Pharmacoscript
Khoiroh et al./Pharmacoscript, Volume 9, No, 2, Agustus 2026, 327-335
P-ISSN : 2622-4941 | E-ISSN : 2685-1121

THE ETHANOL EXTRACT OF *Piper sarmentosum* Roxb. IMPROVES METABOLIC PARAMETERS IN HIGH-FAT DIET-INDUCED MICE

Itsna Rofiidatul Khoiroh[1*], Sri Adi Sumiwi[2], Jutti Levita[2]

[1]Master Program in Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Indonesia

[2]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Indonesia

*Email: itsna24001@mail.unpad.ac.id

Received: 18/06/2026 Revised: 02/07/2026 Accepted: 06/07/2026 Published: 31/08/2026

ABSTRACT

A high-fat diet (HFD) is a major risk factor for insulin resistance (IR), obesity, and metabolic disorders. Flavonoids have been reported to attenuate IR and obesity-related metabolic alterations in experimental animal models. As *Piper sarmentosum* Roxb. leaves are rich in flavonoids and phenolic compounds, this study aimed to evaluate the effects of the ethanol extract of *P. sarmentosum* leaves (EEPS) on body weight, abdominal circumference, and liver index in HFD-fed mice. Mice were fed an HFD to induce IR and treated with EEPS at different doses. EEPS at 250 mg/kg significantly attenuated HFD-induced increases in body weight gain (12.80%), abdominal circumference (8.44%), and liver index (6.87%) compared with the negative control group ($p < 0.05$). These findings suggest that EEPS attenuates HFD-induced increases in body weight, abdominal adiposity, and liver index in mice.

Keywords: Diabetes Mellitus, Flavonoids, Insulin Resistance, Phenolics, Piperaceae, *Piper sarmentosum*

INTRODUCTION

Diabetes mellitus (DM) is considered one of the leading health problems worldwide, with a rapid increase in incidence rate and mortality as reported by the International Diabetes Federation. In Southeast Asian countries, the prevalence of DM reached 106.9 million; of those, 20.4 million were in Indonesia (International Diabetes Federation, 2025). The development of DM is strongly linked to insulin resistance (IR). Most patients with chronic disease, e.g., DM and cardiovascular disease (CVD), have shown a history of meal imbalances, such as excessive carbohydrate and high-fat diet (HFD) intakes (Chen et al., 2023).

High-fat diet (HFD) has been reported to contribute to the development of insulin resistance (IR) through multiple proposed mechanisms, including reductions in short-

chain fatty acids (SCFAs), impaired activation of free fatty acid receptors (FFARs), and increased oxidative stress and hyperglycemia. IR is also linked with the buildup of abdominal fat and increased body weight (Abbasi & Khodadadi, 2025). An increase in body weight is a parameter for IR development in metabolic disorders. Among middle-aged adults in the Netherlands, greater accumulation of visceral and liver fat was observed in individuals who experienced weight gain and IR (Verkouter et al., 2019).

Flavonoids have shown inhibition against IR development in rodents. Supplementation with flavonoids, such as anthocyanidins and hesperidin, has resulted in a significant restoration in glucose uptake in the IR group, suggesting IR amelioration (Sui et al., 2025). *Piper sarmentosum* Roxb. (Piperaceae) leaves are traditionally used in Sundanese medicine. Studies have shown they contain flavonoids and phenolic compounds (Azelan et al., 2020). Previous studies indicate that the ethanol extract contains high levels of phenolic and flavonoid compounds, with total phenolic content (TPC) of 60.61 ± 0.96 mg GAE/g and a flavonoid content (TFC) of 70.14 ± 0.38 mg QE/g (Nguyen et al, 2020). In a fructose-induced obesity model, treatment with the methanol extract of *P. sarmentosum* at a dose of 125 mg/kg exhibited anti-obesity and antihyperlipidemic effects, which were attributed to decreased adipocyte accumulation and suppression of HMG-CoA reductase activity (Kumar et al., 2021). This study aimed to evaluate the effects of the ethanol extract of *Piper sarmentosum* leaves (EEPS) on body weight gain, abdominal circumference, and liver index in mice fed a HFD.

METHODS

Tools and Materials

Tools were an analytical digital balance (Ohaus Pioneer PA 114), a rotary evaporator (RV 8, IKA), a water bath (Memmert), macerator, oral gavage (sonde), volumetric flasks and other chemical glasswares (Pyrex), blender (Sharp), evaporating disk, steel cabinet oven (Shel Lab), surgical blade (GEA), mice cage equipped with drink bottle, disposable syringe 1 mL (OneMed).

Materials included *Piper sarmentosum* Roxb. leaves, metformin tablets (500 mg, Hexfarm), sodium carboxymethyl cellulose (Na-CMC, technical grade), 70% ethanol (technical grade), and distilled water.

Animals were Swiss-Webster mice with a weight of approximately 20 g. A commercial high-fat diet (HFD-60%; BioCalorix, Faculty of Pharmacy, Widya Mandala Catholic University Surabaya,

Indonesia) was used to induce IR. The HFD contained approximately 338.9 g fat, 180.2 g protein, and 340.0 g carbohydrates per kilogram of diet, corresponding to 60%, 14%, and 26% of total energy, respectively, with a metabolizable energy of approximately 5,131 kcal/kg.

Research Path

1. Extract Preparation

Fresh leaves were collected Balai Penelitian Tanaman Rempah dan Obat, Kota Bogor, Jawa Barat, Indonesia and were authenticated by Arifin Surya Dwipa Irsyam (https://www.scopus.com/authid/detail.uri?authorId=57211286941; https://herbarium.sith.itb.ac.id/profil-kurator/), a certified botanist at the School of Life Sciences and Technology, Bandung Institute of Technology (Bandung, West Java, Indonesia), (https://herbarium.sith.itb.ac.id/koleksi/). The material was confirmed to be *Piper sarmentosum* Roxb. of the family Piperaceae (document number 7696/IT1.C11.2/TA.00/2025) with characteristics that matched those described in the reference.

The dried materials (1700 g) were put in a blender and ground. The powdered material was extracted by maceration using 70% ethanol for 3 x 24 hours. The extract was collected, filtered, rotary evaporated at 50 °C and 40 rpm, and further heated with a water bath set at 50 °C until the ethanol extract of *P. sarmentosum* (EEPS) was obtained (279.085 g).

2. Determination of Total Phenol Content (TPC) and Total Flavonoid Content (TFC)

TPC and TFC were analyzed based on the procedures specified in the Indonesian Herbal Pharmacopoeia (Farmakope Herbal Indonesia, 2nd Edition, 2017). For TPC determination, gallic acid solutions at concentrations of 20, 40, 60, 80, and 100 were employed as calibration standards, while quercetin at the corresponding concentrations was used for TFC determination. TPC was measured using a spectrophotometer at 765 nm, while TFC was measured at 410 nm. The results were expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract) for TPC and milligrams of quercetin equivalents per gram of extract (mg QE/g extract) for TFC.

3. Effects of EEPS on the Weight Gain, Abdominal Circumference, and Liver Index in Mice Fed a High-Fat Diet

The animal handling procedure is approved by the Research Ethics Committee, Universitas Padjadjaran, Indonesia

(https://kep.unpad.ac.id/; approval document number 147/UN6.KEP/EC/2026).

A total of 25 adult male Swiss-Webster mice weighing 20-30 g were randomly divided into five groups (n = 5/group). IR was induced by feeding the mice an HFD for 14 days. The animals were subsequently assigned to five groups: (1) the normal control group, which received standard feed and Na-CMC suspension; (2) the negative control group, which received a high-fat diet along with Na-CMC suspension; (3) the positive control group, which was administered a high-fat diet and metformin as the standard treatment in Na-CMC suspension; (4) the test I group, which received a high-fat diet and EEPS at a dose of 250 mg/kg in Na-CMC suspension; and (5) the test II group, which received a high-fat diet and EEPS at a dose of 500 mg/kg in Na-CMC suspension.

After 14 days of HFD induction, mice received EEPS or metformin once daily by oral gavage during the treatment period, while continuing the HFD. At the end of the experimental period, the animals were sacrificed, and the liver was excised and weighed for analysis.

$$\text{Liver index (\%)} = \frac{liver\ weight\ (g)}{body\ weight\ (g)}\ x\ 100$$

4. Statistical Analysis

Data were tested for normality and homogeneity of variance and were found to meet the assumptions required for ANOVA. One-way ANOVA was then employed to assess differences among the study groups, followed by post hoc comparisons using the least significant difference (LSD) test. Data are presented as mean ± SD, with statistical significance established at $p < 0.05$.

RESULTS AND DISCUSSION

The preparation of *P. sarmentosum* extract yielded 279.085 g, or 16.06%, which fulfills the requirement for *Piper* extract in the Indonesian Herbal Pharmacopoeia 2017 (Kementerian Kesehatan Republik Indonesia, 2017).

TPC and TFC of EEPS were determined to be 72.5106 mg GAE/g extract and 27.3398 mg QE/g extract, respectively. Compared with the findings of Nguyen et al. (2020), our extract exhibited a higher TPC but a lower TFC, whereas the previously reported values were 60.61 ± 0.96 mg GAE/g and 70.14 ± 0.38 mg QE/g, respectively (Nguyen et al., 2020).

The 14-day experimental period was selected based on preliminary optimization conducted before the main study, which showed that BioCalorix HFD increased body weight after 7 days of feeding. This approach

was further supported by previous studies demonstrating that short-term HFD feeding is sufficient to induce early metabolic alterations. For example, Dedual et al. (2019) reported that four days of HFD feeding impaired glucose tolerance and induced hepatic steatosis in C57BL/6J mice. Although metabolic alterations may vary depending on the duration of HFD feeding, diet composition, and animal model used, a 14-day experimental period was considered sufficient to evaluate the effects of EEPS on HFD-induced metabolic alterations. In the present study, these alterations were assessed through changes in body weight, abdominal circumference, and liver index (Dedual et al., 2019).

The percentage change in body weight among the different treatment groups is presented in Figure 1.

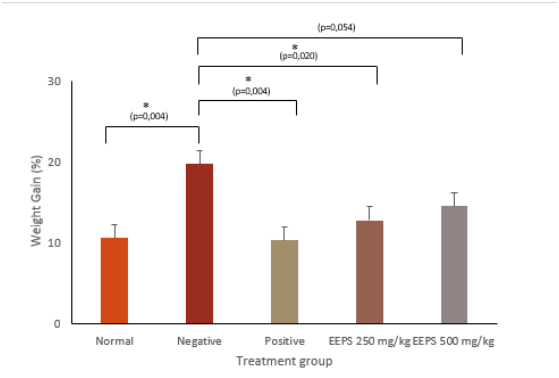

Figure 1. Effects of EEPS on the % weight gain of mice fed a high-fat diet

Excessive body weight gain is considered an important factor in the onset and progression of IR. This condition is generally linked to increased adipose tissue accumulation and obesity due to impaired insulin action, specifically its inability to inhibit hepatic glucose production and to enhance glucose uptake by adipose tissue.

During the 14-day induction period, the mice exhibited an increase in body weight, indicating that the induction protocol not only induced insulin resistance but also promoted body weight gain in the experimental animals. As depicted in Figure 1, EEPS-treated mice displayed a tendency toward lower body weight gain compared with the negative control group (Yan, 2024).

Body weight increased by 12.80% and 14.50% in the groups treated with EEPS at doses of 250 mg/kg and 500 mg/kg, respectively, compared with 19.70% in the negative control group. Notably, the lower dose was more effective in attenuating body weight gain than the higher dose. However, administration of EEPS at 500 mg/kg did not result in a statistically significant reduction in body weight gain compared with the negative control group ($p > 0.05$).

Overall, EEPS treatment did not show a proportional dose–response effect on body weight gain. The higher dose did not provide additional benefit compared with the lower dose, suggesting a non-linear dose–response relationship. These findings may indicate

that EEPS at 250 mg/kg is more effective in limiting body weight gain in HFD-fed mice.

Dose escalation of EEPS did not result in enhanced efficacy in attenuating body weight gain. This observation may reflect the occurrence of a ceiling effect, whereby increasing the dose beyond an optimal range fails to elicit further biological responses. In addition, the diverse bioactive constituents contained within the extract may interact antagonistically at higher concentrations, potentially reducing the overall therapeutic effect and preventing a proportional increase in efficacy (Lema, Ningsih, and Nopiyanti, 2015).

The significant difference observed between the positive and negative control groups ($p < 0.05$) may reflect the pharmacological effects of metformin. One proposed mechanism involves the upregulation of circulating Growth Differentiation Factor 15 (GDF15), formerly termed macrophage inhibitory cytokine-1 (MIC-1), a stress-responsive member of the transforming growth factor beta (TGF-β) superfamily. Produced by multiple tissues, GDF15 interacts with Glial Cell-Derived Neurotrophic Factor Family Receptor Alpha-Like (GFRAL) in the brainstem to regulate appetite and energy intake. This signaling pathway decreases food consumption and may consequently contribute to the attenuation of body weight gain in metformin-treated animals (Al-Kuraishy and Batiha, 2022; Kaneto et al., 2021).

A hallmark of obesity is the excessive accumulation of visceral adipose tissue, especially in the abdominal cavity. Excessive visceral fat accumulation, accompanied by reduced leptin responsiveness and cytokine infiltration within adipose tissue, promotes an increase in intracellular free fatty acid concentrations. Metabolic abnormalities, such as hyperinsulinemia and IR, have been associated with increased concentrations of circulating FFA (Bilbina, Zurriyani, and Feriyani, 2024). These findings suggest that EEPS may reduce abdominal fat accumulation associated with the HFD feeding group, as shown in Figure 2.

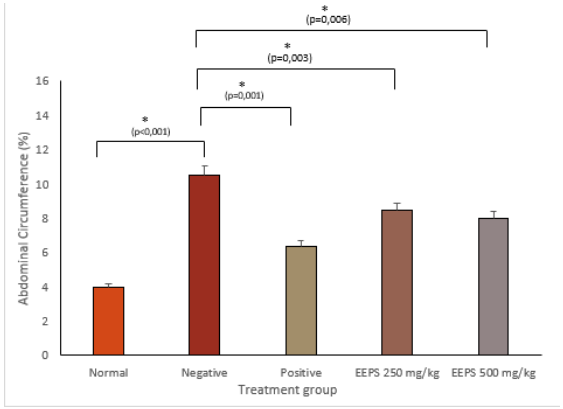

Figure 2. Effects of EEPS on the % abdominal circumference of mice fed a high-fat diet

These results indicate a positive association between body weight and abdominal circumference, whereby higher body weight is accompanied by increased

abdominal circumference in experimental animals. EEPS at a dose of 250 mg/kg reduced the increase in abdominal circumference by 8.44%, while EEPS at 500 mg/kg showed an 8.36% change. In contrast, the negative control group exhibited a 10.53% increase. These findings suggest that the treatment may have attenuated abdominal fat accumulation, which is commonly observed under diabetic conditions (Zhao et al., 2023).

The liver index was evaluated as an additional parameter in this study. Variations in liver index appeared to be associated with differences in body weight among the experimental animals. Figure 3 presents the percentage of liver weight relative to body weight in each treatment group.

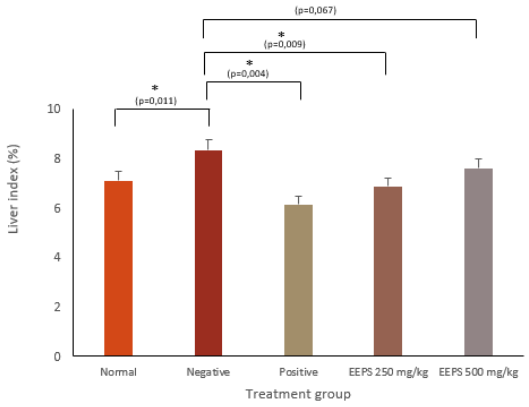

Figure 3. Effects of EEPS on the liver index of mice fed a high-fat diet

The elevated liver index observed in the negative control group may indicate morphological alterations in the liver, potentially resulting from lipid accumulation induced by prolonged consumption of an HFD. This observation is consistent with the characteristics of insulin resistance, which is frequently associated with increased ectopic lipid deposition in hepatic tissue (Deng et al., 2024).

Treatment with EEPS at 250 mg/kg significantly reduced liver index compared with the negative control group, whereas the 500 mg/kg dose showed no significant effect. A reduction in liver index of 6.87% was observed at the 250 mg/kg dose, while a reduction of 7.61% was observed at the 500 mg/kg dose. Overall, changes in liver index following EEPS administration did not show a clear dose–response relationship, suggesting that increasing the dose does not necessarily result in a greater effect (Sukandar, Qowwiyah and Larasati, 2011).

CONCLUSION

This study demonstrated that EEPS at 250 mg/kg significantly attenuated body weight gain, reduced abdominal circumference, and lowered liver index in mice fed a high-fat diet.

ACKNOWLEDGEMENT

The authors thank Dr. apt. Tita Nofianti, M.Si., at the Faculty of Pharmacy, Bakti Tunas Husada University, for all the support and facilitation in this study.

REFERENCES

Abbasi, E., & Khodadadi, I. (2025). High-fat diet may increase the risk of insulin resistance by inducing dysbiosis. *Metabolism Open*, *27*, 100381. https://doi.org/10.1016/j.metop.2025.100381

Al-Kuraishy, H. M., Al-Gareeb, A. I., Alexiou, A., Papadakis, M., Nadwa, E. H., Albogami, S. M., Alorabi, M., Saad, H. M., & Batiha, G. E. (2022). Metformin and growth differentiation factor 15 (GDF15) in type 2 diabetes mellitus: A hidden treasure. *Journal of Diabetes*, *14*(12), 806–814. https://doi.org/10.1111/1753-0407.13334

Azelan, A., Taher, Z. M., Sasano, S., Ariga, T., & Aziz, A. A. (2020). Chemical constituents and bioactivity of Piper sarmentosum: A Mini Review. *Food Research, 4*, 14–18. https://doi.org/10.26656/fr.2017.4(S2).S10

Bilbina, A., Zurriyani, Z., & Feriyani, F. (2024). Hubungan antara lingkar perut dengan diabetes melitus pada pasien Poli Penyakit Dalam di RS Meuraxa. *Media Kesehatan Masyarakat Indonesia, 23*(1), 18-23. https://doi.org/10.14710/mkmi.23.1.18-23

Chen, J., Xiao, Y., Li, D., Zhang, S., Wu, Y., Zhang, Q., & Bai, W. (2023). New insights into the mechanisms of high-fat diet-mediated gut microbiota in chronic diseases. *iMeta*, *2*(1), e69. https://doi.org/10.1002/imt2.69

Dedual, Mara A., Wueest, Stephan., Borsigova, Marcela., Konrad, Daniel. (2019). Intermittent fasting improves metabolic flexibility in short-term high-fat diet-fed mice. *American Journal of Physiology-Endocrinology and Metabolism, 317:5,* E773-E782. https://doi.org/10.1152/ajpendo.00187.2019

Deng, M., Li, Z., Chen, S., Wang, H., Sun, L, Tang, J., Luo, L., Zhang, X., Xu, H., & Dai, Z. (2024). Exploring the heterogeneity of hepatic and pancreatic fat deposition in obesity: implications for metabolic health. *Frontiers in Endocrinology, 15*, 1447750. https://doi.org/10.3389/fendo.2024.1447750

International Diabetes Federation. (2025). *IDF Diabetes Atlas (11th ed.)*. International Diabetes Federation. https://diabetesatlas.org

Kementerian Kesehatan Republik Indonesia. 2017. Farmakope Herbal Indonesia Edisi II.

Kumar, S. R., Mohd Ramli, E. S., Abdul

Nasir, N. A., Mohd Ismail, N., & Mohd Fahami, N. A. (2021). Methanolic Extract of *Piper sarmentosum* Attenuates Obesity and Hyperlipidemia in Fructose-Induced Metabolic Syndrome Rats. *Molecules (Basel, Switzerland)*, *26*(13), 3985. https://doi.org/10.3390/molecules26133985

Lema, A. E., Ningsih, D., & Nopiyanti, V. (2015). Aktivitas antihiperglikemik ekstrak air daun sukun (Artocarpus altilis (Park.) Fosberg) terhadap tikus diabetes yang diinduksi aloksan. *J Farm Indones*, *12*, 94-101.

Nguyen, N. Q., Nguyen, V. T., Van, N. T. H., & Vo., T. N. M. (2020). Bioactive compounds and antioxidant activity of leaves from Piper sarmentosum Piperaceae. *IOP Conference Series: Materials Science and Engineering 991*(1). https://doi.org/10.1088/1757899X/991/1/012028

Sui, X., Liu, Y., Zhao, J., Wang, Z., & Zhang, G. (2025). Dietary flavonoids may improve insulin resistance: NHANES, network pharmacological analyses and in vitro experiments. PloS one, 20(12), e0338100. https://doi.org/10.1371/journal.pone.0338100

Sukandar, E. Y., Qowiyyah, A., & Larasari, L. (2011). Effect of methanol extract hearhleaf madeiravine (Anredera Cordifolia (Ten.) Steenis) leaves on blood sugar in diabetes mellitus model mice. *Jurnal Medika Planta*, *1*(4), 1-10.

Verkouter, I., Noordam, R., le Cessie, S., van Dam, R. M., Lamb, H. J., Rosendaal, F. R., van Heemst, D., & de Mutsert, R. (2019). The Association between Adult Weight Gain and Insulin Resistance at Middle Age: Mediation by Visceral Fat and Liver Fat. *Journal of Clinical Medicine*, *8*(10), 1559. https://doi.org/10.3390/jcm8101559

Yan K. (2024). Recent advances in the effect of adipose tissue inflammation on insulin resistance. *Cellular signalling*, *120*, 111229. https://doi.org/10.1016/j.cellsig.2024.111229

Zhao, X., An, X., Yang, C., Sun, W., Ji, H., & Lian, F. (2023). The crucial role and mechanism of insulin resistance in metabolic disease. *Frontiers in endocrinology*, *14*, 1149239. https://doi.org/10.3389/fendo.2023.1149239.