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Research Article

Garcinia mangostana and *Orthosiphon stamineus* Extracts Attenuate Insulin Resistance and Adipokine Dysregulation via SIRT1–PPARγ Signaling in High-Fat, High-Sucrose Diet-Induced Rats

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Abstract | This study evaluated the effects of combined *Garcinia mangostana* (GM) and *Orthosiphon stamineus* (OS) extracts on insulin resistance, adipokine profiles, and metabolic regulatory gene expression in high-fat, high-sucrose diet (HFHSD)-induced rats. Thirty male Wistar rats were randomly divided into five groups (n = 6/group): normal control (NC), vehicle control (VC), and three treatment groups receiving combined GM–OS extracts at doses of 46.25 mg/kg BW/day (T1), 92.5 mg/kg BW/day (T2), and 185 mg/kg BW/day (T3), respectively, administered orally for 12 weeks following HFHSD induction. Compared with the NC group, the VC group demonstrated significantly increased fasting blood glucose (FBG), insulin concentration, HOMA-IR, and visfatin levels, accompanied by decreased associated with decreased adiponectin concentrations, SIRT1, and PPARγ expression ($p < 0.05$). Administration of combined GM–OS extracts significantly improved these metabolic parameters. The greatest dose (T3) produced the greatest reductions in FBG, insulin concentration, HOMA-IR, and visfatin levels, while significantly increasing adiponectin concentration and expression of SIRT1 and PPARγ compared with VC ($p < 0.05$). For several parameters, T3 exhibited values comparable to those of the NC group, indicating no significant differences between the groups ($p > 0.05$). One-way ANOVA demonstrated significant differences among groups for metabolic, adipokine, and gene expression parameters ($p < 0.05$). In conclusion, combined GM and OS extracts improved insulin resistance, adipokine imbalance, and expression of metabolic regulatory genes in HFHSD-induced rats. These findings suggest potential metabolic benefits associated with modulation of adipose tissue function and insulin sensitivity. However, because this study evaluated only mRNA-level expression in an animal model using a single fixed extract ratio, further mechanistic studies and clinical investigations are required before translation into human therapeutic applications.

Keywords | *Garcinia mangostana*, *Orthosiphon stamineus*, insulin resistance, adiponectin, visfatin, SIRT1-PPARγ

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INTRODUCTION

Insulin resistance (*IR*) is a central pathological feature underlying obesity, type 2 diabetes mellitus (*T2DM*), and cardiovascular diseases, and its prevalence continues to increase globally due to excessive intake of high-fat and high-sugar diets combined with sedentary lifestyles (Ahn, 2025; Yan, 2024). Recent evidence shows that obesity-associated adipose tissue inflammation plays a critical role in systemic metabolic dysregulation and insulin resistance development (Yan, 2024). In insulin-resistant states, adipose tissue undergoes functional impairment characterized by chronic low-grade inflammation and dysregulated adipokine secretion, leading to impaired insulin-mediated regulation of glucose metabolism (Ahn, 2025).

This adipokine imbalance is characterized by decreased adiponectin and altered visfatin levels, both of which are associated with metabolic dysfunction. Adiponectin is widely recognized as an insulin-sensitizing adipokine with anti-inflammatory and metabolic protective effects (Kim *et al*., 2022). In contrast, visfatin has been reported to exhibit dual biological roles, acting as an insulin-mimetic molecule in certain physiological contexts, while in obesity and T2DM it is more consistently associated with chronic inflammation and worsening insulin resistance. Therefore, changes in visfatin levels should be interpreted in relation to overall metabolic and inflammatory status rather than as a unidirectional biomarker.

At the molecular level, insulin sensitivity is closely regulated by the Sirtuin 1 (SIRT1) and Peroxisome Proliferator-Activated Receptor gamma (PPARγ) signaling pathways. SIRT1 is an NAD+-dependent deacetylase that regulates mitochondrial biogenesis, oxidative stress, inflammation, and energy metabolism (Nemeth *et al*., 2023; Palabiyik and Palabiyik, 2025). However, its metabolic effects are context-dependent, varying according to tissue type, disease stage, and metabolic status. In obesity and insulin-resistant conditions, reduced SIRT1 expression contributes to impaired insulin signaling and metabolic inflammation, whereas restoration of SIRT1 activity may improve adiponectin expression and insulin sensitivity (Ahn, 2025). Nevertheless, experimental evidence suggests that SIRT1-related metabolic outcomes are not uniformly beneficial and may differ depending on tissue-specific and physiological contexts (Nemeth *et al*., 2022).

PPARγ plays a central role in adipocyte differentiation, lipid metabolism, and adipokine regulation. Although it is an established therapeutic target for insulin resistance, full PPARγ agonists such as thiazolidinediones are associated with adverse effects including weight gain and fluid retention. This has led to increasing interest in natural compounds with potential PPARγ-modulating effects, potentially providing metabolic benefits with fewer side effects, although their exact agonistic profiles remain incompletely characterized (Montaigne *et al*., 2021).

Current pharmacological management of insulin resistance often requires long-term and combination therapy, which may reduce patient adherence and increase the risk of adverse effects. Consequently, increasing attention has been directed toward plant-derived bioactive compounds with multitarget metabolic actions capable of modulating interconnected pathways involved in insulin resistance. *Garcinia mangostana* L. (GM) pericarp contains xanthones, particularly α-mangostin, which exhibit antioxidant, anti-inflammatory, and insulin-sensitizing properties. Recent studies indicate that α-mangostin modulates SIRT1-related signaling pathways and improves oxidative stress and inflammatory status. Furthermore, α-mangostin has been reported to enhance insulin sensitivity through regulation of AMPK, SIRT1, and inflammatory mediators (John *et al*., 2022; Soetikno *et al*., 2023).

Similarly, *Orthosiphon stamineus* Benth. (OS) contains bioactive compounds such as sinensetin and has been associated with beneficial effects on glucose metabolism, lipid regulation, and inflammatory responses (Prasetyanti *et al*., 2021; Wang *et al*., 2022). The different pharmacological properties of GM and OS suggest potential complementary metabolic effects in improving insulin resistance through multitarget metabolic modulation. Previous *in silico* and molecular docking studies have shown that phytochemicals from GM and OS interact with key proteins involved in insulin signaling and adipokine regulation, including AMPK and PPAR isoforms. These computational findings were used to guide the selection of mechanistic biomarkers, including adiponectin, visfatin, SIRT1, and PPARγ, in the present *in vivo* study. Importantly, dose selection in this study was based on the human-equivalent dose of the registered GM–OS herbal formulation, with safety considerations, rather than computational predictions (Prasetyanti *et al*., 2021; Indiastuti *et al*., 2024, 2026).

Despite growing evidence on the individual effects of GM and OS, there is still a lack of *in vivo* studies investigating their combined effects on insulin resistance, particularly through simultaneous modulation of adipokine balance and the SIRT1–PPARγ signaling axis. Therefore, this study aimed to evaluate the effects of combined *Garcinia mangostana* and *Orthosiphon stamineus* using a single fixed extract ratio on insulin resistance in a high-fat, high-sucrose diet-induced rat model, with particular emphasis on adipokine regulation and the SIRT1–PPARγ signaling axis.

MATERIALS AND METHODS

Study design

This study was a randomized controlled laboratory experimental study using a high-fat, high-sucrose diet (HFHSD)-induced Wistar rat model to evaluate insulin resistance. The experimental duration was 24 weeks, consisting of 12 weeks of induction and 12 weeks of treatment. All experimental groups were age-matched at baseline; therefore, potential age-related metabolic changes were evenly distributed across groups and did not introduce differential bias in comparative analyses.

All procedures were approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia (No. 283/EC/KEPK/FKUA/2023) and conducted in accordance with the Guide for the Care and Use of Laboratory Animals. The study was reported following the ARRIVE 2.0 guidelines.

Animals and housing

Sample size was calculated using PASS software (version 2021) with a statistical power of 95% and α = 0.05. The assumed effect size was derived from published HOMA-IR data in an insulin-resistant rat model reported by Safwat *et al*. (2013). Based on the sample size calculation, six rats were required per group, and the number was increased to eight rats per group to anticipate possible drop out during the study. After the induction period, 30 male Wistar rats that met the model criteria were randomly allocated into five groups, with six rats per group. Although each group initially consisted of eight rats at the beginning of the study and six rats at the start of the treatment period, one animal in the VC group and one animal in the T3 group were excluded due to mortality during the intervention period. The specific cause of death could not be confirmed, as no further diagnostic examination was performed. Thus, the final number of animals analyzed was n= 6 in the NC, T1, and T2 groups, and n = 5 in the VC and T3 groups. At baseline, the rats were 7–8 weeks old and weighed 180–200 g. Male rats were selected to minimize hormonal variability associated with the estrous cycle, which may influence insulin sensitivity and adipokine expression. The use of male-only animals was acknowledged as a limitation regarding sex-based generalization of the findings. Animals were acclimatized for 7 days and maintained under controlled environmental conditions (23 ± 2 °C, 50–60% humidity, 12-hour light/dark cycle) with ad libitum access to standard chow and water.

Herbal preparation and dose determination

The combined extract of Garcinia mangostana (GM) and Orthosiphon stamineus (OS) was obtained as a registered herbal product (BPOM No. POM TR 182310261), containing a fixed 1:1 weight ratio of GM extract and OS extract, corresponding to 150 mg GM extract and 150 mg OS extract per unit dose. The tested batch was chemically characterized using α-mangostin as a marker compound for GM and sinensetin as a marker compound for OS. However, quantitative batch-to-batch analysis of marker compounds was not performed in this study; therefore, the consistency of marker compound levels across different production batches could not be evaluated and is acknowledged as a limitation. Phytochemical profiling using UPLC-QToF-MS/MS identified multiple compounds from GM (including α-mangostin, γ-mangostin, mangostanol, and related xanthones) and OS (including sinensetin and other polymethoxylated flavones).

The human equivalent dose (900 mg/day) was converted to the rat dose using body surface area (BSA) normalization (Reagan-Shaw *et al*., 2008), resulting in 92.5 mg/kg BW/day. No pharmacokinetic validation (plasma exposure of active compounds) was performed; therefore, dose equivalence is based on BSA conversion rather than confirmed systemic exposure equivalence. Three dose levels were used in a ½:1:2 ratio to evaluate dose-dependent biological responses: 46.25 mg/kg BW/day (low dose), 92.5 mg/kg BW/day (medium dose) and 185 mg/kg BW/day (high dose). This range was selected to capture biological response trends around the human-equivalent dose while minimizing toxicity risk.

Induction of metabolic disorder

Metabolic syndrome-like conditions were induced using HFHSD for 12 weeks. Successful induction was defined by the following criteria compared with normal controls: Fasting blood glucose > 125.54 ± 10.32 mg/dL, and Lee index > 300 (Vigneshwar *et al*., 2021; Syarif *et al*., 2024).

After induction, rats that met the MetS model criteria were randomized into four groups (n= 6 per group): Vehicle control (VC), Treatment 1 (T1), Treatment 2 (T2) and Treatment 3 (T3). During the treatment phase, HFHSD was continued in all induced groups to maintain a persistent metabolic stress condition. This design ensures that observed effects reflect therapeutic activity under ongoing metabolic insult rather than recovery from diet withdrawal. However, this may either attenuate or stabilize observed treatment effects depending on the stage of metabolic plateau.

Anthropometric measurements

Body weight and naso-anal length were measured using calibrated instruments. The Lee index was calculated as:

$$Lee\ Index = \frac{\sqrt[3]{Body\ weight\ (g)}}{Naso\text{-}anal\ length\ (cm)} \times 1000$$

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Blood and tissue collection

Blood samples were collected after a 12-hour fast. At the end of the experiment, animals were anesthetized using ketamine–xylazine and euthanized via intracardiac puncture. Retroperitoneal adipose tissue was collected due to its metabolic relevance as a visceral fat depot strongly associated with insulin resistance, dyslipidemia, and systemic inflammation. This depot is also relatively easy to identify and collect consistently in rats, which helps maintain uniformity in sample collection across animals.

Biochemical and hormonal analyses

Serum insulin, adiponectin, and visfatin were measured using rat-specific ELISA kits according to the manufacturer's instructions. Serum insulin was measured in pg/mL according to the rat insulin ELISA kit output and converted to μIU/mL only for HOMA-IR calculation by multiplying the pg/mL value by 0.0287; this conversion was based on the relationship that 1 μIU/mL insulin ≈ 34.84 pg/mL, where 0.0287 ≈ 1/34.84. This factor was not provided by the kit manufacturer but was derived from a general insulin mass-to-activity unit conversion; therefore, the original pg/mL values were retained as the primary ELISA output. Insulin resistance was assessed using HOMA-IR:

$$HOMA\text{-}IR = \frac{Fasting\ insulin\ (\mu IU/mL) \times Fasting\ glucose\ (mg/dL)}{405}$$

Gene expression analysis (RT-qPCR)

Total RNA was extracted from retroperitoneal adipose tissue using a commercial RNA purification kit (Jena Bioscience, Germany). Equal amounts of total RNA from each sample were reverse-transcribed into complementary DNA (cDNA) using the GoScript™ Reverse Transcription System (Promega, USA) according to the manufacturer's protocol. Quantitative real-time PCR (RT-qPCR) was performed using GoTaq® qPCR Master Mix (Promega, USA) for all target genes (Table 1). The amplification protocol consisted of initial denaturation at 95°C for 2 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min.

Melt curve analysis was conducted at the end of each amplification run to confirm the specificity of PCR products. A single dominant peak at the target-specific melting temperature was observed for each target gene, supporting specific amplification without strong evidence of non-specific amplification or primer-dimer formation (Table 1). Primer specificity was further evaluated using in silico NCBI Primer-BLAST analysis. All reactions were performed in duplicate.

SIRT1 and PPARγ gene expression levels were normalized to β-actin as the internal control. β-actin Ct values did not differ significantly across experimental groups and were therefore used for normalization. However, additional reference genes were not evaluated. Raw β-actin Ct values and group-level comparison results are provided in Supplementary Table 1. Primer sets were adopted from previously published studies (Table 1). Relative gene expression was calculated using the 2^(-ΔΔCt) method. Primer amplification efficiencies were not experimentally determined using serial dilution standard curves, therefore, this method was applied under the standard assumption of approximately equivalent amplification efficiencies across all primer sets.

Statistical analysis

Data are presented as mean±standard deviation (SD). SD was used to describe the variability of individual values within each experimental group, whereas the standard error of the mean (SEM) reflects the precision of the estimated mean and can provide a visual impression of smaller data dispersion, especially in studies with small sample sizes. Normality was assessed using the Shapiro–Wilk test and homogeneity of variance using Levene's test. One-way ANOVA was applied for group comparisons. Although the final group sizes were slightly unequal, one-way ANOVA was deemed acceptable, as the assumptions of normality and homogeneity of variance were assessed prior to analysis. Tukey's post hoc test was used for parameters with homogeneous variances, while the Games–Howell post hoc test was used for parameters with violated homogeneity of variance, namely HOMA-IR and SIRT1. Statistical analysis was performed using SPSS version 27, with significance set at $p < 0.05$.

Table 1: Primer sequences used for RT-qPCR analysis.

Primer names	Primer sequences (5' - 3')	Product length (bp)	NCBI ID	Melting curve Tm (°C)	Reference
β-actin	F: TTCTTGGGTATGGAATCCTGT R: AGCACTGTGTTGGCATAGAG	101 bp	NM_031144.3	80.25 -80.53	(Wang *et al.*, 2018)
SIRT1	F: GGCACCGATCCTCGAACAAT R: CGCTTTGGTGGTTCTGAAAGG	119 bp	NM_001414959.1	78.83 - 79.06	(Luo *et al.*, 2017)
PPARγ	F: CGGAGTCCTCCCAGCTGTTCGCC R: GGCTCATATCTGTCTCCGTCTTC	117 bp	NM_001145367.1	84.06 – 84.24	(Grasselli *et al.*, 2012)

Table 2: Effects of GM–OS extracts on fasting blood glucose, insulin, HOMA-IR, and Lee index.

Parameter	NC	VC	T1	T2	T3
FBG (mg/dL)	119.400 ± 9.295^{a}	235.000 ± 14.771^{d}	205.933 ± 7.362^{c}	172.800 ± 14.475^{b}	133.520 ± 13.642^{a}
Insulin (μIU/mL)	4.957 ± 1.217^{a}	13.771 ± 2.702^{c}	9.718 ± 2.596^{b}	6.546 ± 1.246^{ab}	4.785 ± 1.554^{a}
HOMA-IR	1.447 ± 0.302^{a}	8.031 ± 1.894^{c}	4.929 ± 1.285^{c}	2.816 ± 0.719^{b}	1.616 ± 0.714^{ab}
Lee Index	275.860 ± 12.436^{a}	316.295 ± 4.484^{d}	308.442 ± 11.167^{cd}	296.259 ± 8.100^{bc}	288.086 ± 9.165^{ab}

Data are presented as mean ± SD. Different superscript letters within the same row indicate significant differences among groups ($p < 0.05$).

Table 3: Effects of GM–OS extracts on adipokine profiles.

Parameter	NC	VC	T1	T2	T3
Visfatin (ng/mL)	3.396 ± 0.630^{a}	7.728 ± 1.380^{c}	6.038 ± 0.984^{bc}	4.662 ± 1.299^{ab}	3.832 ± 0.857^{a}
Adiponectin (μg/mL)	10.201 ± 2.066^{ab}	5.297 ± 0.779^{c}	7.850 ± 1.051^{bc}	9.235 ± 1.762^{ab}	10.876 ± 0.969^{a}

Data are presented as mean ± SD. Different superscript letters within the same row indicate significant differences among groups ($p < 0.05$).

Table 4: Effects of GM–OS extracts on SIRT1 and PPARγ gene expression.

Parameter	NC	VC	T1	T2	T3
Relative SIRT1 mRNA expression	1.000 ± 0.255^{a}	0.481 ± 0.082^{b}	0.751 ± 0.233^{ab}	0.868 ± 0.158^{a}	0.990 ± 0.098^{a}
Relative PPARγ mRNA expression	1.000 ± 0.177^{a}	0.564 ± 0.053^{b}	0.749 ± 0.145^{ab}	0.871 ± 0.160^{a}	0.947 ± 0.191^{a}

Data are presented as mean ± SD. Different superscript letters within the same row indicate significant differences among groups ($p < 0.05$).

RESULTS

The complete pairwise post hoc comparison matrix supporting the superscript annotations in Tables 2–4 is provided in Supplementary Table 2.

Effects of *Garcinia mangostana* and *Orthosiphon stamineus* extracts on fasting blood glucose, insulin, HOMA-IR and Lee index

The HFHSD-fed vehicle control (VC) group exhibited significantly higher fasting blood glucose (FBG), insulin concentration, and HOMA-IR values compared with the normal control (NC) group ($p < 0.05$). Administration of combined *Garcinia mangostana* and *Orthosiphon stamineus* extracts significantly improved these metabolic parameters, as shown in Table 2.

FBG levels in the VC group (235.000 ± 14.771 mg/dL) were significantly higher than those in the NC group (119.400 ± 9.295 mg/dL). Treatment with the extracts significantly reduced FBG levels in T1 (205.933 ± 7.362 mg/dL), T2 (172.800 ± 14.475 mg/dL), and T3 (133.520 ± 13.642 mg/dL) compared with VC ($p < 0.05$). The lowest FBG level was observed in T3 and the lowest FBG level was observed in T3 and did not differ significantly from the NC group ($p > 0.05$).

Serum insulin concentrations were significantly elevated in VC (13.771 ± 2.702 μIU/mL) compared with NC (4.957 ± 1.217 μIU/mL). Administration of the extracts significantly reduced insulin concentrations in T1 (9.718 ± 2.596 μIU/mL), T2 (6.546 ± 1.246 μIU/mL), and T3 (4.785 ± 1.554 μIU/mL) relative to VC ($p < 0.05$). Insulin concentration in T3 was comparable to NC.

HOMA-IR values were significantly increased in the VC group (8.031 ± 1.894) compared with NC (1.447 ± 0.302), indicating a marked insulin resistance induced by the high-fat diet. Treatment with the combined extracts significantly reduced HOMA-IR in T2 (2.816 ± 0.719) and T3 (1.616 ± 0.714) compared with VC ($p < 0.05$), whereas T1 (4.929 ± 1.285) did not differ significantly from VC. The T3 group exhibited HOMA-IR values similar to those of NC, suggesting a near-complete restoration of insulin sensitivity.

Additional assessment using the Lee index supported these findings. The NC group (n = 6) had a mean Lee index of 275.400 ± 9.295, while the VC group (n = 5) showed a higher mean of 316.295 ± 4.484. The T1 group (n = 6) had a mean of 308.442 ± 11.167, whereas T2 (n = 6) and T3 (n = 5) groups had lower means of 296.259 ± 8.100 and 288.086 ± 9.165, respectively. These results indicate that medium and high doses of treatment effectively reduced obesity-related parameters compared with VC.

Effects of *Garcinia mangostana* and *Orthosiphon stamineus* extracts on adipokine profiles

The VC group demonstrated significantly higher visfatin concentrations and lower adiponectin levels compared

with the NC group ($p < 0.05$). Administration of combined *Garcinia mangostana* and *Orthosiphon stamineus* extracts significantly improved adipokine profiles, as presented in Table 3.

Visfatin concentration in VC (7.728 ± 1.380 ng/mL) was significantly higher than in NC (3.396 ± 0.630 ng/mL). Treatment significantly reduced visfatin levels in T2 (4.662 ± 1.299 ng/mL) and T3 (3.832 ± 0.857 ng/mL) compared with VC ($p < 0.05$), whereas T1 (6.038 ± 0.984 ng/mL) did not significantly differ from VC. The lowest visfatin concentration was observed in T3 and was comparable to NC.

Adiponectin concentration was significantly lower in VC (5.297 ± 0.779 µg/mL) compared with NC (10.201 ± 2.066 µg/mL). Treatment significantly increased adiponectin concentrations in T2 (9.235 ± 1.762 µg/mL) and T3 (10.876 ± 0.969 µg/mL) compared with VC ($p < 0.05$). Although adiponectin concentration increased in T1 (7.850 ± 1.051 µg/mL), the difference was not statistically significant. T3 showed adiponectin levels comparable to NC.

Effects of *Garcinia mangostana* and *Orthosiphon stamineus* extracts on SIRT1 and PPARγ gene expression

Gene expression analysis demonstrated significantly lower SIRT1 and PPARγ expression levels in the VC group compared with the NC group ($p < 0.05$). Administration of combined *Garcinia mangostana* and *Orthosiphon stamineus* extracts increased the expression of both genes, as presented in Table 4.

SIRT1 expression in the VC group (0.481 ± 0.082) was significantly lower than that in the NC group (1.000 ± 0.255). Treatment significantly increased SIRT1 expression in T2 (0.868 ± 0.158) and T3 (0.990 ± 0.098) compared with the VC group ($p < 0.05$), whereas T1 (0.751 ± 0.233) did not differ significantly from VC. SIRT1 expression in T3 did not differ significantly from the NC group ($p > 0.05$).

Similarly, PPARγ expression was significantly lower in the VC group (0.564 ± 0.053) than in the NC group (1.000 ± 0.177). Treatment significantly increased PPARγ expression in T2 (0.871 ± 0.160) and T3 (0.947 ± 0.191) relative to the VC group ($p < 0.05$). Although PPARγ expression in T1 (0.749 ± 0.145) showed an increasing trend, the difference was not statistically significant. PPARγ expression in T3 was comparable to that in the NC group.

DISCUSSION

The present study demonstrated that administration of combined *Garcinia mangostana* and *Orthosiphon stamineus* extracts improved insulin resistance parameters in HFHSD-induced rats, as reflected by significant reductions in fasting blood glucose (FBG), insulin concentration, and HOMA-IR values (Table 2). Insulin concentrations were also significantly lower in treatment groups, accompanied by progressive reductions in HOMA-IR values. The HOMA-IR value in the NC group was comparable to the lower range reported in Wistar rats by Imam *et al*. (2022), whereas the elevated value observed in the VC group supports the presence of insulin resistance after HFHSD induction. Notably, HOMA-IR and FBG values in the T3 group did not differ significantly from those in the NC group ($p > 0.05$), suggesting that the highest dose of GM–OS improved insulin sensitivity and glycemic regulation toward normal control levels. However, this finding should be interpreted as the absence of a statistically significant difference, rather than as confirmed statistical equivalence.

These findings suggest that the combined extracts improved glucose homeostasis and insulin responsiveness despite continued exposure to a high-fat, high-sucrose diet. Previous studies have shown that impairment of insulin signaling pathways, particularly PI3K/Akt signaling, plays an important role in obesity-associated insulin resistance and glucose dysregulation (Su *et al*., 2021; Ahn, 2025).

Improvement in insulin resistance in the treatment groups was accompanied by increased expression of metabolic regulatory genes, particularly SIRT1 and PPARγ (Table 4). Post hoc analysis demonstrated no significant difference between the T3 and NC groups for PPARγ expression ($p > 0.05$), although the study was not specifically powered to detect very small differences of approximately 5% between groups. The observed increases in SIRT1 and PPARγ expression were consistent with improvements in glucose metabolism and insulin sensitivity observed in treatment groups. The approximately 50% reduction of SIRT1 expression in the VC group is biologically plausible, as high-fat diet feeding has been reported to reduce SIRT1 mRNA levels by approximately two-fold in white adipose tissue. Studies from 2012 further support the role of adipose SIRT1 in obesity-related inflammation and insulin resistance (Chalkiadaki and Guarente, 2012).

The increased mRNA expression of Sirt1 in this study may support improved metabolic regulation in HFHSD-induced obese/insulin-resistant rat models. However, since SIRT1 exerts tissue-specific effects and is disease-context dependent, these results do not imply that increased SIRT1 expression is universally beneficial under all metabolic conditions (Fu and Hao, 2025; Sola-Sevilla *et al*., 2025). Experimental studies have demonstrated that SIRT1 modulates insulin sensitivity through regulation of IRS–

PI3K–Akt signaling and suppression of proinflammatory mediators that contribute to adipose tissue inflammation and insulin resistance. Therefore, increased SIRT1 expression observed following administration of the combined extracts may contribute to improved insulin signaling and reduced HOMA-IR values in HFHSD-induced rats.

Increased PPARγ expression was also accompanied by improvements in adipocyte metabolic regulation and glucose homeostasis. PPARγ is a central transcription factor involved in adipocyte differentiation, lipid metabolism, insulin sensitivity, and adipokine regulation. Reduced PPARγ expression is commonly associated with adipose tissue dysfunction and impaired glucose metabolism during obesity and insulin resistance (Kim *et al*., 2022). Increased PPARγ expression observed particularly in T2 and T3 groups suggests improvement of adipocyte metabolic responsiveness and glucose utilization following treatment. The exact post hoc p-value for the T3 vs NC comparison was 0.978, indicating no statistically significant difference; however, this finding should be interpreted as statistical non-significance rather than confirmed equivalence. Previous studies have demonstrated functional interactions between SIRT1 and PPARγ, in which SIRT1 regulates adipogenic transcriptional activity through deacetylation-related mechanisms involved in adipose tissue metabolism and insulin sensitivity (Kounatidis *et al*., 2025). However, the present study evaluated only mRNA expression levels; therefore, the molecular interactions, protein expression, and functional activities underlying these responses require further investigation.

Although increased PPARγ expression is generally associated with improved insulin sensitivity, excessive PPARγ-related signaling has also been linked to adipogenesis, lipid accumulation, fluid retention, and weight gain. Therefore, changes in *Pparγ* mRNA expression in this study are interpreted as modulation of PPARγ-related pathways at the transcriptional level, rather than direct evidence of PPARγ agonism. Accordingly, the observed effect is more appropriately described as a PPARγ-modulating effect. Full PPARγ agonists, such as thiazolidinediones, have been reported to promote adipose tissue expansion during long-term administration (Montaigne *et al*., 2021; Zhang *et al*., 2024). The present study did not evaluate body composition, adipocyte size, fat pad weight, or adipose tissue redistribution; therefore, further studies are required to determine whether prolonged administration of the combined extracts influences adiposity or lipid storage despite improvements in insulin resistance parameters.

Alterations in adipokine profiles further supported improvement of adipose tissue metabolic function in the present study (Table 3). HFHSD induction significantly increased visfatin concentrations while reducing adiponectin levels in the VC group compared with NC, suggesting adipose endocrine dysfunction. Moreover, adiponectin and visfatin levels in T3 did not differ significantly from those in the NC group ($p > 0.05$), suggesting restoration toward the normal control range of adipokine homeostasis following treatment. Although adiponectin concentration in T3 was numerically higher than in NC, the difference was not statistically significant and was therefore interpreted as restoration toward the normal control range. The higher SD observed in the NC group may reflect basal inter-individual variation among untreated normal rats, although this should be interpreted cautiously given the small sample size. Reduction in visfatin concentration should be interpreted as significant only in T2 and T3 groups, while T1 showed a non-significant intermediate response between VC and NC. Adipokines play critical roles in obesity-associated inflammation, insulin resistance, and metabolic syndrome progression (Dakroub *et al*., 2020; Roy *et al*., 2023).

Visfatin has been associated with obesity, chronic inflammation, endothelial dysfunction, and insulin resistance, although its biological role remains complex because several studies have also reported insulin-mimetic effects under specific physiological conditions (Abdalla, 2022). In the present study, reduction in visfatin concentrations following treatment was interpreted as part of improved metabolic and inflammatory. Meanwhile, adiponectin functions as an insulin-sensitizing adipokine with anti-inflammatory and anti-atherogenic properties that contribute to improved glucose uptake and fatty acid oxidation (Cersosimo *et al*., 2020). Therefore, concurrent reduction in visfatin and increase in adiponectin concentrations indicate improvement of adipose tissue endocrine regulation following administration of the combined extracts.

The increase in adiponectin concentration observed in this study coincided with increased SIRT1 and PPARγ expression. Previous studies have suggest that SIRT1 regulates adiponectin secretion through FoxO1- and PPARγ-dependent pathways that influence insulin sensitivity and fatty acid metabolism (Chen *et al*., 2022). However, these molecular mechanisms were not evaluated directly in adipose tissue in the present study; therefore, interpretation should be considered supportive rather than definitive. The coordinated increase in SIRT1 expression, PPARγ expression, and adiponectin concentration observed particularly in T3 suggests integrated modulation of adipose tissue metabolism following administration of the combined extracts.

Interestingly, the lowest dose group (T1) showed significant reductions in FBG and insulin concentrations compared with the vehicle control, whereas HOMA-IR was numerically lower but not significantly different. These findings suggest that the lower dose may have been sufficient to improve systemic glucose metabolism but insufficient to substantially modulate adipokine regulation and adipose tissue endocrine function.

Assessment of obesity-related parameters using the Lee index further supported these findings. The NC group had a mean Lee index of 275.86 ± 12.44, while the VC group showed a higher mean of 316.29 ± 4.48, consistent with HFHSD-induced obesity. The T1 group had a mean Lee index of 308.44 ± 11.17, which was not significantly different from VC. In contrast, T2 and T3 groups exhibited lower mean Lee indices of 296.26 ± 8.10 and 288.09 ± 9.17, respectively, indicating that medium and high doses of the combined extracts effectively mitigated obesity-related parameters. These results demonstrate a dose-dependent effect of the extracts on body composition and support the improvements in insulin sensitivity and adipokine profiles observed in treated groups.

The beneficial metabolic effects observed in the present study are likely attributed to bioactive compounds present in Garcinia mangostana and Orthosiphon stamineus. *Garcinia mangostana* pericarp contains xanthones, particularly α-mangostin, which possess antioxidant, anti-inflammatory, and antihyperglycemic properties. Previous studies have reported that α-mangostin improves glucose metabolism and suppresses inflammatory pathways associated with insulin resistance and adipose tissue dysfunction (Li *et al*., 2019; Labban *et al*., 2021). Meanwhile, *Orthosiphon stamineus* contains phenolic compounds and polymethoxylated flavones such as sinensetin, which demonstrate antidiabetic and antioxidant activities through modulation of oxidative stress and glucose uptake pathways (Ahda *et al*., 2023; Bassalat *et al*., 2023). Although the present study did not directly evaluate synergistic interactions, compare single-extract treatments, or assess alternative extract ratios, administration of the combined extracts was associated with improvements in insulin sensitivity, adipokine profiles, and metabolic regulatory gene expression in HFHSD-induced rats.

Several limitations of this study should be acknowledged. First, this study included only male rats; therefore, potential sex-specific responses should be considered. In addition, the slightly reduced sample size in the VC and T3 groups may have reduced the statistical power of the analysis. Second, the molecular analysis was limited to mRNA expression of SIRT1 and PPARγ, without assessment of protein expression, enzyme activity, downstream signaling pathways, multiple reference genes, or primer amplification efficiency using serial dilution standard curves. Thus, the findings should be interpreted as changes in gene expression rather than definitive evidence of pathway activation. Third, this study did not include single-extract groups, alternative GM:OS ratios, or a positive control drug, so synergistic effects and comparative efficacy against standard therapy could not be directly determined. Fourth, quantitative batch-to-batch standardization of specific phytochemical markers and pharmacokinetic evaluation were not performed. Finally, adipose-related findings were limited to retroperitoneal adipose tissue, and adipocyte morphology, fat pad weight, body composition, and long-term safety parameters were not evaluated. Further studies are needed to confirm the molecular mechanisms, tissue-specific effects, safety, and translational relevance of the combined extracts.

CONCLUSION

In conclusion, administration of combined *Garcinia mangostana* and *Orthosiphon stamineus* extracts improved insulin resistance parameters, adipokine imbalance, and expression of metabolic regulatory genes in HFHSD-induced rats. The combined extracts reduced fasting blood glucose, insulin concentration, HOMA-IR, and visfatin levels while increasing adiponectin levels as well as SIRT1 and PPARγ expression, particularly at medium and high doses. These findings suggest that the combined extracts may modulate metabolic pathways associated with insulin sensitivity and adipose tissue function under conditions of diet-induced metabolic stress. However, because the present study was limited to an animal model using a single fixed extract ratio, the findings should not be directly extrapolated to humans. Additional mechanistic studies, pharmacokinetic evaluations, and well-designed clinical studies are required to determine safety, efficacy, optimal dosing, and translational applicability in human metabolic disorders.

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NOVELTY STATEMENT

This study provides novel in vivo evidence on the combined effects of *Garcinia mangostana* and *Orthosiphon stamineus* extracts in improving insulin resistance, adipokine dysregulation, and metabolic regulatory gene expression

in a high-fat, high-sucrose diet-induced rat model. Unlike previous studies that mainly evaluated the individual effects of these plants or focused on computational predictions, the present study investigated a fixed-ratio combination based on a registered herbal formulation and assessed its effects on fasting blood glucose, insulin concentration, HOMA-IR, adiponectin, visfatin, SIRT1, and PPARγ expression. These findings contribute to the understanding of the potential metabolic benefits of combined GM–OS extracts through modulation of adipose tissue function and the SIRT1–PPARγ signaling axis.

AUTHOR'S CONTRIBUTION

Danti Nur Indiastuti contributed to the conceptualization, study design, experimental work, data collection, data analysis, interpretation of results, and preparation of the manuscript. Arifa Mustika contributed to the study design, supervision, methodological guidance, and critical revision of the manuscript. Suharjono contributed to the pharmacological concept, dose determination, data interpretation, and manuscript review. Chrismawan Ardianto contributed to experimental design, statistical analysis, and interpretation of pharmacological findings. I Nengah Budi Sumartha contributed to methodological review, data interpretation, and critical revision of the manuscript. Lita Rakhma Yustinasari contributed to animal handling, veterinary-related procedures, and review of animal experimental aspects. Ratna Wardani contributed to data interpretation, manuscript review, and public health-related perspective. Sukardiman contributed to the conceptualization, supervision, phytochemical and pharmacological interpretation, manuscript review, and final approval of the manuscript. All authors read and approved the final version of the manuscript.

Generative AI and AI-assisted technology statement

The authors used generative AI and AI-assisted technology only to support language editing, grammar improvement, and manuscript clarity. The authors did not use AI tools to generate research data, perform statistical analysis, interpret experimental results, or draw scientific conclusions. All scientific content, data interpretation, and final manuscript decisions were reviewed, verified, and approved by the authors.

Conflicts of interest

The authors have declared no conflict of interest.

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Supplementary Table 1: Raw β-actin Ct values and group comparison across experimental groups.

Group	Sample ID	β-actin Ct			
		Replicate 1	Replicate 2	Mean	Group Mean ± SD
NC	NC-1	23.774	23.794	23.784	23.509 ± 0.554
	NC-2	23.972	23.883	23.928	
	NC-3	23.985	24.050	24.017	
	NC-4	23.038	23.077	23.057	
	NC-5	22.664	22.566	22.615	
	NC-6	23.685	23.624	23.654	
VC	VC-1	22.948	22.900	22.924	22.862 ± 0.234
	VC-2	22.565	22.401	22.483	
	VC-3	23.003	23.066	23.034	
	VC-4	23.016	23.105	23.061	
	VC-5	22.789	22.824	22.806	
T1	T1-1	22.545	22.677	22.611	23.273 ± 0.479
	T1-2	23.317	23.348	23.333	
	T1-3	22.867	22.888	22.878	
	T1-4	23.602	23.446	23.524	
	T1-5	23.250	23.389	23.320	
	T1-6	23.968	23.972	23.970	
T2	T2-1	23.638	23.647	23.643	23.278 ± 0.416
	T2-2	23.149	23.131	23.140	
	T2-3	23.608	23.463	23.536	
	T2-4	22.838	22.992	22.915	
	T2-5	23.731	23.707	23.719	
	T2-6	22.667	22.760	22.713	
T3	T3-1	22.892	22.845	22.869	23.279 ± 0.479
	T3-2	23.195	23.009	23.102	
	T3-3	22.926	22.813	22.870	
	T3-4	23.976	23.915	23.945	
	T3-5	23.585	23.633	23.609	

Values represent raw β-actin Ct values obtained from duplicate technical reactions. The mean β-actin Ct value of each biological sample was used for statistical analysis. Group mean ± SD values were calculated from the mean Ct values of biological samples. One-way ANOVA showed no significant difference in β-actin Ct values among groups ($p = 0.254$), supporting the stability of β-actin as the reference gene under the present experimental conditions. NC, normal control; VC, vehicle control; T1–T3, treatment groups.

Supplementary Table 2: Pairwise post-hoc comparison matrix for metabolic, adipokine, and gene expression parameters.

Group	FBG	Insulin	HOMA-IR	Lee Index	Visfatin	Adiponectin	SIRT1	PPARγ
NC-VC	< 0.0001	< 0.0001	0.0062	< 0.0001	< 0.0001	0.0001	0.0173	0.0009
NC-T1	< 0.0001	0.0027	0.0050	0.0001	0.0021	0.0689	0.4410	0.0669
NC-T2	< 0.0001	0.6284	0.0227	0.0103	0.2663	0.7775	0.8126	0.6030
NC-T3	0.3319	0.9999	0.9845	0.2570	0.9588	0.9375	1.0000	0.9782
VC-T1	0.0050	0.0177	0.0913	0.6675	0.0967	0.0562	0.1703	0.3062
VC-T2	< 0.0001	< 0.0001	0.0113	0.0176	0.0007	0.0015	0.0056	0.0244
VC-T3	< 0.0001	< 0.0001	0.0043	0.0010	0.0001	< 0.0001	0.0002	0.0056
T1-T2	0.0008	0.0674	0.0472	0.2199	0.1971	0.4821	0.8398	0.6542
T1-T3	< 0.0001	0.0031	0.0042	0.0155	0.0172	0.0172	0.2543	0.2475
T2-T3	0.0002	0.5798	0.1221	0.6344	0.6975	0.3628	0.5505	0.9239

Values represent post-hoc pairwise p-values. Games–Howell test was used for HOMA-IR and SIRT1 because of unequal variance, whereas Tukey's test was used for the remaining parameters.