Original Article

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Centella asiatica (L.) extract attenuates inflammation and improve insulin sensitivity in a coculture of lipopolysaccharide (LPS)-induced 3T3-L1 adipocytes and RAW 264.7 macrophages

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Summary **Insulin resistance in obese condition is related to chronic low-grade inflammation which leads to insulin signaling impairment. *Centella asiatica* (L.) is an herb that exhibits anti-inflammatory and blood sugar-lowering activity (hypoglycemia). The study aims to investigate the molecular mechanism of *C. asiatica* extract in insulin sensitivity improvement in a coculture of lipopolysaccharide (LPS)-induced 3T3-L1 adipocytes and RAW 264.7 macrophages. A coculture of 3T3-L1 adipocytes and RAW 264.7 macrophages were incubated with LPS to induce insulin resistance in the adipocytes. An extract of *C. asiatica* was added to coculture cells and after 24 hours, insulin sensitivity and inflammatory response were determined, including glucose consumption, glucose transporter-4 (GLUT-4), insulin receptor substrate-1 (IRS-1), and interleukin-6 (IL-6) mRNA expression. *C. asiatica* extract at a concentration of 500 µg/mL increased glucose consumption and induced GLUT-4 and IRS-1 mRNA expression significantly in a coculture of LPS-induced 3T3-L1 adipocytes and RAW 264.7 macrophages. The pro-inflammatory cytokines IL-6 mRNA expression was decreased in the coculture cells after treatment with *C. asiatica* extract at a concentration of 500 µg/mL. This result indicates that *C. asiatica* has an effect to stimulate glucose consumption in the coculture cells that might be mediated via GLUT-4/IRS-1 pathway as a result of IL-6 inhibition. These findings suggest that the *C. asiatica* extract inhibits inflammation and improves insulin sensitivity in a coculture of LPS-induced 3T3-L1 adipocytes and RAW 264.7 macrophages.**

Keywords: *Centella asiatica* L., insulin sensitivity, coculture, 3T3-L1 adipocytes, RAW 264.7 macrophages

1. Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disorder caused by a defect in insulin action called insulin resistance (*1*). Insulin resistance in obesity is associated with chronic low-grade inflammation resulting from an increase of macrophages infiltration into adipose tissues (*2*). An interaction between excessive adipocytes and the higher amount of macrophages promotes the macrophage activation into inflammatory macrophages (M2) followed by the secretion of pro-inflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and monocyte chemoattractant protein-1 (MCP-1) (*3*). Pro-inflammatory cytokines secreted from both adipocytes and activated macrophages may alter insulin sensitivity in adipocytes through the degradation and deactivation of insulin receptor substrate-1 (*IRS-1*), one of essential protein in the insulin receptor substrate-1/phosphoinositide 3-kinases/Akt (IRS-1/PI3K/Akt) insulin signaling pathway (*3,4*). The degradation of IRS-1 may trigger the downregulation of proteins downstream in the IRS-1/PI3K/Akt pathway, including glucose transporter-4 (*GLUT-4*) expression leading to

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the disruption of glucose uptake in adipocytes (*5*).

Anti-diabetic drugs efficacy in controlling blood glucose in diabetic patients has been established. However, they also have undesirable side effects such as weight gain, bone loss and increased cardiovascular risk (*6*). Therefore, safer and more effective alternative agents are needed and developing new drugs from herbal is a promising method.

Centella asiatica (L.) or Pegagan in Bahasa Indonesia is one of the most important herbal medicine used empirically in Indonesia. The herb has been proven to possess hypoglycemic activity in in-vivo models. Recent studies reported that *C. asiatica* extract decreased glucose plasma levels in high fat diet (HFD)-induced and streptozotocin-induced diabetic mice in dose- dependent manner (*7,8*). One of the active compounds in *C. asiatica*, asiatic acid has also been shown as an anti-inflammatory and a hypoglycemic agent (*10,11*). Recent studies reported that asiatic acid decrease secretion of pro-inflammatory cytokines in the liver tissues of diabetic mice and inhibit LPS-induced inflammatory response in endometrial epithelial cells (*12,13*). However, there is minimal information about molecular mechanism of *C. asiatica* extract in improving insulin sensitivity related to insulin resistance caused by inflammation. In the present study, we investigated the effect of *C. asiatica* extract on the insulin sensitivity of adipocytes using a coculture of LPS-induced RAW 264.7 macrophages and 3T3-L1 adipocytes. This study also evaluated involvement of the IRS-1/GLUT-4 pathway in relation to insulin sensitivity effect. The expression of the pro-inflammatory cytokine IL-6 was also investigated to determine *C. asiatica* effect on the inflammatory changes related to insulin sensitivity impairment.

2. Materials and Methods

2.1. *C. asiatica extract preparation*

A standardized *C. asiatica* extracts was produced by Javaplant PT, Tri Rahardja Tawangmangu Surakarta Indonesia, in September of 2016. Extraction was performed using water as a solvent and following good manufacturing practices. *C. asiatica* herbs were soaked in water at boiling point followed by stirring with a magnetic stirrer for 5 h. Herbs were removed from the heat sources then filtered to obtain the concentrated extract. The freeze-drying method was used to obtain dried powder and then extract powder was dissolved in a Dimethyl Sulfoxide (DMSO) to prepare samples for testing.

2.2. *Cell lines and culture*

3T3-L1 preadipocyte cells and RAW 264.7 murine macrophage cells were obtained from the Food and Science Technology Department of National Pingtung Science Technology (NPUST, Pingtung, Taiwan). Both cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco, New York, USA) containing 1% penicillin/streptomycin (PS, 100 units of penicillin/mL and 100 pg streptomycin/mL, Gibco, New York, USA) and supplemented with 10% New-born Calf Serum (NBCS, Gibco, New York, USA) to culture the 3T3-L1 cells and 10% Fetal Bovine Serum (FBS, Gibco, New York, USA) to culture RAW 264.7 cells. The cells were incubated at 37°C in a humidified 5% CO_2 atmosphere and the medium was replaced every 2 days.

2.3. *Viability assay*

RAW 264.7 macrophages were seeded at 2.4×10^4 cells/well and 3T3-L1 pre-adipocytes were seeded at 1.5×10^4 cells/well. Cells were cultured in a various concentrations (62.5-1,000 µg/mL) of the *C. asiatica* extract and then, the cells were incubated at 37°C in a humidified 5% CO_2 atmosphere for 24 h. Viability assay of the 3T3-L1 pre-adipocyte cells and RAW 264.7 macrophage cells were performed using a previously described 3-[4,5- dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay protocol (*14*). Safe concentrations of the extract were determined and used in further experiment.

2.4. *Coating the plates*

Gelatin 1.3% (Sigma Aldrich, Saint Louis, USA) was dissolved in phosphate buffered saline (PBS). Transferred 2 mL of gelatin working solution into each well and then, plates were incubated at 37°C for 1 h. The plates were dried for 30 min and sterilized by an Ultraviolet (UV) lamp for 15 min.

2.5. D*ifferentiation and coculture of 3T3-L1 adipocyte cells and RAW 264.7 macrophage cells*

3T3-L1 pre-adipocytes were differentiated into mature adipocytes in gelatin-coated 6 well plate using a previously described protocol (*15*). RAW 264.7 macrophages were plated onto differentiated 3T3-L1 in serum-starved DMEM and incubated at 37°C in a humidified 5% CO_2 atmosphere for 3 h. Lipopolysaccharide (Sigma Aldrich, Saint Louis, USA) at a concentration of 0.125 µg/mL was added to the cells in the FBS-supplemented DMEM medium and the cells were incubated at 37°C in a humidified 5% CO_2 atmosphere for 48 h. As a control, equal numbers of RAW 264.7 macrophages and differentiated 3T3-L1 cells were cultured separately in the same medium with a coculture. After 48 h, *C. asiatica* extract dissolved in DMEM containing FBS 2% was added to a coculture cells at three different concentration (125, 250 and 500

µg/mL) followed by incubation at 37°C in a humidified 5% CO_2 atmosphere for an additional 24 h. Cells were incubated in serum-starved DMEM containing 1 µg/mL insulin (Sigma Aldrich, Saint Louis, USA) for 30 min. The medium and cells were collected and stored at -20°C until use.

2.6. *Determination of glucose consumption*

Glucose concentration contained in the cell medium was measured using Glucose GOD FS glucose kit assay (Diasys Diagnostics, Holzheim, Germany) and was performed following the manufacturer's protocol with slight modifications. Reagents were added after 20 µL of cells media was plated onto 96 well-plate. The plates were then incubated at 25°C for 20 min. Absorbance at 540 nm was recorded using a microplate reader (Thermo Fisher Scientific Multiskan, Ratastie, Finland). All the samples were measured in triplicate.

2.7. *Quantitative of polymerase chain reaction*

A coculture of adipocytes and macrophages treated with the extract was harvested after 24 h of incubation. Control of RAW 264.7 macrophages and differentiated 3T3-L1 were harvested separately and then mixed in a single tube as a control without a coculture. Total RNA was isolated in each group according to manufacturer protocol (Geneaid biotech Ltd, New Taipei, Taiwan). Expression of GLUT-1, IRS-1 and IL-6 mRNA were measured by quantitative polymerase chain reaction (qPCR) using a Sensifast SYBR No-ROX One-Step Kit Master Mix Kit (Bioline, London, United Kingdom) and analyzed on an Eco™ Real-Time PCR instrument (Illumina Inc, San Diego, USA). The following primers were used for the qPCR: GLUT-4, 5'-TTGCACACGGCTTCCGAACG-3' (forward) and 5'-GATCTGCTGGAAACCCGACGG-3'; IRS-1 5'-CCATGAGCGATGAGTTTCGC-3' (reverse) and 5'- GCAGTGATGCTCTCAGTTCG; IL-6 5'-GAGTCACAGAAGGAGTGGCTAAG-3' (forward) and 5'-ACCACAGTGAGGAATGTCCAC-3' (reverse); and β-actin 5'-CTCTGGCTCCTAGCACCATGAAGA-3' (forward) and 5'-GTAAAACGCAGCTCAGTAAC AGTCCG-3' (reverse). The qPCR reaction cycling parameters for all genes were performed at 45°C for 10 min for reverse transcriptase activation, followed by a pre-incubation condition step at 95°C for 2 min and amplification for 40 cycles at 95°C for 5 sec, 62°C for 15 sec and 72°C for 5 sec. Gene expression was calculated using the relative expression ΔΔCq method and β-actin was used as a normalizer. All samples were amplified in duplicate.

2.8. *Statistical analysis*

Data were expressed as the mean ± SD. Statistical analysis were performed using the unpaired Student t-test and analysis of variance (ANOVA) followed by post-hoc tests. A value of $p < 0.05$ was considered significant.

3. Results

3.1. *Viability assay of the C. asiatica extract on 3T3-L1 preadipocytes and RAW 264.7 macrophages*

To investigate *C. asiatica* extract effect on insulin sensitivity related to inflammatory changes in a coculture of differentiated 3T3-L1 and RAW 264.7 macrophages, we first evaluated its cytotoxicity at various concentration. Cells were treated with *C. asiatica* extract at concentrations up to 500 µg/mL showed viability more than 90% (Figure 1), suggesting that *C. asiatica* extract is safe and non-toxic up to a concentration of 500 µg/mL in both of 3T3-L1 pre-adipocytes and RAW 264.7 macrophages.

3.2 *Coculture of RAW 264.7 macrophages in contact with 3T3-L1 adipocytes induced insulin resistance in adipocytes and altered macrophages morphology*

Before we examine insulin sensitivity effect of *C. asiatica* extract on coculture cells, we evaluated adipocytes insulin responsiveness in a direct coculture of LPS-induced 3T3-L1 adipocytes and RAW 264.7 macrophages. Cellular contact in direct coculture under LPS treatment triggered morphological changes in the macrophages. RAW 264.7 macrophages appeared rounded without the LPS treatment and the macrophages became elongated with short extension appearance after LPS treatment. With 48 hours of the direct coculture, macrophages showed an elongated appearance with a long cellular extension between and

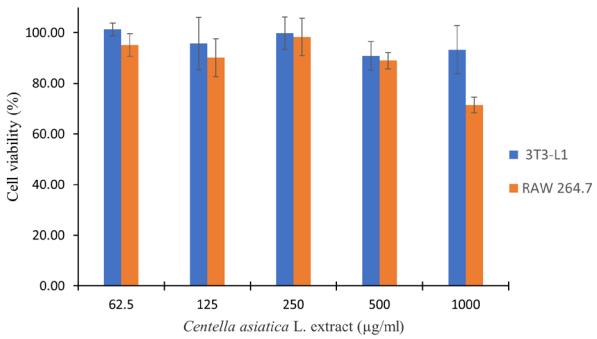

Figure 1. Effects of *Centella asiatica* (L.) extract on RAW 264.7 macrophages and 3T3-L1 pre-adipocytes viability. RAW 264.7 macrophages and 3T3-L1 preadipocytes were culturing in DMEM high glucose supplemented by New Calf Bovine Serum (NCBS) 10% for culturing 3T3-L1 pre-adipocytes and Fetal Bovine Serum (FBS) 10% for culturing RAW 264.7 macrophages in 96 well plate for 24 h. The cells then were incubated at 37°C in a humidified 5% CO_2 with various concentration of *Centella asiatica* (L.) extract for 24 h and cell viability was determined using the MTT assay. Extract at a concentration up to 500 µg/mL showed cell viability more than 90% in both of the cells. Values are expressed as means ± SD.

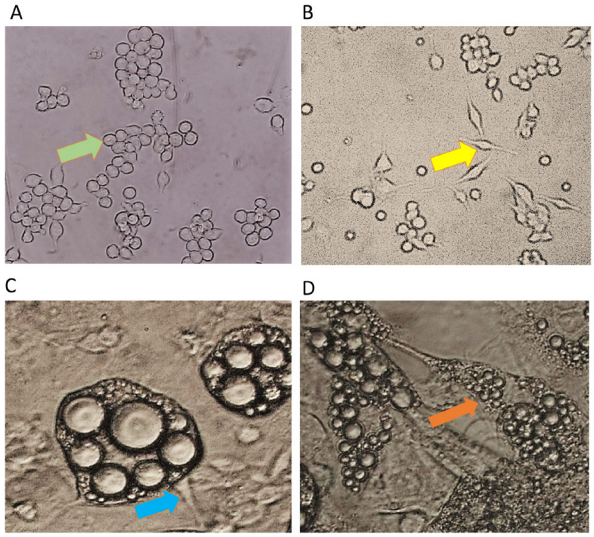

Figure 2. Morphological changes of RAW 264.7 macrophages without coculture and a direct coculture with adipocytes. Both of cells were culturing in DMEM high glucose supplemented by fetal bovine serum (FBS) 10% in 6 well plate and incubated at 37°C in a humidified 5% CO_2 for 48 h. Morphology of the cells were observed under microscope: **(A)** RAW 264.7 macrophages without LPS appeared small rounded (green arrow); **(B)** RAW 264.7 macrophages under LPS treatment showed elongated appearance with a short extension (yellow arrow); **(C)** Culture RAW 264.7 macrophages in contact with adipocytes under LPS treatment showed elongated appearance with a long cellular extension of RAW 264.7 macrophages between and along the surrounding adipocytes (blue arrow) and **(D)** Direct coculture between RAW 264.7 macrophages and differentiated 3T3-L1 adipocytes under LPS treatment developed lipid vacuoles accumulated in the macrophages cytoplasm (orange arrow).

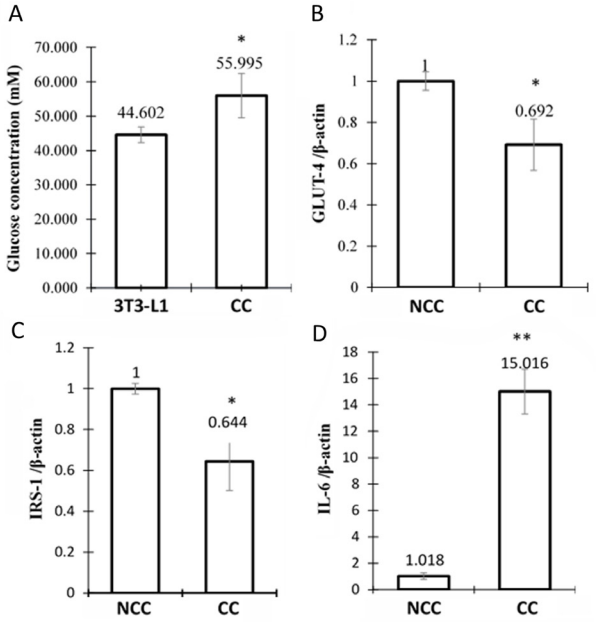

Figure 3. Insulin sensitivity and inflammatory response in control of coculture of LPS-induced adipocytes and macrophages (CC) and control of adipocytes and macrophages without co-culture (NCC). Parameters were measured after cells were incubated at 37°C in a humidified 5% CO_2 for 48 h and insulin induction for 30 minutes. **(A)** glucose concentration; **(B)** GLUT-4 mRNA; **(C)** IRS-1 mRNA; **(D)** IL-6 mRNA. Glucose concentration were measured using glucose assay kit and expression of target genes were quantified using qPCR. Values are mean ± Standard Deviation. *$p < 0.05$, *vs*. NCC; **$p < 0.01$, *vs*. NCC.

along the surrounding adipocytes (Figure 2) resembling the morphology of adipose tissue macrophages (ATM) isolated from obese mice (*16*). However, direct contact between 3T3-L1 adipocytes and RAW 264.7 macrophages developed an accumulation of lipid vacuoles in the macrophages cytoplasm referred to as lipid-laden giant multinucleated. The results showed that a direct coculture of 3T3-L1 adipocytes and RAW 264.7 macrophages under LPS induction for 48 h stimulates changes in the macrophages behavior.

Insulin sensitivity was assessed by evaluating insulin-stimulated glucose consumption in a coculture of 3T3-L1 adipocytes and RAW 264.7 macrophages. Glucose concentration contained in a coculture medium indicates how much glucose was taken up by the coculture cells. Our study showed there were higher glucose concentration contained in the coculture of adipocytes and macrophages medium compared to the adipocytes medium only ($p = 0.046$, Figure 3A) indicating that an interaction between the adipocytes and macrophages under LPS treatment stimulated glucose uptake impairment in the adipocytes.

In this study, we determined insulin-stimulated GLUT-4 mRNA expression as a glucose transporter in adipocytes cells in contact with RAW 264.7 macrophages. We observed significantly lower GLUT-4 mRNA expression in a coculture of LPS-induced 3T3-L1 adipocytes and RAW 264.7 macrophages compared to the sum of the GLUT-4 mRNA expression by adipocytes and macrophages without a co-culture ($p = 0.016$, Figure 3A). This result is consistent with the glucose consumption data previously.

Glucose transport involves insulin signaling pathways after insulin stimulation. The IRS1/PI3K/Akt pathway is essential for insulin-regulated glucose transport by activating and translocating GLUT-4 from intracellular vesicles to the plasma membrane. This study determined the mRNA expression of IRS-1. The result showed that IRS-1 mRNA expression in coculture of LPS-induced adipocytes and macrophages was significantly lower ($p = 0.045$) compared to the sum of the IRS-1 mRNA expression by adipocytes and macrophages without a co-culture (Figure 3C).

To evaluate inflammatory changes caused by the interaction between adipocytes and macrophages under LPS treatment, we assessed pro-inflammatory cytokine IL-6 mRNA expression in a coculture of 3T3-L1 adipocytes and RAW 264.7 macrophages. The coculture cells showed significantly higher of IL-6 mRNA expression compared to the sum of the IL-6 mRNA expression by adipocytes and macrophages without a co-culture ($p < 0.01$, Figure 3D). This result suggested that direct contact between adipocytes and macrophages under LPS treatment induced an

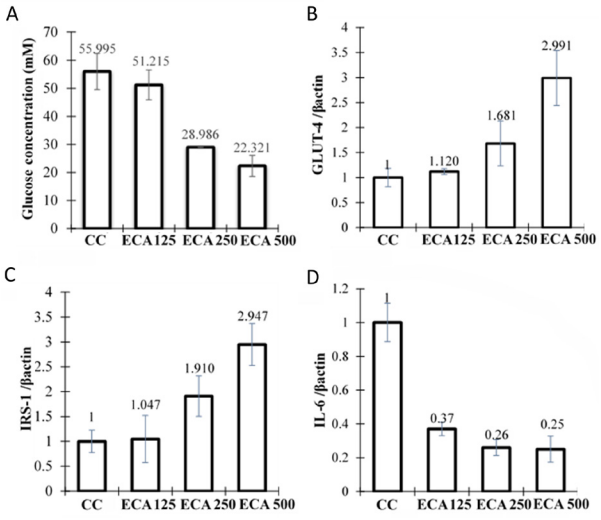

Figure 4. Insulin sensitivity and inflammatory response in control of coculture (CC) and coculture after treatment with *Centella asiatica* (L.) extract (ECA). Parameters were measured after both of cells incubated with a various concentration of *Centella asiatica* L. extract (125, 250, and 500 µg/mL) at 37°C in a humidified 5% CO_2 for 24 h, followed by insulin induction for 30 min. **(A)** glucose concentration; **(B)** GLUT-4 mRNA; **(C)** IRS-1 mRNA; **(D)** IL-6 mRNA. Glucose concentration were measured using glucose assay kit and expression of target genes were quantified using qPCR. Values are mean ± Standard Deviation. *$p < 0.05$, *vs*. CC; ***$p < 0.001$, *vs*. CC.

inflammatory response, which in turn, influence the adipocytes insulin sensitivity that might have mediated *via* the IRS-1/GLUT-4 pathway.

3.3. *Glucose consumption in a coculture of LPS-induced 3T3-L1 adipocytes and RAW 264.7 macrophages after 24 h treatment of C. asiatica extract*

To evaluate *C. asiatica* extract effect to improve insulin sensitivity, we determined glucose concentration contained in a coculture of LPS-induced adipocytes and macrophages medium after treated by *C. asiatica* extract at a various concentration (125, 250, and 500 µg/mL) for 24 h. Glucose concentration contained in a coculture LPS-induced of adipocytes and macrophages medium after treated with *C. asiatica* extract at concentration 250 and 500 µg/mL showed significantly lower concentrations compared to a differentiated adipocytes in a dose-dependent manner ($p < 0.01$ and $p < 0.01$, respectively, Figure 4A). This result indicated that *C. asiatica* extract improved glucose consumption in a coculture cells.

3.4. *GLUT-4 and IRS-1 mRNA expression in a coculture of LPS-induced 3T3-L1 adipocytes and RAW 264.7 macrophages after a 24 h treatment with C. asiatica extract*

A co-culture of adipocytes and macrophages treated with *C. asiatica* extract at a concentration of 500 µg/mL showed significantly higher GLUT-4 and IRS-1 mRNA expression compared to a control of coculture of adipocytes and macrophages ($p < 0.01$ and $p < 0.01$, respectively) as shown in Figures 4B and 4C. This result suggested that improvement of glucose consumption in a coculture cells after treated with *C. asiatica* extract at a concentration of 500 µg/mL might be related to the upregulation of GLUT-4 and IRS-1 mRNA expression in the adipocytes.

3.5. *Pro-inflammatory cytokine IL-6 mRNA expression in a coculture of LPS-induced 3T3-L1 adipocytes and RAW 264.7 macrophages after 24 h treatment with C. asiatica extract*

A direct interaction between adipocytes and macrophages aggravates inflammatory changes in adipose tissue marked by the increased production of pro-inflammatory cytokines such as IL-6, TNF-α and IL-1β. This study determined pro-inflammatory cytokine IL-6 mRNA expression in a coculture of LPS-induced adipocytes and macrophages after treatment with *C. asiatica* extract. We observed that extract at a concentration of 500 µg/mL attenuates inflammatory response in cells marked by significantly lower IL-6 mRNA expression in a coculture of adipocytes and macrophages compared to control of coculture of adipocytes and macrophages as shown in Figure 4D ($p = 0.026$).

4. Discussion

C. asiatica is a promising herb with anti-inflammatory and hypoglycemic activity. This study aimed to investigate the effect of *C. asiatica* extract on insulin sensitivity improvement related to the inflammatory response and its insulin signaling mechanism in a coculture of LPS-induced 3T3-L1 adipocytes and RAW 264.7 macrophages.

Direct contact between adipocytes and macrophages stimulates cytokines pro-inflammatory production such as IL-6, TNF-α, MCP-1, and IL-1β which may, in turn alter insulin sensitivity in adipocytes (*16,17*). In this study, a direct coculture of 3T3-L1 adipocytes and RAW 264.7 macrophages treated with TLR-4 lipopolysaccharide (LPS) showed that direct contact between adipocytes and macrophages under LPS induction stimulated insulin resistance in the adipocytes. A coculture of adipocytes and macrophages showed glucose consumption impairment followed by degradation of GLUT-4 and IRS-1 mRNA. The coculture of adipocytes and macrophages under LPS induction also elevated IL-6 mRNA expression compared to the sum of IL-6 expression in adipocytes and macrophages without a co-culture. This result suggested that upregulated of *IL-6* gene expression caused by direct contact and LPS induction in a

coculture cells might lead to IRS-1 degradation resulting in GLUT-4 downregulation. GLUT-4 as a glucose transport is a key protein in the regulation of glucose in adipose tissue thus decreasing GLUT-4 mRNA expression should affect insulin sensitivity in adipocytes. It means that higher expression of pro-inflammatory cytokines in adipocyte tissue plays a pivotal role in the disruption of insulin sensitivity.

Insulin signaling impairment caused by pro-inflammatory cytokines might be influenced by upregulation of suppressor of cytokine signaling (SOCS)1/3 protein. A study conducted by Rui found that the activation and upregulation of the SOCS1/3 protein caused by pro-inflammatory cytokines altered insulin sensitivity in inflammation-induced adipocytes that have been shown to reduce IRS1/2 proteins by targeting them through ubiquitin-mediated proteasomal degradation (*18*).

C. asiatica extract at a concentration of 500 µg/mL was shown to improve insulin sensitivity in a coculture of LPS-induced 3T3-L1 adipocytes and RAW 264.7 macrophages. Increasing glucose consumption in a coculture of LPS-induced adipocytes and macrophages after treatment with the extract at a concentration of 500 µg/mL was followed by upregulation of *GLUT-4* and *IRS-1* gene expression in adipocytes. This result indicates that upregulation of *GLUT-4* and *IRS-1* gene expression in a coculture cells after treated with extract at a concentration of 500 µg/mL might be contributed to insulin sensitivity improvement in adipocytes.

C. asiatica extract at a concentration of 500 µg/mL attenuated inflammatory response in a coculture of LPS-induced 3T3-L1 adipocytes and RAW 264.7 macrophages by inhibiting the pro-inflammatory cytokine IL-6. This finding is consistent with the gene expression of *GLUT-4*/*IRS-1* and the glucose consumption results, indicating that the effect of *C. asiatica* extract to improve insulin sensitivity might be mediated through the IRS-1/GLUT-4 pathway as a result of inhibiting the pro-inflammatory cytokine IL-6.

Recent studies reported that leaf aqueous extract of *C. asiatica* at a concentration of 50 mg/kg of body weight has a glucose-lowering effect in alloxan-induced diabetic rats compared to diabetic and anti-diabetic drug groups (*19*). Other studies have also shown that an ethanol extract of *C. asiatica* has an anti-diabetic effect at a concentration of 200 mg/kg of body weight based on blood-glucose serum level compared to the control group (*20*). However, asiatic acid, as an active compound in *C. asiatica*, has anti-inflammatory activity by downregulating the pro-inflammatory cytokines IL-6, IL- 1β, and TNF-α in human corneal epithelial cells and the liver tissue of diabetic-induced mice (*12,13*). In addition, asiatic acid exerts anti-hyperglycemia effect in high fat diet (HFD)-induced diabetes mice through the PI3K/Akt/GSKβ signaling pathway (*21*). Another previous study showed that asiatic acid at a dose of 20 mg/kg body decreased blood-glucose level in diabetic-induced rat using streptozotocin (STZ) followed by improved glucose uptake into skeletal muscle tissues *via* the IRS-1/PI3K-Akt signaling pathway (*22*).

IRS-1 is a key intracellular molecule that mediates insulin signaling through the IRS-1/PI3K/Akt pathway. Defects in or degradation of IRS-1 in adipocytes may be causes of insulin resistance (*23*). *IRS-1* gene disruption in obese mice impaired glucose transports, suggesting that insulin sensitivity in adipocytes is dependent on the expression of the IRS-1 protein (*24-26*). Upregulated *IRS-1* gene expression after treatment with *C. asiatica* at a concentration of 500 µg/mL might be related to its inflammation inhibition effect inducing macrophages to polarize into "macrophage alternatively activated" M2. A study conducted by Hawas found that treatment with *C. asiatica* at doses of 500 and 100 mg/kg of body weight prevented M1/M2 ratio from increasing in diabetic rats (*27*). This effect might trigger the deactivation of c-Jun-NH terminal kinase (JNK) and decrease SOC1/3 expression which in turn affects IRS-1 upregulation. Increasing *GLUT-4* and *IRS-1* gene expression in adipocytes after treatment with *C. asiatica* might contribute to the adipocytes ability to take up glucose upon insulin stimulation.

In conclusion, *C. asiatica* extract alleviated the inflammatory response by inhibiting the pro-inflammatory cytokine IL-6 and improved insulin sensitivity in adipocytes marked by stimulated glucose consumption in a coculture cells that might be mediated *via* IRS-1/GLUT-4 insulin signaling pathway. However, further studies are required to evaluate more fully understanding about molecular actions of *C. asiatica* extract in insulin sensitivity improvement in adipocytes related to inflammatory changes.

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