PHARMACIA

Pharmacia 71: 1–10
DOI 10.3897/pharmacia.71.e139050

Research Article

In silico investigation and *in vivo* effect of Berastagi orange (*Citrus sinensis*) peel extract on male obese rats

Andika Prasetyo[1], Winda Rizki Pebrina Batubara[2,3], Dono Indarto[4], Tri Nugraha Susilawati[5]

1 *Undergraduate Program in Medicine, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia*
2 *Clinical Nutrition Postgraduate Program, School of Graduates, Universitas Sebelas Maret, Surakarta, Indonesia*
3 *Nutrition Science Department, Faculty of Public Health, Medistra Institute of Health Lubuk Pakam, Deli Serdang, Indonesia*
4 *Biomedical Laboratory, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia*
5 *Department of Microbiology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia*

Corresponding author: Tri Nugraha Susilawati (tri.susilawati@staff.uns.ac.id)

Received 11 October 2024 ♦ **Accepted** 25 November 2024 ♦ **Published** 18 December 2024

Citation: Prasetyo A, Batubara WRP, Indarto D, Susilawati TN (2024) *In silico* investigation and *in vivo* effect of Berastagi orange (*Citrus sinensis*) peel extract on male obese rats. Pharmacia 71: 1–10. https://doi.org/10.3897/pharmacia.71.e139050

Abstract

Our previous studies show that Berastagi orange peel extract (BOPE) has anti-obesity properties, indicated by its active compounds and effects on reducing body weight gain and body fat percentage. This present study further analysed the effects of BOPE by evaluating docking analysis of BOPE's phytochemicals and measuring lipid profiles, atherogenic indexes, triglyceride metabolism-related proteins (CD36, DGAT1, and DGAT2) levels, and dipeptidyl peptidase-4 (DPP4) activity in high-fat high-fructose (HFHFr)-induced obese rats. Docking analysis shows that BOPE's phytochemicals, i.e., naringenin and hesperidin, potentially inhibit CD36 and DGAT1. BOPE significantly reduced LDL cholesterol, triglyceride levels, and DPP4 activity while increasing HDL cholesterol levels. BOPE also improved atherogenic indexes, indicating that BOPE has anti-atherosclerotic and cardioprotective effects. However, no significant effect was observed in the levels of triglyceride metabolism-related proteins, thus warranting further research towards the development of BOPE as a nutraceutical product for obesity prevention and treatment.

Keywords

atherogenic index, Berastagi orange peel extract, lipid metabolism, lipid profiles, obesity

Introduction

Globally, 38% of people are overweight (BMI ≥ 25 kg/m^2), and it will increase to 51% in 2025 (Lobstein et al. 2023). Obesity poses risks for various complications, including dyslipidaemia, metabolic syndrome, diabetes mellitus, cardiovascular disease, and hypertension (Bischoff et al. 2017). An increasing prevalence of obesity and its complications leads to an economic burden for the country (Lam et al. 2023).

Obesity is a complex, multifactorial disease due to excess lipid accumulation as a result of energy imbalance. The excess energy will be stored as triacylglycerol as an energy reserve (Blüher 2019; Lin and Li 2021). Triacylglycerol is a major compound in dietary fat that will be hydrolysed into fatty acid and monoacylglycerol by gastric and duodenal lipases. Fatty acids are subsequently transported into enterocytes facilitated by the cluster of differentiation 36 (CD36) and utilised in the

triacylglycerol resynthesis via monoacylglycerol and the glyceraldehyde-3-phosphate pathways. Diacylglycerol acyltransferase (DGAT), which has two isoforms: DGAT1 and DGAT2, resynthesises triacylglycerol in the final step of both pathways (Carreiro and Buhman 2018).

Dipeptidyl peptidase-4 (DPP4) is identified as a serine protease that cleaves the incretin hormone, thereby influencing glucose and lipid metabolism. Recent classification categorises DPP4 as an adipokine with higher expression in obese individuals. This elevation correlates positively with increasing adipocyte size and insulin resistance. Consequently, DPP4 likely contributes to obesity pathogenesis and insulin resistance, suggesting that DPP4 activity has become a focal point of interest in managing obesity and its associated metabolic disorders (Love and Liu 2021).

Orange is Indonesia's fourth largest fruit commodity, producing 2.7 million tonnes in 2023 (Indonesia Central Bureau of Statistics 2023). Unfortunately, oranges are used only for pulp, and the peels are discarded as waste. In fact, our prior studies show that Berastagi orange peel extract (BOPE) has good nutrition, such as magnesium, vitamin C, and phytochemicals like naringenin and hesperidin, which could reduce body weight gain and body fat percentage in obese rats (Batubara et al. 2023a, 2023b). Considering BOPE's therapeutic properties in obesity, this present study aimed to further analyse its effect by evaluating docking analysis of BOPE's phytochemicals on triglyceride metabolism-related proteins, lipid profiles, atherogenic indexes, triglyceride metabolism-related proteins (CD36, DGAT1, and DGAT2) levels, and DPP4 activity in male obese rats.

Methods

Molecular docking analysis

The identified phytochemicals in BOPE from previous research by Batubara et al. (2023), including naringenin and hesperidin, were the ligands for molecular docking analysis. The crystal structures of target proteins were obtained from the RCSB PDB database: CD36 (PDB ID: 5LGD), DGAT1 (PDB ID: 6VP0), and DPP4 (PDB ID: 1X70), while the DGAT2 structure was obtained from the AlphaFold protein structure database (www.alphafold.ebi.ac.uk). These protein structures were prepared by removing co-ligands, undesirable protein chains, and water molecules and adding hydrogen and charges using AutoDock Tools version 1.5.7 (Scripps Research, USA). For validation, we redocked using the native ligand of the crystal structures and measured the root mean standard deviation, which was ≤ 2.00 Å. Molecular structures of naringenin (CID: 439246), hesperidin (CID: 10621), pradigastat (DGAT1 inhibitor with CID: 53387035), and ervogastat (DGAT2 inhibitor with CID: 134262752) were retrieved from NCBI PubChem. The ligands were prepared by adding charges and hydrogen and making all flexible bonds rotatable using the AutoDock Tools. The prepared proteins and ligands were docked using AutoDock Vina, and the results were visualised using Biovia Discovery Studio Visualiser 2024 (Biovia, USA).

Animal study

BOPE was made using the previous protocol by Batubara et al. (2023b) and was used in the animal study involving 30 male Wistar albino rats aged 4–5 weeks and weighing 100–200 g. The protocols for animal study and the use of leftover biological materials were approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Sebelas Maret (No. 89/UN27.06.11/KEP/EC/2023 and 53/UN27.06.11/KEP/EC/2024).

Measurement of lipid profiles

Plasma samples were used to measure the lipid profiles, i.e., total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) levels. TC levels were measured using the cholesterol oxidase-peroxidase aminoantipyrine (CHOD-PAP) method, HDL-C and LDL-C were analysed by the precipitation method, and TG levels were measured using the glycerophosphate oxidase-*p*-aminophenazone (GPO-PAP) method. The optical density of the lipid profiles measurement was assessed at a 546 nm wavelength using a semi-automated clinical chemistry analyser (Microlab 300, VitalScientific, Netherlands).

Atherogenic indexes

After measuring lipid profiles, we calculated atherogenic indexes consisting of the atherogenic index of plasma (**AIP**), atherogenic coefficient (**AC**), cardiac risk ratio (**CRR**), and cardioprotective index (**CPI**) using the following formulas:

$$\text{AIP} = \text{Log (TG/HDL-C)}$$

$$\text{AC} = \text{(TC-HDL-C)/HDL-C}$$

$$\text{CRR} = \text{TC/HDL-C}$$

$$\text{CPI} = \text{HDL-C/LDL-C}$$

Preparation of tissue homogenates

Cold PBS-washed duodenum and white adipose tissues were cut into 100 mg and homogenised in 900 µL of radioimmunoprecipitation (RIPA) buffer (Thermo Scientific, USA), which was added with protease cocktail inhibitor (Thermo Scientific, USA), using an ultrasonic homogeniser (Omni, USA). The homogenates were centrifuged at 11,500 rpm for 20 minutes at 4 °C, and the supernatant was collected into a microtube. In addition, the centrifugation process for adipose tissues was repeated once more to remove fat contaminants. Finally, the supernatants were stored at -20 °C until used.

Immunoassay of CD36, DGAT1, and DGAT2 levels

CD36, DGAT1, and DGAT2 levels were measured using the enzyme-linked immunosorbent assay (ELISA) commercial kits (catalogue numbers: BZ-08180023-EB, BZ-08183092, and BZ-22183566-EB; Bioenzy, Indonesia). The ELISA assays were performed according to the manufacturer's instructions, and the optical density was measured using a spectrophotometer (BioRad, USA) at a 450 nm wavelength.

DPP4 activity assay

DPP4 activity was measured using a colorimetric assay referring to the previous protocol (Indarto et al. 2022). This measurement was read every 10 minutes for an hour and calculated using the Beer-Lambert formula and normalised against the total protein contents, which were determined using the bicinchoninic acid (BCA) assay method (Lot. UJ295773; Thermo Scientific, USA).

Statistical analysis

Data were analysed using the statistical program for social science (SPSS) version 25 (IBM, USA). Numerical data were presented as the mean ± standard deviation (SD). The differences in the levels of lipid profiles, atherogenic indexes, CD36, DGAT1, DGAT2, and DPP4 among groups were compared using the one-way ANOVA and the Kruskal-Wallis test for parametric and non-parametric data, respectively. The changes in the levels of lipid profiles and DPP4 activity before and after intervention were analysed using the paired student's T-test and the Wilcoxon signed rank test for parametric and nonparametric data, respectively. A p-value of < 0.05 was considered statistically significant.

Results

Docking analysis of hesperidin and naringenin

In this study, we performed molecular docking to assess the potential of BOPE's phytochemicals in inhibiting CD36, DGAT1, and DGAT2, which are involved in triglyceride metabolism, as well as DPP4, which is involved in insulin secretion. Table 1 shows the docking score and binding site of naringenin and hesperidin on the target proteins. Our results showed that naringenin and hesperidin were able to bind to CD36, DGAT1, and DGAT2, while only naringenin could bind to DPP4. For CD36, only naringenin had lower binding energy than palmitic acid, the CD36 standard ligand. Regarding DGAT1, only hesperidin had a lower binding energy than pradigastat. As with DGAT2 and DPP4, naringenin and hesperidin did not exceed ervogastat and sitagliptin binding energy.

Table 1. Docking of naringenin and hesperidin into the CD36, DGAT1, and DGAT2.

Compound	Docking score (kcal/mol)			
	CD36	DGAT1	DGAT2	DPP4
Naringenin	-8.6	-8.2	-7.8	-8.1
Hesperidin	-4.5	-9.4	-6.8	4.5
Palmitic acid	-6.8	-	-	-
Pradigastat	-	-8.6	-	-
Ervogastat	-	-	-9.2	-
Sitagliptin	-	-	-	-8.6

Fig. 1 represents the three-dimensional docking structures and two-dimensional interactions between naringenin, hesperidin, and palmitic acid with CD36. Naringenin built up one carbon-hydrogen bond, four hydrophobic bonds, and one electrostatic bond, which are the same as the palmitic acid interactions with CD36, except the electrostatic bond with Lys385 (Fig. 1A, C). Hesperidin afforded 15 interactions consisting of three conventional hydrogen bonds and thirteen hydrophobic interactions (Fig. 1B). Seven amino acid residues that interacted with hesperidin were the same as the palmitic acid interactions. Based on ligand interactions, naringenin and hesperidin show an inhibitory effect on CD36.

Fig. 2 shows the three-dimensional docking structures and two-dimensional interactions between naringenin, hesperidin, and pradigastat with DGAT1. Naringenin afforded three bonds to DGAT1: one conventional hydrogen bond and two hydrophobic bonds (Fig. 2A). Nevertheless, there was no similar binding as the pradigastat interactions toward DGAT1 (Fig. 2C). Hesperidin had four hydrogen bonds and five hydrophobic bonds with DGAT1 (Fig. 2B). In addition, hesperidin interactions with DGAT1 had three identical amino acid residues. Hence, only hesperidin shows inhibitory potential with the target DGAT1 protein.

Fig. 3 represents the three-dimensional docking structures and two-dimensional interactions between naringenin, hesperidin, and ervogastat with DGAT2. Naringenin established four interactions, which are a conventional hydrogen bond and three hydrophobic bonds (Fig. 3A). Two of these interactions were the same as ervogastat interactions toward DGAT2 (Fig. 3C). Hesperidin afforded eleven interactions to DGAT2: two carbon-hydrogen bonds, eight hydrophobic bonds, and an electrostatic interaction (Fig. 3B). Five bonds on hesperidin interactions toward DGAT1 were the same as DGAT2 inhibitor interactions. This molecular docking study showed that naringenin and hesperidin potentially inhibit DGAT2 protein.

Fig. 4 shows the three-dimensional docking structures and two-dimensional interactions between naringenin, hesperidin, and sitagliptin with DPP4. Naringenin afforded two hydrogen bonds and five hydrophobic bonds (Fig. 4A). Five of these bonds were similar to sitagliptin interactions with DPP4 (Fig. 4C). Thus, naringenin showed an inhibitory effect on DPP4. This characteristic is not shown in hesperidin (Fig. 4B).

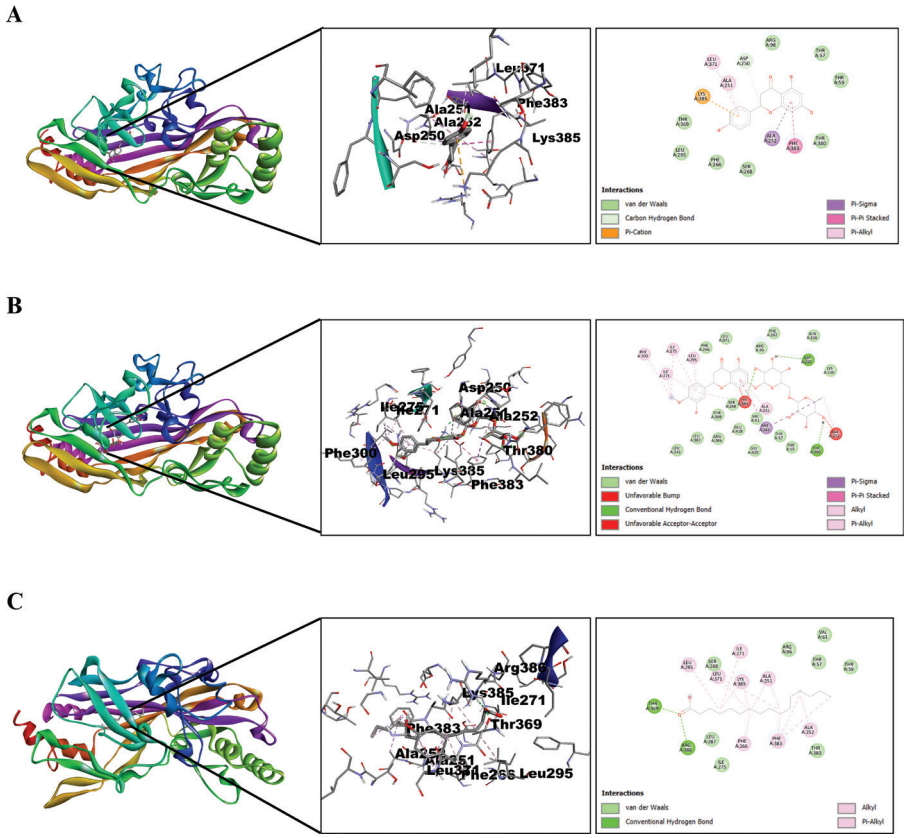

Figure 1. Three-dimensional docking structures and two-dimensional interactions between **A.** Naringenin; **B.** Hesperidin; and **C.** Palmitic acid with the amino acid residues of CD36.

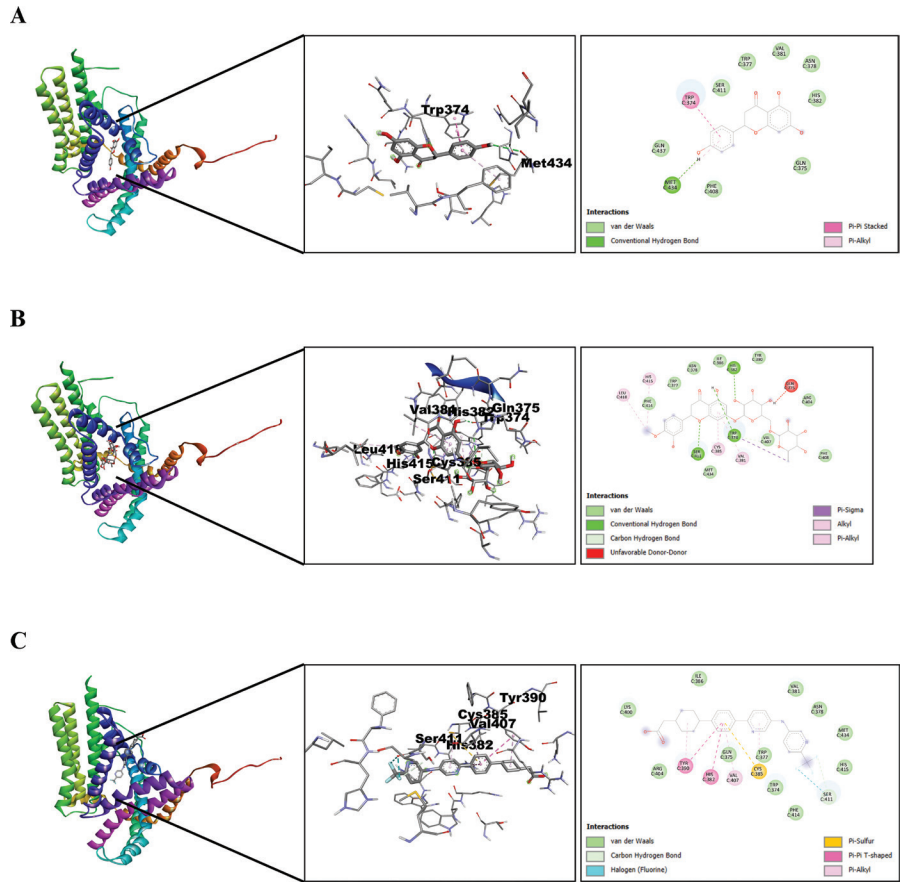

Figure 2. Three-dimensional docking structures and two-dimensional interactions between **A.** Naringenin; **B.** Hesperidin; and **C.** Pradigastat with the amino acid residues of DGAT1.

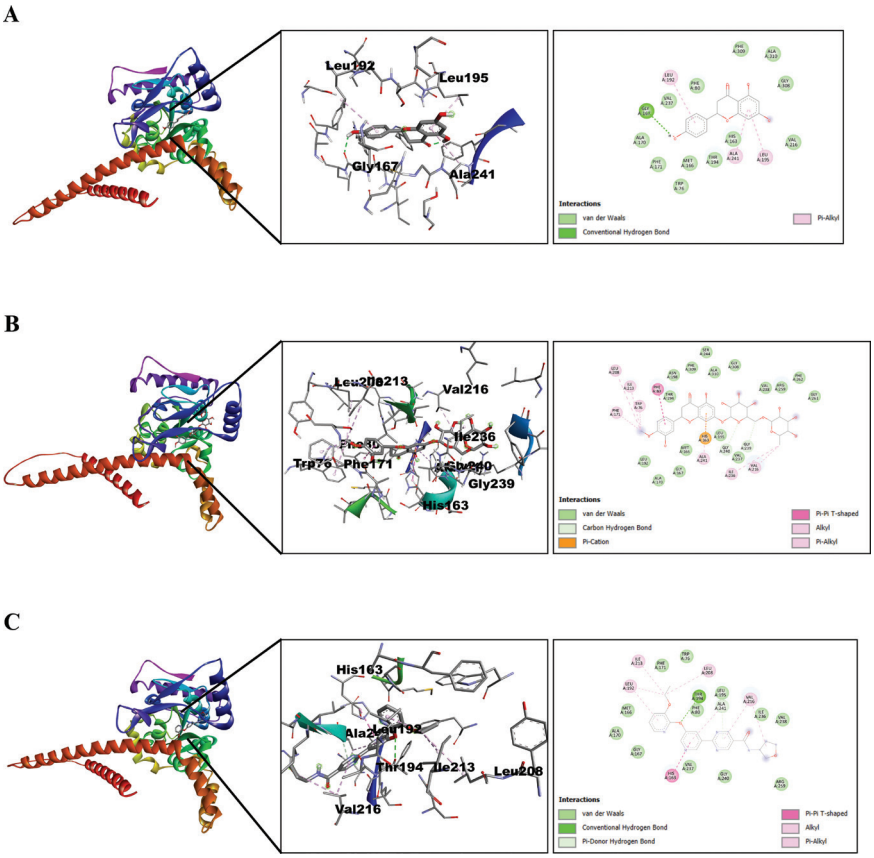

Figure 3. Three-dimensional docking structures and two-dimensional interactions between **A.** Naringenin; **B.** Hesperidin; and **C.** Ervogastat with the amino acid residues of DGAT2.

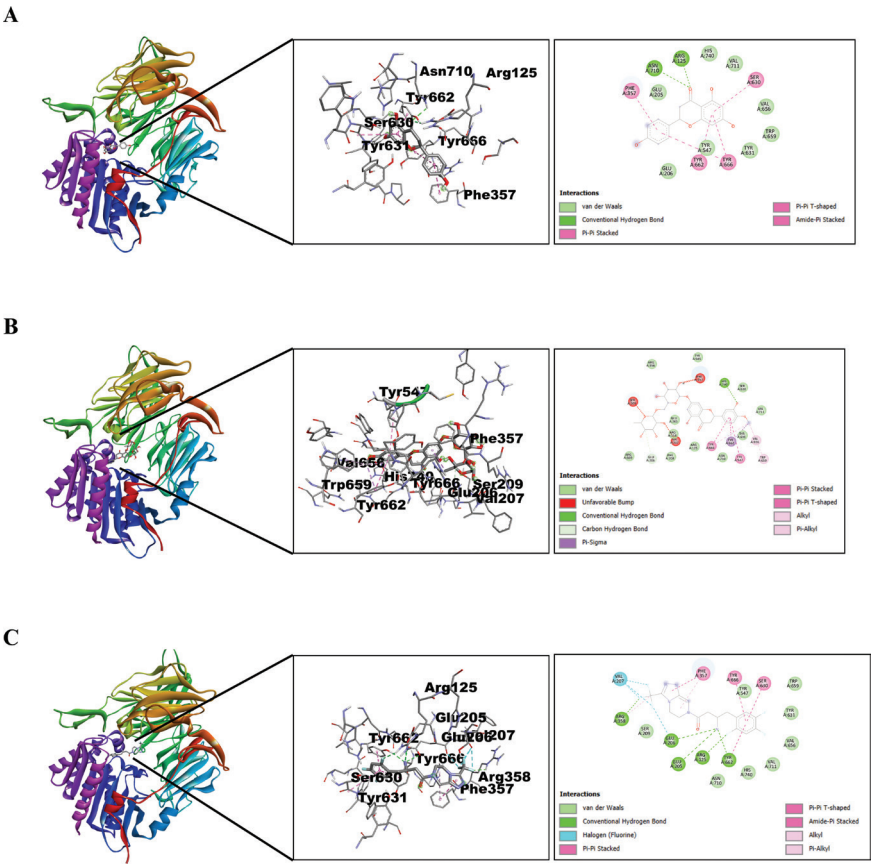

Figure 4. Three-dimensional docking structures and two-dimensional interactions between **A.** Naringenin; **B.** Hesperidin; and **C.** Sitagliptin with the amino acid residues of DPP4.

Effect of BOPE on lipid profiles of obese rats

Fig. 5 represents the changes in lipid profiles after intervention compared to baseline. After 28 days of intervention, the administration of 250/kgBW and 500/kgBW of BOPE per day increased HDL-C levels by 36.6% ($p = 0.026$) and 19.6% ($p = 0.031$), respectively. Furthermore, significantly higher HDL-C levels were observed in the BOPE250 ($p = 0.006$) and BOPE500 ($p = 0.028$) groups, compared to the control group. Compared to the orlistat group, both the BOPE250 and BOPE500 groups demonstrated higher changes in HDL-C level, but it was not statistically significant (Fig. 5A). LDL-C reduction was only observed in the orlistat group after 28 days of intervention. In contrast, the administration of BOPE increased LDL-C levels, particularly in the BOPE500 group ($p = 0.017$). Nevertheless, LDL-C levels in all BOPE groups were lower than those in the control group (Fig. 5B). It was also demonstrated that BOPE administration resulted in a dose-dependent reduction in TG levels. Only the BOPE750 ($p = 0.028$) group showed a significant reduction of TG levels from baseline. Regarding TC level changes, we observed TC level reduction in the orlistat, control, and BOPE750 groups. However, the TC level change in the BOPE750 was higher than that of the control and orlistat groups, but there was no statistical significance ($p = 0.430$).

Anti-atherosclerotic effect of BOPE

To evaluate the anti-atherosclerotic effect of BOPE, we calculated atherogenic indexes consisting of AIP, AC, CRR, and CIP. We observed that the control group was at the highest risk for atherosclerotic events compared to the other groups. In contrast, the groups receiving BOPE had significantly lower AIP, AC, and CRR ($p = 0.06$, $p = 0.010$, and $p = 0.06$, respectively) compared to control (data from ANOVA is not shown). Further analysis showed that compared to control, a significantly lower AIP was only observed in the BOPE750 group ($p = 0.06$) (Fig. 6A), while remarkably lower AC and CRR were observed in the BOPE250 ($p = 0.04$ and $p = 0.04$, respectively) and BOPE750 ($p = 0.032$ and $p = 0.032$, respectively) groups (Fig. 6B, C). Furthermore, the BOPE250 administration caused a significantly higher CPI ($p = 0.004$) compared to the control (Fig. 6D). Our findings indicated that BOPE has an anti-sclerotic effect.

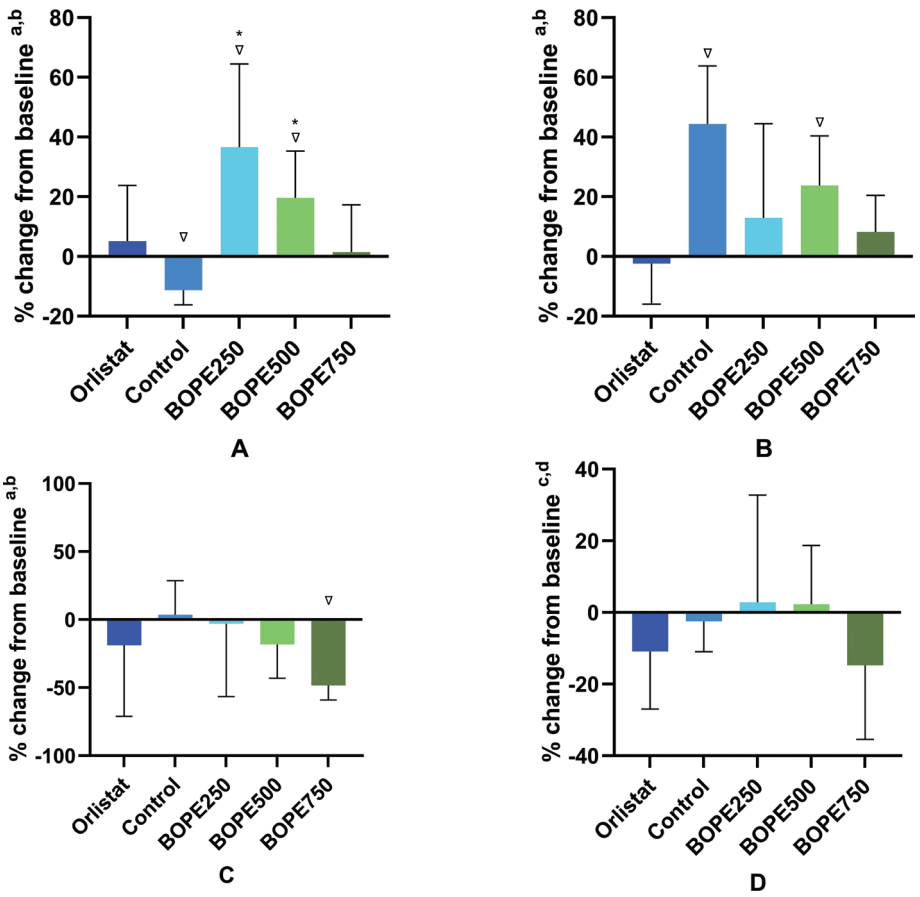

Figure 5. The effect of BOPE on the changes of **A.** HDL cholesterol; **B.** LDL cholesterol; **C.** Triglyceride; and **D.** Total cholesterol levels. The orlistat group was given 12.3 mg/kgBW of orlistat per day, the control group was given 1% Na-CMC, and the BOPE groups were given 250, 500, and 750 mg/kgBW of BOPE per day (the BOPE250, BOPE500, and BOPE750 groups, respectively) for 28 days. [a] Student's paired T-test; white down-pointing triangle $p < 0.05$. [b] Kruskal-Wallis test; *$p < 0.05$ (compared to control). [c] Wilcoxon signed rank test; white down-pointing triangle $p < 0.05$. [d] One-way ANOVA test; *$p < 0.05$ (compared to control).

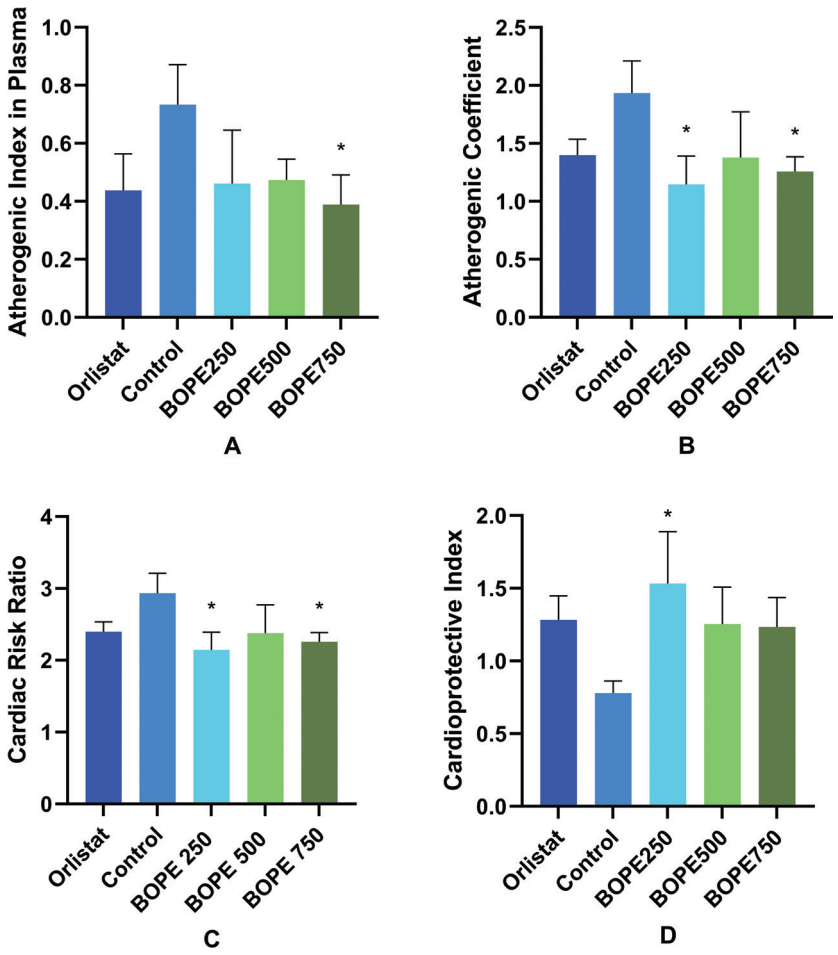

Figure 6. The effect of BOPE on atherogenic indexes; **A.** Index of plasma or AIP; **B.** Atherogenic coefficient or AC; **C.** Cardiac risk ratio or CRR; and **D.** Cardioprotective index or CPI. Statistical significance was calculated using Kruskal-Wallis; *p < 0.05 (compared to control).

Effect of BOPE on CD36, DGAT1, and DGAT2 levels

We have shown that the BOPE administration improved lipid profiles; thus, we sought to evaluate several proteins involved in lipid metabolism, such as CD36, DGAT1, and DGAT2, in the duodenum and white adipose tissues. Compared to the control group, the BOPE250 and BOPE750 groups had higher levels of CD36 in the duodenum and white adipose tissues, although the differences were not statistically significant (Fig. 7A). DGAT1 levels were lower in all BOPE groups than those in the control group in the duodenum tissues, whereas in white adipose tissues, a low DGAT1 level was only observed in the BOPE500 group (Fig. 7B). In contrast to the decreased DGAT1 levels in the duodenum tissue of the BOPE groups, the DGAT2 level was higher than that in the control group. In the adipose tissue, DGAT2 levels of BOPE groups were higher than those in the control group except in the BOPE500 group (Fig. 7C).

Effect of BOPE on DPP4 activity in obese rats

DPP4 activity was evaluated to determine the BOPE effect on glucose homeostasis in obese rats. After 28 days of intervention, the DPP4 activity in all groups was significantly decreased except in the BOPE750 group (Fig. 8). Despite a remarkable decrease in DPP4 activity, there were no differences in DPP4 activity compared to the control. These findings indicate that BOPE administration did not modulate DPP4 activity in obese rats.

Discussion

This study used molecular docking to evaluate the binding affinity and interaction between the BOPE's phytochemicals and three target proteins involved in triglyceride metabolism (CD36, DGAT1, and DGAT2) and DPP4 for glucose homeostasis. CD36 facilitates fatty acid transport in many tissues, while DGAT1 and DGAT2 are involved in converting diacylglycerol into triacylglycerol, especially in the gastrointestinal tract and adipose tissues (Carreiro and Buhman 2018). Thus, inhibiting these proteins would be beneficial in blocking lipid uptake. Our previous research shows that BOPE consists of naringenin and hesperidin (Batubara 2023). Our docking investigation showed that naringenin and hesperidin could bind to CD36, but only naringenin had lower binding energy than the palmitic acid as the native ligand of CD36, indicating that naringenin potentially inhibits CD36. Both narin-

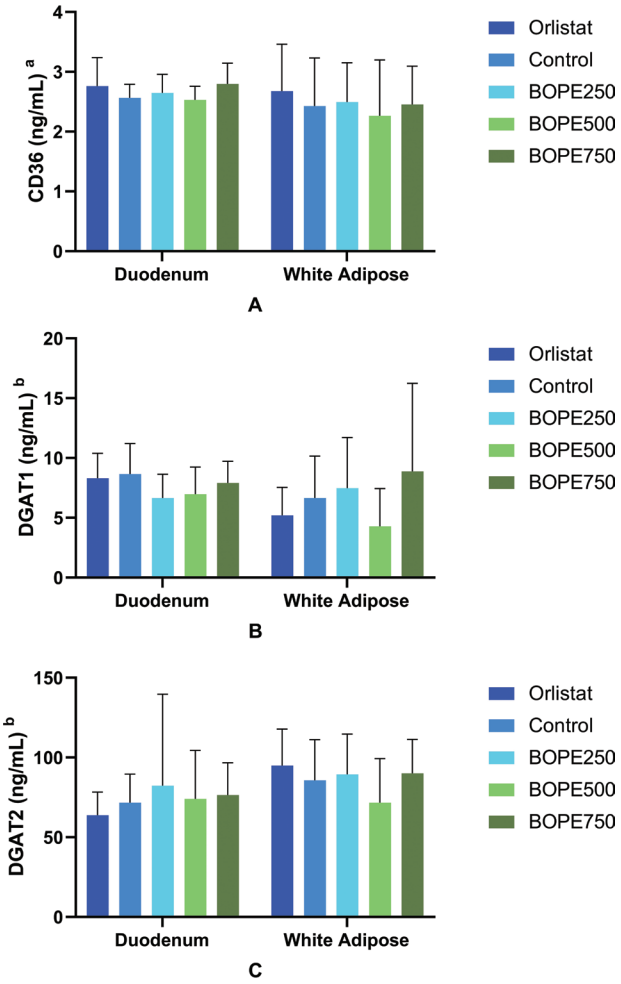

Figure 7. The effect of BOPE on the levels of **A.** CD36; **B.** DGAT1; and **C.** DGAT2. [a] One-way ANOVA test; *p < 0.05 vs. the control group. [b] Kruskal-Wallis test; *p < 0.05 vs. the control group.

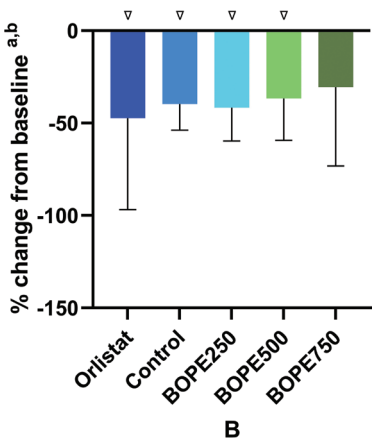

Figure 8. The effect of BOPE on DPP4 activity. [a] Kruskal-Wallis test; *p < 0.05 (compared to control). [b] Wilcoxon signed rank test; white down-pointing triangle p < 0.05.

genin and hesperidin could also bind to DGAT1, yet only hesperidin binding energy was lower than pradigastat binding energy and had three identical bonds, similar to the pradigastat and DGAT1 interactions. A previous study also found that hesperidin had nine interactions, and its binding energy to DGAT1 was -7.07 kcal/mol (Khalil et al. 2022). Our docking showed that hesperidin had a lower binding energy to DGAT1 than that in the Khalil *et al.* study and had different binding pockets. Furthermore, naringenin and hesperidin bound to DGAT2, but neither had lower binding energy than the ervogastat binding energy. Regarding DPP4, only naringenin bound to DPP4, while hesperidin had positive binding energy. Moreover, all naringenin interactions were bound to the active site of DPP4, but the naringenin binding energy was not lower than the sitagliptin binding energy (Kalhotra et al. 2018). So, our docking study suggests that BOPE potentially inhibits CD36 and DGAT1.

We examined the lipid profiles of obese rats to evaluate the hypolipidemic effects of BOPE. Obesity-induced dyslipidaemia is characterised by hypertriglyceridemia, hypercholesterolaemia, and reduced HDL-C (Nussbaumerova and Rosolova 2023). Our study showed that BOPE administration for 28 days resulted in lower LDL-C and TG levels and significantly increased HDL-C levels. In addition, BOPE reduced atherogenic indexes and increased the cardioprotective ratio. These results align with prior studies in which orange peels were beneficial in improving lipid profiles (Ding et al. 2012; Samsudin et al. 2017). We suspected these positive effects were due to BOPE flavonoids—naringenin and hesperidin—that up-regulated adenosine monophosphate-activated protein kinase (AMPK) levels (Lee et al. 2020; Chen et al. 2022; Batubara 2023; Pan et al. 2024). AMPK plays an essential role in regulating lipid and glucose metabolism. Activated AMPK inhibits sterol regulatory element binding protein 1c (SREBP-1c) function as the transcription factor of proprotein convertase subtilisin/kexin type 9 (PCSK9), fatty acid synthase (FAS), and acetyl-CoA carboxylase (ACC) (Han et al. 2019; Cai et al. 2023). Specifically, PCSK9 regulates LDL receptors, which bind to LDL for lysosomal degradation, decreasing the amount of LDL-C (Xia et al. 2021). Moreover, FAS and ACC are responsible for promoting fatty acid synthesis (Batchuluun et al. 2022). Previous studies showed that naringin, the aglycone of naringenin, increased hepatic *Scarb1* and *Lcat* gene expression, which are crucial for HDL-C recycling and formation (Duan et al. 2022; Yang et al. 2022). Thus, our study demonstrated that BOPE has hypolipidemic and anti-atherosclerotic agents, suggesting its potential therapeutic use in managing obese-induced dyslipidaemia.

The duodenum and white adipose tissues play a critical role in lipid absorption and metabolism. Our present study focused on the role of CD36, DGAT1, and DGAT2 in improving lipid profile. We showed that BOPE administration for 28 days slightly lowered DGAT1 and DGAT2 levels while not altering CD36 levels in the duodenum and white adipose tissues. The hypothesis of DGAT1 and DGAT2 reduction may be caused by naringenin and hesperidin. Our molecular docking study revealed that hesperidin potentially inhibits DGAT1. Furthermore, *in vitro* studies on 3T3-L1 adipocytes given hesperidin and naringenin showed lower DGAT1 mRNA levels (Dayarathne et al. 2021; Khalil et al. 2022). As for DGAT2, naringenin supplementation in obese ovariectomised mice resulted in

lower DGAT2 gene expression (Ke et al. 2016). In contrast, BOPE supplementation did not affect CD36 levels in both tissues, although our docking simulation showed that naringenin potentially inhibits CD36. A prior study showed that 20 mg and 100 mg of hesperidin decreased CD36 mRNA levels (Sukkasem et al. 2021). The fluctuation in DGAT1 and DGAT2 levels and the consistent CD36 levels may be due to the limited quantity of bioactive compounds present in BOPE (Batubara 2023). Hence, an optimised method of extraction is needed to get optimum phytochemicals from Berastagi orange peel.

DPP4 is a prolyl oligopeptide family member that regulates glucose and lipid metabolism and is associated with obesity. Circulating DPP4 levels were higher in obese individuals, mostly in the presence of metabolic syndrome. We examined DPP4 activity to determine DPP4 inhibition due to BOPE administration. Our study showed a significant decrease in DPP4 activity in all groups, including the control group. It is hypothesised that this reduction could be attributed to dietary adjustments during BOPE administration. Supporting this hypothesis, a prior study has documented a significant decline in circulating DPP4 levels following two weeks of caloric restriction (Yamauchi et al. 2021). This evidence suggests that dietary interventions may impact DPP4 activity. Our findings from the docking study showed that naringenin was afforded to the DPP4 active site, while hesperidin was not. However, a previous study showed that naringenin and hesperidin had a low inhibitory effect on DPP4 (Pan et al. 2022). Thus, it is indicating that naringenin and hesperidin in BOPE are not potential candidates for DPP4 inhibitors.

Conclusions

This study shows that BOPE effectively improves lipid profiles and atherogenic indexes in HFHFr-induced obese rats. In particular, BOPE could reduce LDL-C and triglyceride levels and increase HDL-C levels. The improvement of atherogenic indexes is characterised by decreased AIP, AC, and CRR and increased CPI. In addition, BOPE slightly decreases DGAT1 and DGAT2 levels, which are involved in lipid metabolism. Consistent with our prior studies, the present results show that BOPE is potentially developed into a nutraceutical product for obesity prevention and treatment. Further research is needed to optimise the orange peel extraction method and fully understand BOPE therapeutic mechanisms in obesity.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that no experiments on humans or human tissues were performed for the present study.

The authors declared that no informed consent was obtained from the humans, donors or donors' representatives participating in the study.

Experiments on animals: The protocols for animal study and the use of leftover biological materials were approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Sebelas Maret (No. 89/UN27.06.11/KEP/EC/2023 and 53/UN27.06.11/KEP/EC/2024).

The authors declared that no commercially available immortalised human and animal cell lines were used in the present study.

Funding

This study is funded by the Directorate of Research, Technology, and Community Service of the Ministry of Education, Culture, Research, and Technology of Indonesia through the Regular Fundamental Research Grants (No. 160/E5/PG.02.00.PL/2023-1280.1/UN27.22/PT.01.03/2023 and No. 086/E5/PG.02.00.PL/2024-1076.1/UN27.22/PT.01.03/2024).

Author contributions

AP: performing molecular docking and ELISA assay, and drafting manuscript; WPPB: performing animal study; DI: conceptualizing research method, supervising data collection and data analysis; TNS: applying for research grant, interpreting results, and editing the manuscript.

Author ORCIDs

Andika Prasetyo https://orcid.org/0009-0008-6226-3670
Winda Rizki Pebrina Batubara https://orcid.org/0009-0001-2320-8678
Dono Indarto https://orcid.org/0000-0001-7420-5816
Tri Nugraha Susilawati https://orcid.org/0000-0003-4428-7894

Data availability

All of the data that support the findings of this study are available in the main text.

References

Batchuluun B, Pinkosky SL, Steinberg GR (2022) Lipogenesis inhibitors: therapeutic opportunities and challenges. Nature Reviews Drug Discovery 21: 283–305. https://doi.org/10.1038/s41573-021-00367-2

Batubara WRP (2023) Effect of administration of sweet orange peel extract (*Citrus sinensis* L) on obesity model male rats: Study on body weight, lee index, body fat percentage, visceral fat mass, lipid profile and dipeptidyl peptidase-4 activity. Thesis, Universitas Sebelas Maret, Surakarta, Indonesia.

Batubara WRP, Susilawati TN, Indarto D (2023a) Nutritional analysis of sweet orange peel extract for development of obesity treatment. Proceedings of the International Conference on Nursing and Health Sciences 4: 311–314. https://doi.org/10.37287/picnhs.v4i1.1820

Batubara WRP, Susilawati TN, Dono I (2023b) The potency of Berastagi sweet orange (*Citrus sinensis*) peel extract for obesity treatment: An experimental study in male rats. Nusantara Bioscience 15: 251–257. https://doi.org/10.13057/nusbiosci/n150212

Bischoff SC, Boirie Y, Cederholm T, Chourdakis M, Cuerda C, Delzenne NM, Deutz NE, Fouque D, Genton L, Gil C, Koletzko B, Leon-Sanz M, Shamir R, Singer J, Singer P, Stroebele-Benschop N, Thorell A, Weimann A, Barazzoni R (2017) Towards a multidisciplinary approach to understand and manage obesity and related diseases. Clinical Nutrition 36: 917–938. https://doi.org/10.1016/j.clnu.2016.11.007

Blüher M (2019) Obesity: global epidemiology and pathogenesis. Nature Reviews Endocrinology 15: 288–298. https://doi.org/10.1038/s41574-019-0176-8

Cai X, Wang S, Wang H, Liu S, Liu G, Chen H, Kang J, Wang H (2023) Naringenin inhibits lipid accumulation by activating the AMPK pathway *in vivo* and *vitro*. Food Science and Human Wellness 12: 1174–1183. https://doi.org/10.1016/j.fshw.2022.10.043

Carreiro AL, Buhman KK (2018) 36 The molecular nutrition of fats absorption of dietary fat and its metabolism in enterocytes. Elsevier Inc., 33–48. https://doi.org/10.1016/B978-0-12-811297-7.00003-2

Chen H, Nie T, Zhang P, Ma J, Shan A (2022) Hesperidin attenuates hepatic lipid accumulation in mice fed high-fat diet and oleic acid induced HepG2 via AMPK activation. Life Sciences 296: 120428. https://doi.org/10.1016/j.lfs.2022.120428

Dayarathne LA, Ranaweera SS, Natraj P, Rajan P, Lee YJ, Han CH (2021) Restoration of the adipogenic gene expression by naringenin and naringin in 3T3-L1 adipocytes. Journal of Veterinary Science 22: 1–17. https://doi.org/10.4142/jvs.2021.22.e55

Ding X, Fan S, Lu Y, Zhang Y, Gu M, Zhang L, Liu G, Guo L, Jiang D, Lu X, Li Y, Zhou Z, Huang C (2012) Citrus ichangensis peel extract exhibits anti-metabolic disorder effects by the inhibition of PPAR γ and LXR signaling in high-fat diet-induced C57BL/6 mouse. Evidence-based Complementary and Alternative Medicine 2012. https://doi.org/10.1155/2012/678592

Duan Y, Gong K, Xu S, Zhang F, Meng X, Han J (2022) Regulation of cholesterol homeostasis in health and diseases: from mechanisms to targeted therapeutics. Signal Transduction and Targeted Therapy 7. https://doi.org/10.1038/s41392-022-01125-5

Han Y, Hu Z, Cui A, Liu Z, Ma F, Xue Y, Liu Y, Zhang F, Zhao Z, Yu Y, Gao J, Wei C, Li J, Fang J, Li J, Fan JG, Song BL, Li Y (2019) Post-translational regulation of lipogenesis via AMPK-dependent phosphorylation of insulin-induced gene. Nature Communications 10: 1–13. https://doi.org/10.1038/s41467-019-08585-4

Indarto D, Dwipajati D, Dirgahayu P, Wibowo YC, Pratama YM (2022) Acute effects of breakfast fruits meal sequence and postprandial exercise on the blood glucose level and DPP4 activity among type 2 diabetes mellitus patients: A Pilot Study. Journal of Obesity 2022: 0–7. https://doi.org/10.1155/2022/4875993

Indonesia Central Bureau of Statistics (2023) Indonesia Statistics 2023. Indonesia Central Bureau of Statistics, Jakarta, 790 pp. https://www.bps.go.id/publication/2020/04/29/e9011b3155d45d70823c141f/statistik-indonesia-2020.html

Kalhotra P, Chittepu VCSR, Osorio-Revilla G, Gallardo-Velázquez T (2018) Structure–activity relationship and molecular docking of natural product library reveal chrysin as a novel dipeptidyl peptidase-4 (DPP-4) inhibitor: An integrated *in silico* and *in vitro* study. Molecules 23: 1368. https://doi.org/10.3390/molecules23061368

Ke JY, Cole RM, Hamad EM, Hsiao YH, Cotten BM, Powell KA, Belury MA (2016) Citrus flavonoid, naringenin, increases locomotor activity and reduces diacylglycerol accumulation in skeletal muscle of obese ovariectomized mice. Molecular Nutrition and Food Research 60: 313–324. https://doi.org/10.1002/mnfr.201500379

Khalil HE, Abdelwahab MF, Emeka PM, Badger-emeka LI, Thirugnanasambantham K, Ibrahim HM, Naguib SM, Matsunami K, Abdel-wahab NM (2022) Ameliorative Effect of Ocimum forskolei Benth on Diabetic, Apoptotic, and Adipogenic Biomarkers of Diabetic Rats and 3T3-L1 Fibroblasts Assisted by *In Silico* Approach. Molecules 27: 2800. https://doi.org/10.3390/molecules27092800

Lam BCC, Lim AYL, Chan SL, Yum MPS, Koh NSY, Finkelstein EA (2023) The impact of obesity: a narrative review. Singapore medical journal 64: 163–171. https://doi.org/10.4103/singaporemedj.SMJ-2022-232

Lee GH, Peng C, Park SA, Hoang TH, Lee HY, Kim J, Kang S Il, Lee CH, Lee JS, Chae HJ (2020) Citrus peel extract ameliorates high-fat diet-induced NAFLD via activation of AMPK signaling. Nutrients 12: 1–17. https://doi.org/10.3390/nu12030673

Lin X, Li H (2021) Obesity: Epidemiology, pathophysiology, and therapeutics. Frontiers in Endocrinology 12: 1–9. https://doi.org/10.3389/fendo.2021.706978

Lobstein T, Jackson-Leach R, Powis J, Brinsden H, Gray M (2023) World Obesity Atlas 2023. World Obesity Federation: 5–25. www.johnclarksondesign.co.uk

Love KM, Liu Z (2021) DPP4 Activity, Hyperinsulinemia, and Atherosclerosis. Journal of Clinical Endocrinology and Metabolism 106: 1553–1565. https://doi.org/10.1210/clinem/dgab078

Nussbaumerova B, Rosolova H (2023) Obesity and dyslipidemia. Current Atherosclerosis Reports 25: 947–955. https://doi.org/10.1007/s11883-023-01167-2

Pan J, Zhang Q, Zhang C, Yang W, Liu H, Lv Z, Liu J, Jiao Z (2022) Inhibition of dipeptidyl peptidase-4 by flavonoids: Structure–activity relationship, kinetics and interaction mechanism. Frontiers in Nutrition 9: 1–17. https://doi.org/10.3389/fnut.2022.892426

Pan T, Lee YM, Takimoto E, Ueda K, Liu PY, Shen HH (2024) Inhibitory effects of naringenin on estrogen deficiency-induced obesity via regulation of mitochondrial dynamics and AMPK activation associated with white adipose tissue browning. Life Sciences 340: 122453. https://doi.org/10.1016/j.lfs.2024.122453

Samsudin RR, Kunsah B, Widyastuti R (2017) The effect of pacitan's sweet orange's (*Citrus sinensis* (L.) osbeck) peel powder on the lipid profile of male dyslipidemia rats (*Rattus novergicus*). Bali Medical Journal 6: 51. https://doi.org/10.15562/bmj.v6i3.720

Sukkasem N, Chatuphonprasert W, Jarukamjorn K (2021) Hesperidin, a novel candidate for the successful treatment of high fat diet plus ethanol-induced fatty liver disease in mice. Journal of Physiology and Pharmacology 72: 217–224. https://doi.org/10.26402/jpp.2021.2.07

Xia XD, Peng ZS, Gu HM, Wang M, Wang GQ, Zhang DW (2021) Regulation of PCSK9 Expression and Function: Mechanisms and Therapeutic Implications. Frontiers in Cardiovascular Medicine 8: 1–13. https://doi.org/10.3389/fcvm.2021.764038

Yamauchi M, Tsuchiya T, Takebayashi K, Inukai T, Hashimoto K (2021) Effects of short-term calorie restriction on circulating DPP-4/sCD26 concentrations and body composition in patients with type 2 diabetes. Diabetology International 12: 286–292. https://doi.org/10.1007/s13340-020-00485-z

Yang Y, Trevethan M, Wang S, Zhao L (2022) Beneficial effects of citrus flavanones naringin and naringenin and their food sources on lipid metabolism: An update on bioavailability, pharmacokinetics, and mechanisms. Journal of Nutritional Biochemistry 104: 108967. https://doi.org/10.1016/j.jnutbio.2022.108967