.

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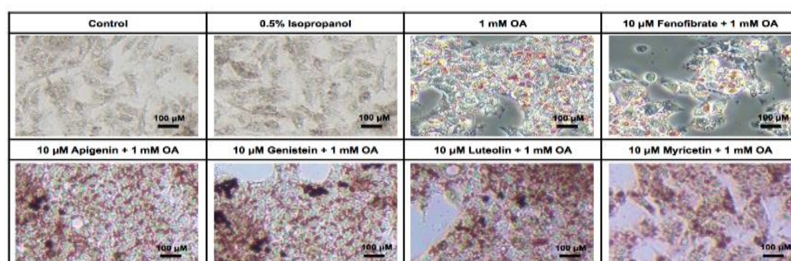
PA-induced NAFLD in HepG2 cells.
The effects of flavonols on histomorphology and metabolic gene expression in PA-induced NAFLD are shown in Figure 7. HepG2 cells treated with PA and kaempferol exhibited cell shrinkage and nuclear fragmentation while galangin, morin, quercetin, and silymarin improved hepatic histomorphology (Figure 7A). The expression of all tested metabolic genes was upregulated by PA (Figure 7B). The PA-induced increases in PPAR-*α*, PPAR-γ, SREBP-1a, SREBP-1c, ACC, ACOX, and FAS expression were down regulated by fenofibrate and all tested flavonols. The PA-induced increase in SREBP-1c expression was suppressed by galangin and kaempferol more than fenofibrate, quercetin lowered the expression of SREBP-1a more than fenofibrate and the PA-induced increases in PPAR-γ, SREBP-1a, and FAS expression were reduced by silymarin more than by fenofibrate.

The histological changes (cell shrinkage and nuclear fragmentation) that were seen in the flavonol treated HepG2 cells might have occurred via flavonols and thiols (organosulfur compounds) formed by flavonols that can increase cell permeability.[2] Interestingly, silymarin Down regulated SREBP-1c expression to a level lower than the control. This over-inhibition of SREBP-1c could decrease thyroid peroxidase levels leading to thyroid hormone compensation. An increase in thyroid hormone level could then increase TG levels.[22]

Previous studies in primary hepatocytes, immortalized hepatic cell lines, mice, and humans have suggested that induction of NAFLD by OA and PA occurs via disruption of lipid metabolism.[23] NAFLD is associated with the consumption of excess dietary fat and OA and PA are the most common dietary fatty acids. PA is a 16-carbon saturated fatty acid and OA is a slightly larger (18-carbon) unsaturated fatty acid with an alkene functional group.[24] OA is found in olive oil, sunflower oil, canola oil, beef, pork, and krill and PA is found in palm oil, lard, and unsalted butter.[25,26] The doses of OA and PA used to induce NAFLD in HepG2 cells in this study were chosen to correlate to human diets.[27] The tested flavonoids were selected as they are commonly found in natural sources, diets, and health supplements.

Previous studies in primary hepatocytes, immortalized hepatic cell lines, mice, and humans have suggested that induction of NAFLD by OA and PA occurs via disruption of lipid metabolism.[23] NAFLD is associated with the consumption of excess dietary fat and OA and PA are the most common dietary fatty acids. PA is a 16-carbon saturated fatty acid and OA is a slightly larger (18-carbon) unsaturated fatty acid with an alkene functional group.[24] OA is found in olive oil, sunflower oil, canola oil, beef, pork, and krill and PA is found in palm oil. The pathogenesis of NAFLD involves the induction of free fatty acid synthetic pathways through PPAR-α, PPAR-γ, SREBP-1a, SREBP-1c, ACC, ACOX, and FAS, which directly affect lipogenesis.[4] In this study, OA or PA dramatically increased expression of these metabolic genes resulting in steatosis. The tested flavonoids showed potential to improve NAFLD via various mechanisms, depending on their structures. Our study found that both PA and OA induced NAFLD via induction of PPARs, SREBP, ACC, ACOX, and FAS, which corresponds with previous reports that PA and OA disturbed lipid metabolism and induced NAFLD in HepG2 cells.[28,29] In one study, PA impaired cell viability and disturbed lipid metabolism in HepG2 cells, but OA robustly rescued cells from cell death by inhibition of endoplasmic reticulum stress and pyroptosis.[28] Moreover, in another study, OA reduced PA-induced oxidative stress, endoplasmic reticulum stress, and mitochondrial dysfunction in rat hepatocytes.[30] Hence, it is of interest to study the impact of flavonoids on endoplasmic reticulum stress, oxidative stress, and pyroptosis in an NAFLD model.

Treatment with fenofibrate or flavonoids reduced lipid deposition in cells without improving histomorphological features. This might be explained by the process of the regenerative response in the cells. There are several factors involved in cell regeneration such as altered gene expression profiles, stimulation of numerous transcription factors and receptors, and secretion of growth-promoting signal molecules.[31] In humans, hepatic histology and functions are normally restored after injury within 8 to 10 days.[32] However, changes at RNA/DNA level begin at 12-16 h and reach the maximum level at 24 to 48 h.[32,33]



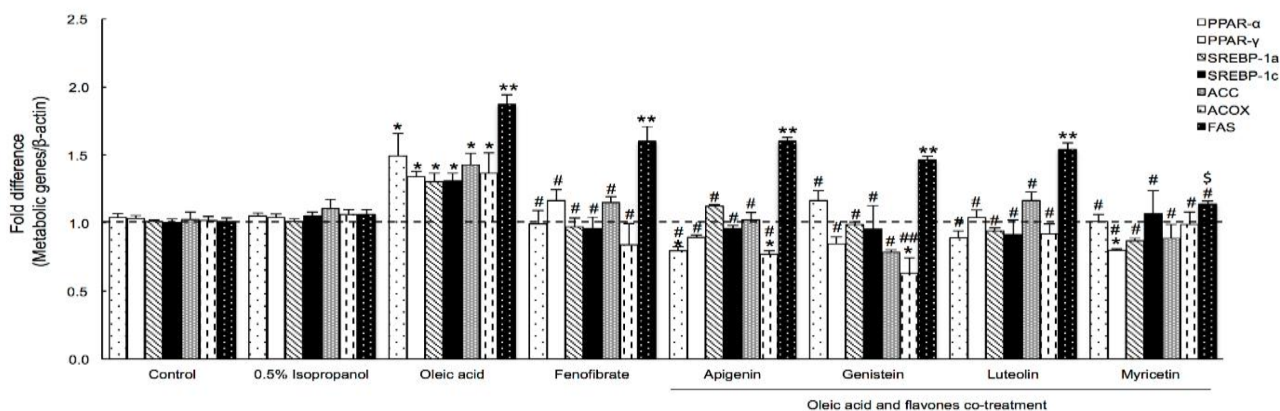


Figure 2: Effect of flavones on histomorphology and the expression of metabolic genes in oleic acid (OA)-induced NAFLD in HepG2 cells. **(A)** Histomorphology of oleic acid (OA)-induced and flavone treated NAFLD in HepG2 cells. Red staining indicates lipid storage. **(B)** The expression of metabolic genes in oleic acid (OA)-induced and flavone treated NAFLD in HepG2 cells.
*$p < 0.05$, **$p < 0.001$ vs. control; $^{\#}p < 0.05$, $^{\#\#}p < 0.001$ vs. OA -induced NAFLD; $^{\$}p < 0.05$ vs. fenofibrate.

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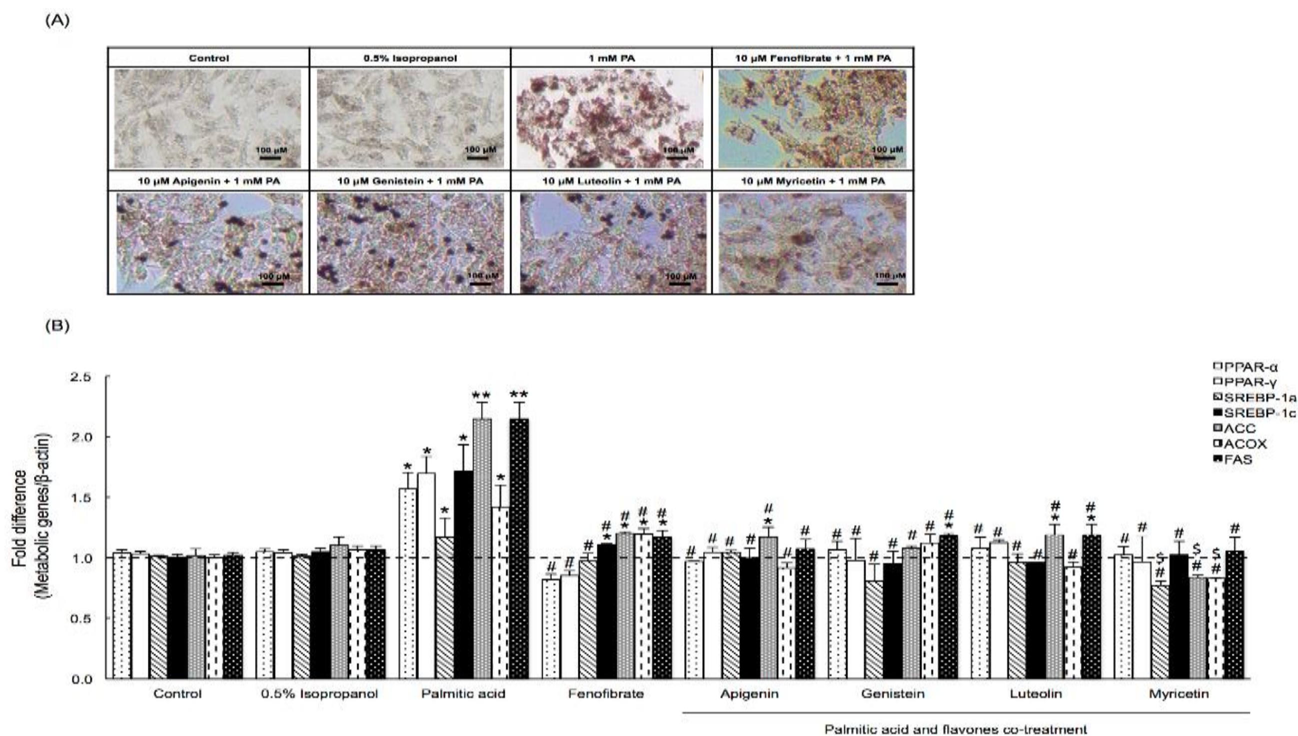

Figure 3: Effect of flavones on histomorphology and the expression of metabolic genes in palmitic acid (PA)-induced NAFLD in HepG2 cells. **(A)** Histomorphology of palmitic acid (PA)-induced and flavone treated NAFLD in HepG2 cells. Red staining indicates lipid storage. **(B)** The expression of metabolic genes in palmitic acid (PA)-induced and flavone treated NAFLD in HepG2 cells.
$*p < 0.05$, $**p < 0.001$ vs. control; $^{\#}p < 0.05$ vs. PA-induced NAFLD; $^{\$}p < 0.05$ vs. fenofibrate

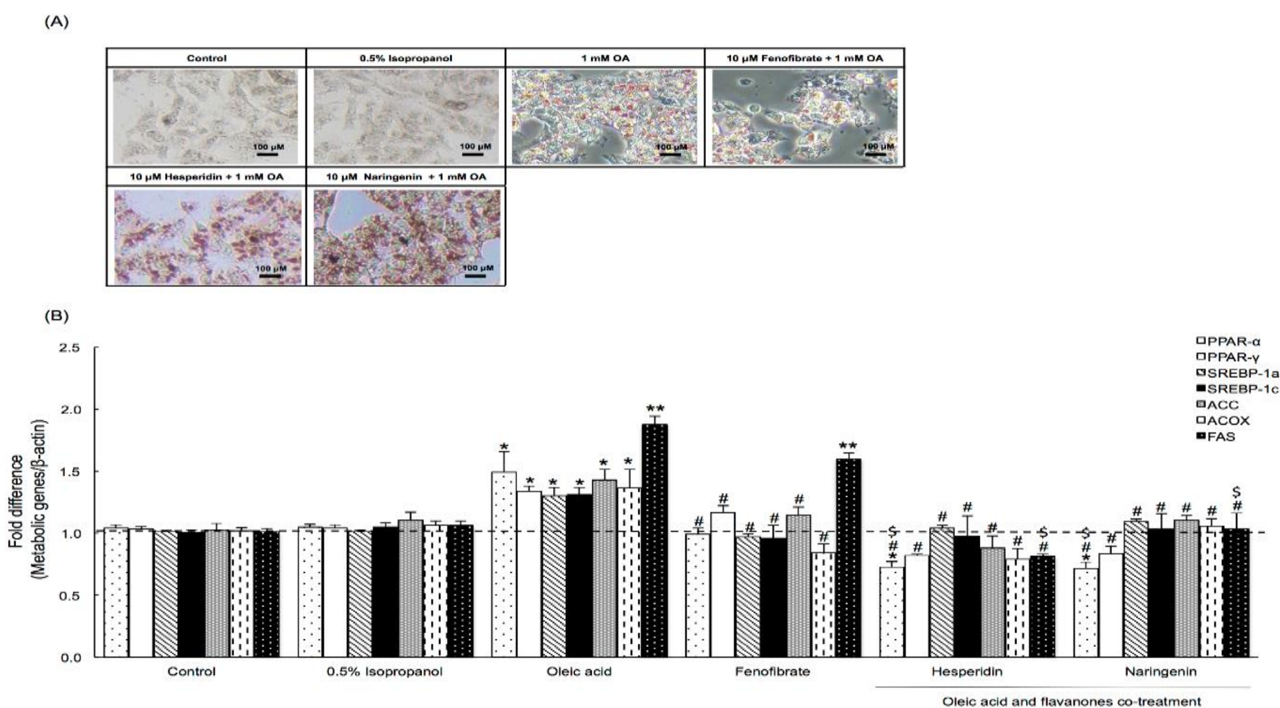

Figure 4: Effect of flavanones on histomorphology and the expression of metabolic genes in oleic acid (OA)-induced NAFLD in HepG2 cells. **(A)** Histomorphology of oleic acid (OA)-induced and flavanone treated NAFLD in HepG2 cells. Red staining indicates lipid storage. **(B)** The expression of metabolic genes in oleic acid (OA)-induced and flavanone treated NAFLD in HepG2 cells. $*p<0.05$, $**p < 0.001$ vs. control; $^{\#}p < 0.05$ vs. OA-induced NAFLD; $^{\$}p < 0.05$ vs. fenofibrate.

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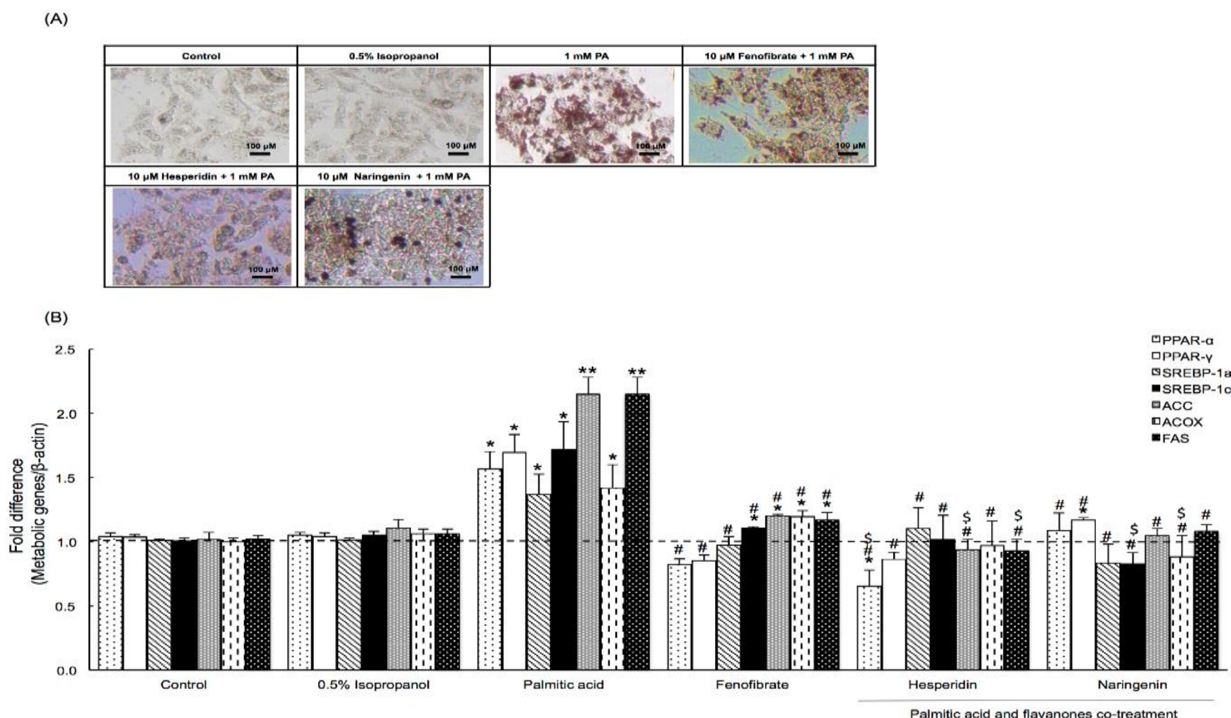

Figure 5: Effect of flavanones on histomorphology and the expression of metabolic genes in palmitic acid (PA)-induced NAFLD in HepG2 cells. **(A)** Histomorphology of palmitic acid (PA)-induced and flavanone treated NAFLD in HepG2 cells. Red staining indicates lipid storage. **(B)** The expression of metabolic genes in palmitic acid (PA)-induced and flavanone treated NAFLD in HepG2 cells.
$^{*}p < 0.05$, $^{**}p < 0.001$ vs. control; $^{\#}p < 0.05$ vs. PA -induced NAFLD; $^{\$}p < 0.05$ vs. fenofibrate.

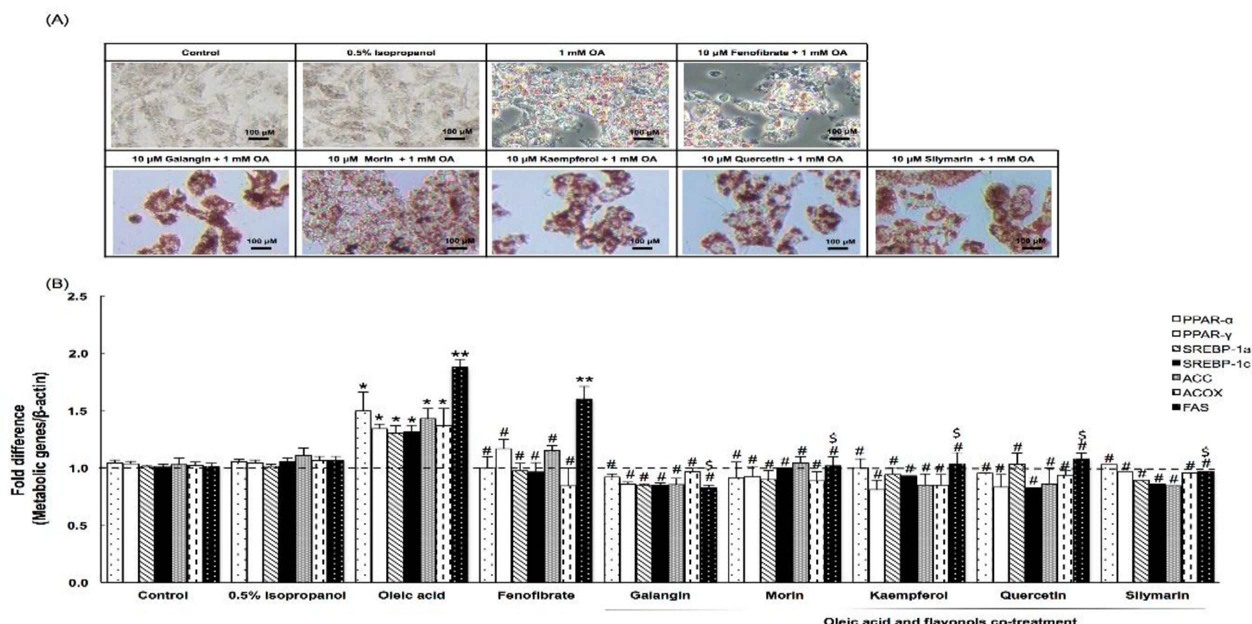

Figure 6: Effect of flavonols on histomorphology and the expression of metabolic genes in oleic acid (OA)-induced NAFLD in HepG2 cells. **(A)** Histomorphology of oleic acid (OA)-induced and flavonol treated NAFLD in HepG2 cells. Red staining indicates lipid storage. **(B)** The expression of metabolic genes in oleic acid (OA)-induced and flavonol treated NAFLD in HepG2 cells.
$^{*}p < 0.05$, $^{**}p < 0.001$ vs. control; $^{\#}p < 0.05$ vs. OA-induced NAFLD; $^{\$}p < 0.05$ vs. fenofibrate.

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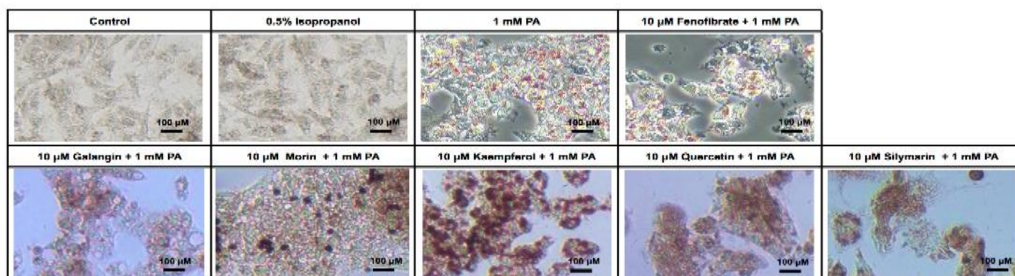

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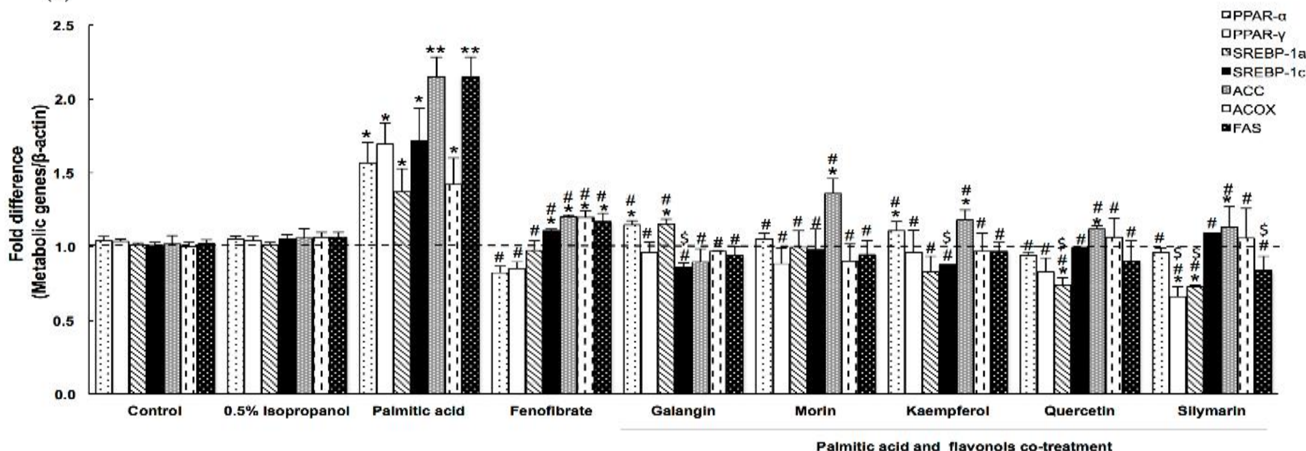

Figure 7: Effect of flavonols on histomorphology and the expression of metabolic genes in palmitic acid (PA)-induced NAFLD in HepG2 cells. **(A)** Histomorphology of palmitic acid (PA)-induced and flavonol treated NAFLD in HepG2 cells. Red staining indicates lipid storage. **(B)** The expression of metabolic genes in palmitic acid (PA)-induced and flavonol treated NAFLD in HepG2 cells.
$^{*}p < 0.05$, $^{**}p < 0.001$ vs. control; $^{\#}p < 0.05$ vs. PA-induced NAFLD; $^{\$}p < 0.05$ vs. fenofibrate.

Therefore, in this study, while the expression of metabolic related genes were improved by the tested flavonoids and the standard drug, recovery in histomorphological features was not yet observed.

According to the results, flavones reduced the OA- and PA-induced increases in PPARs, SREBPs, ACC, and FAS expression, flavanones reduced expression of PPAR-α and FAS, while flavonols mainly down regulated ACC, ACOX, and FAS, which are related to insulin resistance. A previous report mentioned the potential of another flavonol, rutin, to prevent OA-induced activation of SREBP-1c in HepG2 cells.[34] Hesperetin, a flavanone, and nobiletinan, an O-methylated flavone, induced SREBP dependent expression of low density lipoprotein receptor (LDLR) mRNA.[35] Several flavonoids such as hesperetin, naringenin, quercetin, genistein, apigenin, and luteolin were reported to be ligands of several nuclear receptors including PPARs, liver X receptor (LXR), estrogen receptor, glucocorticoid receptor, and pregnane X receptor.[36] In mouse, LXR is normally activated during feeding to induce fatty acid synthesis and cholesterol transport, and its targets include ABC proteins, and pro-lipogenic transcription factor SREBP-1c.[37] Therefore, the impact of these flavonoids on other nuclear receptors related to NAFLD or in *in vivo* studies for systemic effects are recommended for further investigation.

Conclusion

Myricetin and hesperidin controlled the progression of NAFLD in an *in vitro* OA/PA-induced NAFLD model in HepG2 cells by decreasing lipid deposition and alleviating OA/PA-induced increases in PPARs, SREBPs, ACC, ACOX, and FAS expression. Hence, myricetin and hesperidin provide potent activities for further study in other NAFLD models such as *in vivo*.

Conflict of interest

The authors report no conflict of interest.

Authors' Declaration

The work presented in this article is original. The authors are liable for any claims relating to the content of this article.

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