



Original Article

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Wedelolactone from *Eclipta prostrata* (L) L. suppresses inflammation and improves insulin resistanceTrinh Tat Cuong^{1✉}, Duong Duc Thien¹, Hoang Hai Yen², Nguyen Anh Duc², Trinh Quang Nam³, Do Viet Khanh⁴¹Key Laboratory for Enzyme and Protein Technology, Vietnam National University University of Science, Hanoi, Vietnam²Faculty of Biology, Vietnam National University University of Science, Hanoi, Vietnam³Hanoi University of Pharmacy, Hanoi, Vietnam⁴Vinschool, Hanoi, Vietnam

ABSTRACT

Objective: To evaluate the effects of wedelolactone, a major flavonoid from Vietnamese *Eclipta prostrata* (L) L., on inflammation and insulin resistance.

Methods: Wedelolactone was extracted from the leaves of Vietnamese *Eclipta prostrata* (L) L. with methanol by Soxhlet. The effects of wedelolactone on lipopolysaccharide (LPS)-induced cytokine production, reactive oxygen species (ROS) generation, and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activities in Raw 264.7 cells were measured by enzyme-linked immunosorbent assay (ELISA), specific immunofluorescent dyes and luminometric analysis, respectively. In addition, its effects on glucose uptake and the protein expression of insulin receptor substrate 1 (IRS1) and glucose transporter 4 (GLUT4) were examined in 3T3-L1 cells by immunofluorescent dyes and Western blot.

Results: Wedelolactone at 30 µg/mL significantly inhibited LPS-induced production of tumor necrosis factor-α, interleukin (IL)-6, and IL-8 ($P < 0.01$) with no noticeable effects on IL-10 level. It also reduced ROS generation and NADPH oxidase activities in LPS-stimulated Raw 264.7 cells ($P < 0.01$). Furthermore, wedelolactone showed anti-insulin resistance activity, as evidenced by improved glucose uptake and the upregulated expression of IRS1 and GLUT4 in 3T3-L1 cells ($P < 0.01$).

Conclusions: Wedelolactone exhibits anti-inflammation and anti-insulin resistance effects, which may be used for the treatment of diabetes and inflammation-associated diseases.

KEYWORDS: Wedelolactone; *Eclipta prostrata*; Inflammatory responses; Anti-insulin resistance; Diabetes; IRS1; GLUT4

1. Introduction

Eclipta prostrata (L.) (*E. prostrata*) L., a traditional medicinal herb, has been employed for centuries in various parts of the world due to its remarkable therapeutic properties. Its applications range from alleviating liver damage resulting from alcohol overdose and jaundice to combating inflammatory conditions[1]. In India, it has been used as a folk remedy for the treatment of infective hepatitis[2], while in China, it is utilized in the management of septic shock[1]. Wedelolactone is a key compound from *E. prostrata* with a wide spectrum of biological effects such as anti-inflammation[3,4], anticancer[4–7], antidiabetes[8,9], anti-pyoptosis[10,11], anti-

Significance

Wedelolactone, as a flavonoid, has a wide range of biological activities. This study demonstrates the role of wedelolactone in anti-inflammation and anti-insulin resistance. Wedelolactone inhibited lipopolysaccharide-induced proinflammatory cytokines and reactive oxygen species in Raw 264.7, and increased glucose uptake and the expression of IRS1 and GLUT4 in 3T3-L1 cells. Further *in vivo* tests need to be carried out to verify its efficacy.

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Alzheimer[12], anti-hepatotoxicity[13], and anti-osteolysis[14].

Chronic inflammation and insulin resistance are two closely intertwined pathophysiological processes that significantly contribute to the development and progression of type 2 diabetes mellitus (T2DM)[15]. This debilitating metabolic disorder, characterized by hyperglycemia and impaired insulin sensitivity, has reached epidemic proportions worldwide, imposing a substantial burden on healthcare systems and affecting individual quality of life[15]. Amidst the quest for effective therapeutic strategies, natural compounds derived from medicinal plants have emerged as promising candidates for the management of chronic inflammation and insulin resistance. Beyond its bioactivity qualities, wedelolactone has exhibited a profound ability to modulate the intricate system of cellular events underpinning inflammation[4]. Chronic inflammation, often characterized by elevated proinflammatory cytokines and aberrant activation of transcription factor NF- κ B, plays a pivotal role in the onset and progression of insulin resistance and T2DM[15]. Wedelolactone has been reported to inhibit NF- κ B-mediated transcription, thereby downregulating the expression of caspase-1[1], a critical regulator of proinflammatory cytokine IL-1 β maturation and pathological apoptosis[11]. This unique mechanism of action positions wedelolactone as a potential game-changer in the battle against chronic inflammation and insulin resistance, offering fresh insights into the management of T2DM. As the prevalence of T2DM continues to rise globally, innovative therapeutic strategies that address the intertwined issues of chronic inflammation and insulin resistance are of paramount importance. In this study, we examined the role of wedelolactone from Vietnamese *E. prostrata* (L.) L. in alleviating inflammation and insulin resistance.

2. Materials and methods

2.1. Plant material

E. prostrata (L.) L. samples were obtained in Thai Binh province, Vietnam. This plant was identified (TTC01) by the Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, Hanoi.

2.2. Chemicals

Lipopolysaccharide (LPS) and wedelolactone were purchased from Sigma-Aldrich (St. Louis, MO). ELISA kits for TNF- α , IL-6, IL-10, and IL-8 were obtained from BD Pharmingen (Franklin Lakes, NJ).

2.3. Extraction and isolation

Wedelolactone was extracted and isolated as previously described[16]. Methanol was added to the leaves of *E. prostrata* (L.) L. The mixture was then extracted using a Soxhlet extractor.

The residues were heated in a steam bath below 80 °C. The filtered aqueous phase was added with ethyl acetate and evaporated to a light brown powder. The powder was eluted with chloroform + methanol (70:30) before subjecting to fractionation by column chromatography on silica gel. The purified fraction of wedelolactone was analyzed by HPLC.

2.4. Cell culture

Raw 264.7 and 3T3-L1 cells (American Type Culture Collection; ATCC) were maintained in complete medium (DMEM with 10% FBS), sodium pyruvate, non-essential amino acids, penicillin G (100 IU/mL), and streptomycin (100 μ g/mL) in 95% humidity and 5% CO₂ at 37 °C. The steps of culture were performed according to the instructions so that cells reached 80%-85% confluency.

2.5. Cell viability assay

Raw 264.7 and 3T3-L1 cell viability was performed using a Cell Counting Kit-8 (CCK-8, Dojindo Laboratories, Kumamoto, Japan) as per the manufacturer's instructions. Briefly, cells at a density of 1×10^6 cells/well were incubated with varying concentrations of wedelolactone (10-40 μ g/mL). Then, 10 μ L of CCK-8 solution was incubated with cells for 1 h. Absorbance was measured at 450 nm using an enzyme-linked immunosorbent assay (ELISA) reader (Molecular Devices, San Jose, CA, USA).

2.6. Enzyme-linked immunosorbent assay (ELISA)

Raw 264.7 cells were treated with wedelolactone at varying concentrations (10, 15, 20, 25, 30, and 40 μ g/mL) in 0.1% dimethyl sulfoxide (DMSO, Sigma) for 45 min before stimulation with LPS. Supernatants were harvested 18 h after stimulation with 1 μ g/mL LPS to induce inflammation. Concentrations of TNF- α , IL-6, IL-8, and IL-10 in the culture supernatants were determined by ELISA reagents (BD Pharmingen).

2.7. Measurement of intracellular ROS

Raw 264.7 cells were incubated with or without wedelolactone (30 μ g/mL) for 45 min. Then, the cells were treated with LPS (60 min) and added with 2 μ M dihydroethidium (Calbiochem) for 15 min at 37 °C in 5% CO₂. The cells were examined by laser-scanning confocal microscopy (LSM 510) and a Carl Zeiss vision system (LSM510) and the mean relative fluorescence intensity for all groups was determined.

2.8. Measurement of NADPH oxidase activity

NADPH oxidase activities were evaluated as described previously[16]. Raw 264.7 cells were treated with wedelolactone (30

$\mu\text{g/mL}$) for 45 min. Then the cells were stimulated with LPS ($1 \mu\text{g/mL}$) for 60 min. NADPH oxidase activity in the cells was measured by a lucigenin chemiluminescence assay (Sigma).

2.9. Determination of insulin-induced glucose uptake

To analyze the anti-insulin resistance effect of wedelolactone, as previously described[17], 3T3-L1 cells were washed with phosphate-buffered saline (PBS, Sigma) after incubation with 50 ng/mL of TNF- α for 24 h and subsequent treatment with wedelolactone for 24 h. The cell was further washed with PBS and added with low-glucose Dulbecco's Modified Eagle Medium (DMEM, Sigma) for 3 h at 37°C . The medium was then changed with 1 mg/mL bovine serum albumin (BSA) containing 100 mmol/L 2-[N-(7-nitrobenz-2-oxa-1, 3-diazol-4-yl) amino]-2-deoxy-glucose (2-NBDG) and 100 nmol/L insulin, and incubated for 1 h at 37°C . A microplate reader (Hidex, USA) measured fluorescence intensity of intracellular 2-NBDG at $\lambda_{\text{ex}} = 485 \text{ nm}$ and $\lambda_{\text{em}} = 535 \text{ nm}$.

2.10. Western blot analysis

3T3-L1 cells were treated as indicated and processed for Western blotting, as previously described[18]. Briefly, 3T3-L1 cells were treated with TNF- α (1 ng/mL) for 4 d to induce insulin resistance. The cells were pretreated with solvent control or wedelolactone

($30 \mu\text{g/mL}$) or rosiglitazone maleate ($10 \mu\text{M}$) for 24 h. Then the cells were stimulated with insulin (100 nmol) for 5 min, harvested and lysed. Primary antibodies were used against the following proteins: insulin receptor substrate-1 (IRS1, Abcam, ab131487; 1:1 000), glucose transporter 4 (GLUT4, Sigma-Aldrich, G4048; 1:1 000), and β -actin (Sigma-Aldrich, A1978; 1:4 000). A secondary antibody was used either HRP-linked anti-rabbit antibodies (Cell signaling Technology). The membranes were developed by a chemiluminescence assay (ECL; Amersham-Pharmacia). The densitometry for each bands was assayed by a ImageLab software version 4.1 (Bio-Rad).

2.11. Statistical analysis

All the data are presented as mean $\pm$ SD of three independent experiments. One-way ANOVA analysis was used to analyze significant differences between groups at $P < 0.05$.

3. Results

3.1. HPLC of wedelolactone

Wedelolactone was isolated from Vietnamese *E. prostrata* (L.) L. as shown in Figure 1.

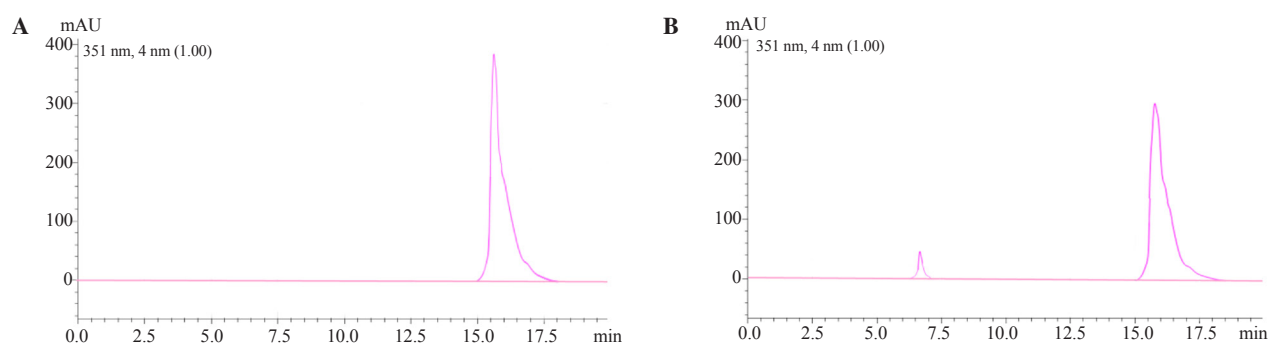


Figure 1. HPLC analysis of wedelolactone isolated from *Eclipta prostrata* (L.) L. (A) Standard wedelolactone; (B) Sample wedelolactone.

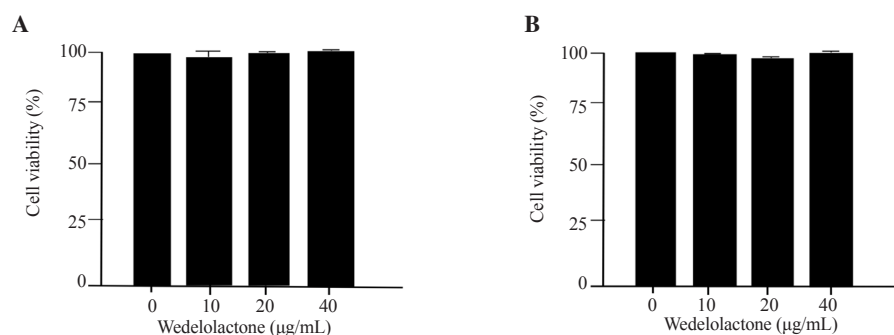


Figure 2. Effect of wedelolactone on the viability of (A) Raw 264.7 and (B) 3T3-L1 cells. Data are presented as mean $\pm$ SD of three independent experiments.

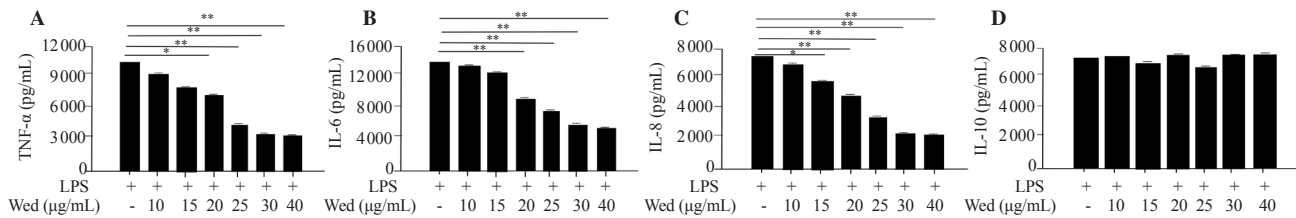


Figure 3. Effect of wedelolactone on (A) TNF- α , (B) IL-6, (C) IL-8, and (D) IL-10 in Raw 264.7 cells. Raw 264.7 cells were treated with wedelolactone at varying concentrations in 0.1% DMSO before stimulation with lipopolysaccharide (LPS). Concentrations of TNF- α , IL-6, IL-8, and IL-10 in the culture supernatants were determined by ELISA. Data are presented as mean $\pm$ SD of three independent experiments and analyzed by one-way ANOVA analysis. * P <0.05, ** P <0.01 compared with the LPS-stimulated group. Wed: wedelolactone.

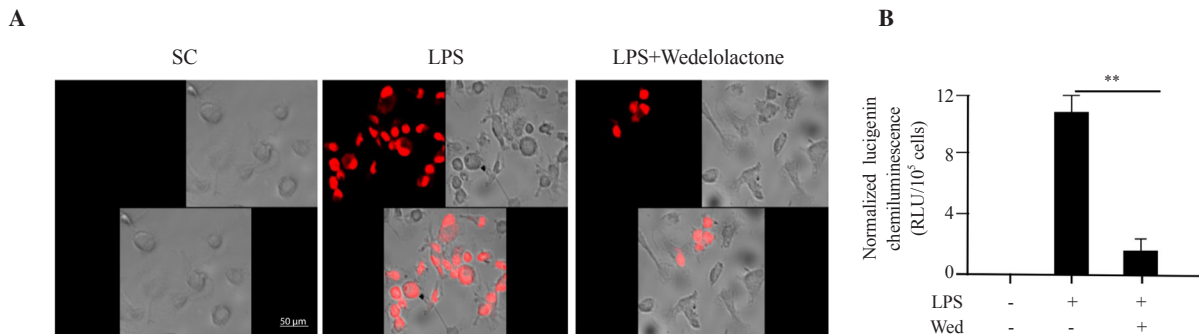


Figure 4. Effect of wedelolactone on the formation of reactive oxygen species and NADPH oxidase activity in LPS-induced Raw 264.7 cells. (A) The fluorescence intensity of the cells (scale bar: 50 μ m). (B) NADPH in the cells. Data are presented as mean $\pm$ SD of three independent experiments and analyzed by one-way ANOVA analysis. ** P <0.01 compared with the LPS-stimulated group. SC: solvent control.

3.2. Effects of wedelolactone on cell viability

The cytotoxicity of wedelolactone in Raw 264.7 cells and 3T3-L1 cells was measured by CCK-8 assay (Figure 2). The results showed that wedelolactone did not affect cell viability at different concentrations for 48 h. Therefore, 10–40 μ g/mL of wedelolactone was used for further experiments.

3.3. Wedelolactone inhibits cytokine production in LPS-stimulated Raw 264.7 cells

Figure 3 indicates that LPS caused a rise in IL-6, TNF- α , and IL-8 levels. Wedelolactone inhibited IL-6, TNF- α , and IL-8 in LPS-stimulated Raw 264.7 cells (P <0.01) in a concentration-dependent manner. However, no remarkable effects on IL-10 levels were observed.

3.4. Wedelolactone inhibits ROS production and NADPH

The production of ROS and NADPH in Raw 264.7 cells was significantly enhanced by LPS (P <0.01). As shown in Figure 4A, wedelolactone markedly suppressed LPS-stimulated ROS generation (P <0.01). It also significantly reduced the NADPH in LPS-treated

Raw 264.7 cells (P <0.01) (Figure 4B).

3.5. Wedelolactone alleviates TNF- α -induced insulin resistance in 3T3-L1 adipocytes

Figure 5 shows that wedelolactone significantly increased glucose uptake in 3T3-L1 cells dose-dependently, with 30 μ g/mL showing the superior effect (P <0.01). Wedelolactone improved TNF- α -induced insulin resistance by enhancing glucose uptake.

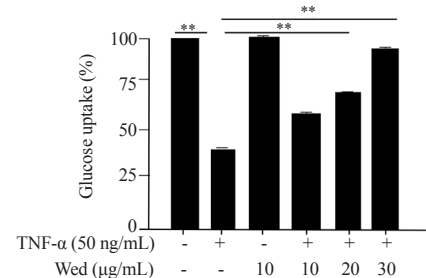


Figure 5. Effect of wedelolactone on glucose uptake in TNF- α -induced 3T3-L1 cells. Data are presented as mean $\pm$ SD of three independent experiments and analyzed by one-way ANOVA analysis. ** P <0.01 compared with the LPS-stimulated group.

3.6. Effect of wedelolactone on the expression of IRS1 and GLUT4 in 3T3-L1 cells

TNF- α is associated with the development of insulin resistance. It disrupts the expression of proteins involved in IRS1 and GLUT4. Therefore, we next examined the effects of wedelolactone on the expression of IRS1 and GLUT4. The results showed that the expressions of IRS1 and GLUT4 were very low in TNF- α -induced 3T3-L1 cells. However, wedelolactone markedly upregulated the expression of IRS1 and GLUT4 ($P < 0.01$, Figure 6).

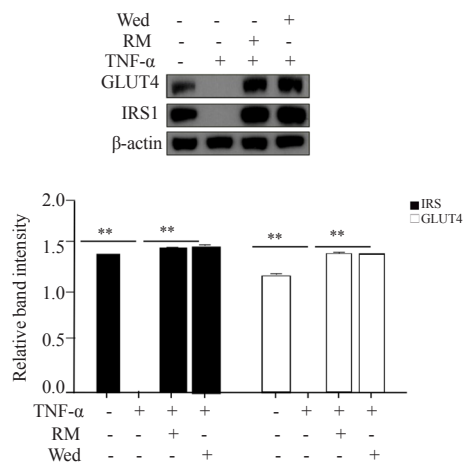


Figure 6. Effect of wedelolactone on the expression of insulin receptor substrate 1 (IRS-1) and glucose transporter 4 (GLUT4) in TNF- α -induced 3T3-L1 cells. The expression of IRS-1 and GLUT4 was determined by Western blot analysis and normalized against β -actin. Data are presented as mean $\pm$ SD of three independent experiments and analyzed by one-way ANOVA analysis. ** $P < 0.01$ compared with the LPS-stimulated group. RM: rosiglitazone maleate.

4. Discussion

In a previous study, we extracted wedelolactone from Vietnamese *E. prostrata* (L.) L, and found it inhibited inflammatory responses through Dectin-1 receptor[16]. In the present study, wedelolactone was isolated from Vietnam *E. prostrata* (L.) L to explore the effects on inflammation and insulin resistance. Overproduction of TNF- α , IL-6, and IL-8 has been implicated in several inflammatory diseases[17]. In the current study, wedelolactone could inhibit LPS-induced proinflammatory cytokines including TNF- α , IL-6, and IL-8 without noticeable effects on IL-10 in LPS-stimulated Raw 264.7 cells, suggesting that inflammation was reduced. However, wedelolactone did not inhibit LPS-induced p38 and ERK1/2 in Raw 264.7 cells[19] in the same way as our previous publication in zymosan-stimulated macrophages[16].

Increasing ROS in the activated macrophages could induce a host-defense mechanism and cellular or tissue damage leading to inflammation. Therefore, the inhibition of ROS could be a therapeutic approach to inflammation and its associated diseases[20].

Our results also indicated that wedelolactone significantly inhibited the LPS-induced ROS generation and NADPH oxidase activities in Raw 264.7. This result is similar to the ability of wedelolactone to inhibit ROS generation in embryonic cells[21]. Besides the ability to scavenge the free radicals and to inhibit inflammation[11], wedelolactone has been announced to support the treatment of rats with streptozotocin-induced diabetes by regulating the markers of oxidative stress as well as serum glycated proteins and blood glucose[8,9]. However, the ability of wedelolactone to protect the glucose absorption in adipose cells through TNF- α has not yet been reported. In this study, we found that wedelolactone (30 μ g/mL) could inhibit TNF- α -induced insulin resistance in adipocytes as shown by increased glucose uptake. Furthermore, IRS are cytoplasmic receptors that determine insulin signaling[22,23]. After this receptor becomes activated, several signaling chains are activated. This leads to many downstream effects being triggered that are directly related to the insulin signals that govern intracellular pathways. When the receptor pairs with insulin on the cell surface, IRS-1 is phosphorylated and activates phosphatidylinositol 3-kinase. The intracellular signals lead to a cascade of cellular responses, including activation of GLUT receptors (glucose transporter) that help cells increase glucose uptake[23]. In clinical treatment, the potency of IRS-1 and IRS-2 in adipocytes has been reduced in people with non-insulin-dependent diabetes mellitus[24]. In the present study, wedelolactone significantly increased IRS and GLUT4 expression in 3T3-L1 cells.

In summary, we demonstrated anti-inflammation and anti-insulin resistance of wedelolactone from Vietnamese *E. prostrata* (L.) L. Together, our data suggest the potential use of wedelolactone for the modulation of acute inflammatory status and anti-insulin resistance. Further investigation is needed to verify its efficacy.

Conflict of interest statement

The authors declare no conflicts of interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding author upon request.

Authors' contributions

TTC and DDT designed the study. HHY, DVK and TQN performed experimental studies. DDT and NAD performed the statistical analysis. TTC and DDT contributed to the final version of the manuscript. TTC supervised the project.

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